



HAL
open science

Latence du VIH-1 et réplication des Coronavirus : nouvelles approches thérapeutiques.

Sébastien Pasquereau

► **To cite this version:**

Sébastien Pasquereau. Latence du VIH-1 et réplication des Coronavirus : nouvelles approches thérapeutiques.. Biologie cellulaire. Université Bourgogne Franche-Comté, 2021. Français. NNT : 2021UBFCE011 . tel-03545680

HAL Id: tel-03545680

<https://theses.hal.science/tel-03545680>

Submitted on 27 Jan 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

THESE DE DOCTORAT DE L'ETABLISSEMENT UNIVERSITE BOURGOGNE FRANCHE-COMTE

PREPAREE A L'UNIVERSITE DE BOURGOGNE FRANCHE-COMTE

Ecole doctorale n°554

Environnement-Santé

Doctorat de Biologie Cellulaire

Par

PASQUEREAU Sébastien

Latence du VIH-1 et réplication des Coronavirus : nouvelles approches thérapeutiques

Thèse présentée et soutenue à Besançon, le 8 Octobre 2021

Composition du Jury :

Toussirost Eric	Professeur, Université de Bourgogne Franche-Comté	Président
Van Lint Carine	Professeure, Université Libre de Bruxelles, Belgique	Rapporteuse
Schwartz Christian	Maitre de conférences, Université de Strasbourg	Rapporteur
Herbein Georges	Professeur, Université de Bourgogne Franche-Comté	Directeur de thèse

Remerciements

Je souhaite remercier en premier lieu le Pr Georges Herbein, qui a accepté de m'accueillir au sein de son laboratoire et de son équipe. Au cours de ces années à travailler pour lui, j'ai côtoyé un scientifique passionné, souhaitant toujours aller plus loin, et voulant transmettre autour de lui ce feu intérieur qui l'anime. Je le remercie pour son aide, ses conseils et sa volonté de nous pousser à donner le meilleur de nous-même. J'ai énormément appris à ses côtés, sur le plan scientifique et sur le plan personnel. Je lui suis reconnaissant de m'avoir donné ma chance quand peu d'autres l'auraient fait. Je le remercie sincèrement de m'avoir donné l'opportunité d'en arriver là où j'en suis aujourd'hui.

Je remercie grandement le Pr Carine Van Lint, le Pr Eric Toussiroot et Dr Christian Schwartz, qui m'ont fait l'honneur d'accepter de composer le jury de cette thèse. Je remercie en particulier le Pr Van Lint et le Dr Schwartz d'avoir accepté d'être rapporteurs de ma thèse.

Je souhaite remercier le Dr Amit Kumar, estimé collègue et ami, qui m'a vu débiter au laboratoire et m'a permis, à travers son aide et ses conseils, de devenir autonome au laboratoire. Sa gentillesse et sa bonne humeur constantes font de lui une des meilleures rencontres que j'ai faites. Je lui suis éternellement redevable de tout ce qu'il m'a appris.

Je souhaite remercier mes autres collègues désormais parties poursuivre leurs routes. Je remercie le Dr Fatima Al Moussawi, dont la gentillesse rendait chaque jour de travail plus facile. Je remercie le Dr Zeina Nehme, avec qui nous avons partagé les bons moments comme les moments de doute. J'ai apprécié les longues discussions que nous avons eues, et le soutien mutuel que nous avons pu nous donner durant les longs mois de pandémie de Covid-19. Son aide a été précieuse, au laboratoire comme en dehors.

Je remercie ma collègue Sandy Haidar Ahmad, pour sa constante bonne humeur et pour son rire qui illumine nos journées de travail. Je la remercie pour toutes les discussions que nous avons eues et pour son aide volontaire. Je remercie ma collègue Ranim El Baba pour sa gentillesse et pour sa disponibilité.

Je remercie vivement le Dr Stéphanie Morot-Bizot pour son soutien.

Je remercie tous mes amis qui m'ont toujours soutenu et avec qui j'ai des souvenirs impérissables. Les copains de la musique, Berkan, Serkan, Faust, Thomas, Thibaud, Florentin, Eric, Kevin, les copains de copains, Alexis, Alex, Thomas (Pipin), Simon, Robin, les copines de copains des copains (ou un truc comme ça) Clara, Raphaëlle, Philomène, Mathilde, Audrey, Camille, Marianne. Merci à vous tous pour votre amitié.

Je remercie ma famille pour leur soutien sans faille, et leurs efforts. Merci Pierre, Alain, Michelle et Dominique. Merci le zinz Jacques-Michel. Merci Papa et Maman. Merci d'être toujours là pour moi, merci d'être aussi patients, compréhensifs, merci pour votre soutien, je ne serais pas allé aussi loin sans vous. Merci Amélie « choupinou » pour ton soutien, malgré la distance. Merci à toi, à Alex et à Hugo de me mettre du baume au cœur quand j'en ai besoin.

Merci au Dr Rémi « Piou » Meyer, le copain sur qui on peut compter pour discuter doctorat, stress, musique, film, bière, l'univers, tout et rien. Merci pour ton amitié et pour ton aide dans la rédaction de cette thèse.

Et enfin un immense merci au Dr Coralie « Nyû » Joucla. Après toutes ces années à se connaître, notre amitié est à toute épreuve. Je ne pense pas que cette thèse aurait été au bout sans toi. Merci d'être là pour me tirer vers le haut quand c'est nécessaire. Tu l'as dit toi-même, tu es de ma famille.

Merci à vous tous que j'ai eu le plaisir de côtoyer durant cette aventure.

Table des matières

1.	Généralités sur les virus.....	3
1.1.	Description et définitions.....	3
1.2.	Classification des virus.....	3
1.3.	Emergence des zoonoses virales.....	4
1.4.	Une zoonose pandémique : le VIH.....	5
1.5.	Nouveaux virus émergents.....	5
1.6.	Emergence des coronavirus.....	6
2.	Virus de l'immunodéficience humaine.....	7
2.1.	Caractéristiques du VIH.....	7
2.2.	Structure du génome viral.....	7
2.3.	Groupes et sous-types.....	8
2.4.	Structure et protéines de la particule virale.....	9
2.5.	Enzymes virales.....	10
2.6.	Protéines régulatrices.....	13
2.7.	Cycle de vie du virus.....	20
2.8.	Tropisme cellulaire et réservoirs.....	21
2.9.	Mécanismes moléculaires de la latence.....	24
2.10.	Rôle d'Akt dans l'infection.....	25
2.11.	Traitements et stratégies thérapeutiques.....	26
3.	Les Coronavirus.....	31
3.1.	Caractéristiques des Coronavirus.....	31
3.2.	Structure du génome viral.....	32
3.3.	Structure et protéines de la particule virale.....	33
3.4.	Cycle de vie des coronavirus.....	36
3.5.	Pathogénicité et interaction avec l'hôte.....	39
3.6.	Traitements.....	39
4.	Ciblage du TNF et des récepteurs TNF dans l'infection par le VIH.....	45
4.1.	Voies de signalisation du TNF et des récepteurs TNF dans l'infection par le VIH.....	45
4.2.	Interaction des protéines du VIH avec les récepteurs TNF et les voies de signalisation en aval.....	45
4.3.	Thérapies anti-TNF et impact sur les réservoirs de latence du VIH.....	46

4.4.	Article paru dans la revue Viruses en 2017 « Targeting TNF and TNF Receptor Pathway in HIV-1 Infection: from Immune Activation to Viral Reservoirs »	46
5.	Traitement des infections virales par des thérapies ciblant la régulation épigénétique.	65
5.1.	VIH et épigénétique	65
5.2.	Cytomégalovirus humain et épigénétique.....	65
5.3.	Thérapie épigénétique des infections virales	65
5.4.	Article paru dans la revue Clinical Epigenetics en 2019 « Control of viral infections by epigenetic-targeted therapy »	66
6.	Etude de la réactivation virale dans les lymphocytes T CD4+ quiescents de patients avirémiques.....	85
6.1.	Comparaison des inhibiteurs de protéase et des inhibiteurs non nucléosidiques de la reverse transcriptase sur la réactivation virale dans les lymphocytes T CD4+ de patients infectées.....	85
6.2.	Impact du type de traitement sur la réactivation virale	85
6.3.	Impact du type de traitement sur la taille du réservoir viral intégré	85
6.4.	Discussion.....	86
6.5.	Article paru dans la revue Scientific Reports en 2016 « Limited HIV-1 Reactivation in Resting CD4 + T cells from Aviremic Patients under Protease Inhibitors »	86
7.	Etude de l'activation de la voie de signalisation Akt par Nef et impact sur la réactivation	95
7.1.	Nef exogène entre dans les lymphocytes et augmente l'activation d'Akt.	95
7.2.	Nef active PI3K et Akt par une interaction directe, entraînant l'activation des voies NFκB et du CD28RE.	95
7.3.	Les inhibiteurs de protéase limitent l'activation d'Akt par Nef.	95
7.4.	Les inhibiteurs de protéase limitent la réactivation du VIH dans les lymphocytes T CD4+ quiescents.	96
7.5.	Discussion.....	96
7.6.	Article paru dans la revue Scientific Reports en 2016 « Tuning of AKT-pathway by Nef and its blockade by protease inhibitors results in limited recovery in latently HIV infected T-cell line »	97
8.	Etude de l'impact des inhibiteurs de protéase sur les réservoirs de latence virale et l'activation d'Akt dans l'infection par le VIH.....	113
8.1.	Impact du type de traitement antiviral sur l'infection par VIH-1	113
8.2.	Effet sur l'activation d'Akt par la protéine Nef du VIH	113
8.3.	Blocage de la réactivation du VIH in vitro	113
8.4.	Réduction de l'activation d'Akt ex vivo.....	114
8.5.	Discussion.....	114

8.6.	Article paru dans la revue Viruses en 2018 « Counteracting Akt Activation by HIV Protease Inhibitors in Monocytes/Macrophages »	115
9.	Etude de la réutilisation de traitements existants sur l'infection par les coronavirus.	137
9.1.	Evaluation de la toxicité des traitements sélectionnés.	137
9.2.	Evaluation de l'effet sur la réplication du HCoV-229E.	137
9.3.	Evaluation de l'effet sur la réplication du SARS-CoV-2.	137
9.4.	Discussion.....	138
9.5.	Article paru dans la revue Viruses en 2021 : « Resveratrol Inhibits HCoV-229E and SARS-CoV-2 Coronavirus Replication In Vitro ».....	138
10.	Extension de la recherche de traitements antiviraux ciblant les coronavirus à de nouvelles molécules.	151
10.1.	Utilisation du modèle de screening HCoV-229E pour l'évaluation de traitements anti-SARS-CoV-2.	151
10.2.	Sélection et évaluation de l'effet cytotoxique de petites molécules.	151
10.3.	Evaluation de l'effet anti-HCoV-229E.	151
10.4.	Evaluation de l'effet sur le SARS-CoV-2	151
10.5.	Discussion.....	152
10.6.	Ferulic acid derivatives block coronaviruses HCoV-229E and SARS-CoV-2 replication in vitro.	152
11.	Discussion générale.....	165
11.1.	Traitement du VIH.....	165
11.2.	Traitement des coronavirus.....	169
12.	Conclusions	173
13.	Références	175
	Liste des publications.....	202

Liste des figures

Figure 1-1 : Classification de Baltimore des virus animaux, tiré de Mahmoudabadi et Philips, 2018 [6]. Les virus sont classés selon le type d'acide nucléique.	4
Figure 2-1 : Structure de l'ARN génomique du VIH, d'après Ryu, 2017 [23].	8
Figure 2-2 : Groupes et sous-groupes du VIH	9
Figure 2-3 : Structure du virion du VIH. Diagramme schématique (A) et imagerie en microscopie électronique (B). [23]	9
Figure 2-4 : Mécanisme moléculaire d'action de la transcriptase inverse, d'après Sarafianos et al., 2009 [46].	11
Figure 2-5 : Mécanisme moléculaire d'action de l'intégrase virale, d'après Ingale et Manish, 2011 [49].	12
Figure 2-6 : Mécanismes moléculaires d'action de la protease, d'après Konvalinka et al., 2015 [53].	13
Figure 2-7 : Mécanismes moléculaires de la transcription virale, sous le contrôle de Tat, d'après Tabarrini et al., 2016 [62].	14
Figure 2-8 : Interactions moléculaires des protéines régulatrices du VIH avec les protéines cellulaires.	19
Figure 2-9 : Cycle de réplication du VIH. National Institute of Allergy and Infectious Diseases (NIAID)	20
Figure 2-10 : Mécanismes moléculaires de la latence virale. L'ADN proviral passe d'un état de transcription active (b) à un état de latence (a) réversible. D'après Churchill et al., 2014 [180].	25
Figure 2-11 : Stratégies épigénétiques de thérapies anti-VIH, selon les modèles Shock and Kill (A) ou Block and Lock (B). [205]	28
Figure 3-1 : Structure de l'ARN génomique des Coronavirus. [23]	32
Figure 3-2 : Structure du virion des coronavirus. Diagramme schématique (A) et imagerie en microscopie électronique (B). [23]	33
Figure 3-3 : Cycle de réplication des coronavirus, adapté de Ryu 2017 [23]	36
Figure 3-4 : Fonctions connues des protéines non structurales (nsp) des coronavirus.	38

Liste des tableaux

Tableau 2-1 : Liste des traitements anti-VIH	27
Tableau 2-2 : Liste des LRA ayant été testés <i>in vitro</i> et <i>in vivo</i> . Adapté de Ait-Ammar et al. 2020 [206].	29
Tableau 3-1 : Protéines non structurales (nsp) et leur fonctions, adapté de Rohaim et al., 2021 [238]	35
Tableau 3-2 : Liste des composés testés contre le SARS-CoV-2. Adapté de Valle et al. 2020 [286]	41

Liste des abréviations

ACE2 : Enzyme de conversion de l'angiotensine

ADN : Acide désoxyribonucléique

AF9 : Sous-unité du complexe de super élongation MLLT3

AFF : Protéine membre de la famille AF4/FMR2

Akt : Protéine kinase B

ARN : Acide ribonucléique

ARN Pol II : ARN polymérase II

ARNt : ARN de transfert

CA : Protéine de la capsid

cART : Thérapies antivirales combinées

CCR5 : Récepteur de chimiokine 5

CDK : Kinase cycline-dépendante

Covid-19 : Maladie à coronavirus 2019

CRF : Circulating recombinant form

CRM1 : Exportine 1

CTIP2 : Chicken ovalbumin upstream promoter transcription factor-interacting protein 2

CXCR4 : Récepteur de chimiokine 4

DNMT : ADN méthyltransférase

EAF : Facteur associé à ELL

ELL : Facteur d'élongation de l'ARN polymérase II

ENL : Sous-unité du complexe de super élongation MLLT1

Env : Protéine précurseur de l'enveloppe

EZH2 : Amplificateur de l'homologue zeste 2

gp120 : Glycoprotéine de 120 kDa

gp41 : Glycoprotéine de 41 kDa

HAT : Histones acétyltransférase

HCoV : Coronavirus humain

HMT : Histone méthyltransférase

IKK : Kinase I κ B

IL : Interleukine

IN : Intégrase virale

INF : Interféron

LRA : Agents d'inversion de la latence

LEDGF : Facteur de croissance dérivé d'épithélium du cristallin

LSF : Facteur de transcription alpha-globine CP2 / Facteur SV40 tardif

LTR : Long terminal repeat

MA : Protéine de la matrice

MBD2 : Protéine 2 du domaine de liaison méthyl-CpG

MERS-Cov : coronavirus du syndrome respiratoire du Moyent-Orient

MHC-I : Complexe majeur d'histocompatibilité de classe I

mTOR : Cible de la rapamycine chez les mammifères

NC : Nucléocapside

Nef : Facteur de régulation négative

NFAT : Facteur nucléaire des lymphocytes T activés

NF κ B : Facteur nucléaire kappa B

NNRTI : Inhibiteurs de la transcriptase inverse non-analogues nucléosidiques

NRTI : Inhibiteurs de la transcriptase inverse analogues nucléosidiques

Nsp : Protéines non structurales

ORF : Cadre de lecture ouvert

PBAF : Facteur associé à BRG/BRM polybromo-associé

Pbs : Primer binding site

PI3K : Phosphoinositide 3-kinase

PIC : Complexe de préintégration

PPT : Tractus polypurine

PR : Protéase virale

P-TEFb : Facteur d'allongement de transcription positif

REAF : Facteur antiviral précoce associé à l'ARN

Rev : Régulateur de l'épissage de l'ARN

RNase H : Ribonucléase H

RRE : Elément de réponse Rev

RT : Transcriptase inverse

RTC : Complexe viral de réplication et de transcription

SAMHD1 : Protéine 1 contenant un domaine HD et un domaine SAM

SARS-CoV : Coronavirus du syndrome respiratoire aigu sévère

SARS-CoV-2 : Coronavirus du syndrome respiratoire aigu sévère 2

SEC : Complexe de super élongation

SIDA : Syndrome d'immunodéficience acquise

SIV : Virus de l'immunodéficience simienne

Sp1 : Facteur de transcription spécificité protéine 1

SP2 : Peptide d'espacement 2

SU : Protéine de surface

TAR : Elément de réponse de transactivation

Tat : Protéine transactivatrice

TBP : Protéine de liaison à TATA

TET2 : Ten-eleven-translocation 2

TM : Protéine transmembranaire

TNF : Facteur de nécrose tumorale

UNG2 : Uracile-ADN glycosylase

Vif : Facteur d'infectivité virale

VIH : Virus de l'immunodéficience humaine

Vpr : Protéine virale r

Vpu : Protéine virale unique

Vpx : Protéine virale x

YY1 : Facteur de transcription Yin Yang 1

I. Introduction

1. Généralités sur les virus

1.1. Description et définitions

Les virus sont des microorganismes composés d'un ou plusieurs acides nucléiques (ADN ou ARN), liés à une capsidite faite de protéines, constituant ainsi la particule virale. Cette particule virale peut également comprendre une enveloppe contenant des protéines, des glucides et des lipides.

Les virus ont été initialement identifiés comme des agents infectieux responsables de maladies, non isolables par filtration, à l'inverse des bactéries, champignons ou parasites. Les virus sont des parasites cellulaires, dont le cycle de réplication utilise les ressources de l'hôte. Cela peut concerner des cellules procaryotes, comme les bactériophages infectant les bactéries, ou des cellules eucaryotes, incluant les cellules animales, végétales ou de mycètes. Il a également été démontré récemment l'existence de virus pouvant parasiter d'autres virus [1].

Bien que la classification et la taxonomie des virus fassent consensus, la définition même d'un virus est régulièrement remise en question, pour pouvoir y inclure les dernières découvertes. La taille constituait un de critères principaux de la définition des virus, jusqu'à la mise en lumière de l'existence de virus de grande taille, répondant aux autres critères, et infectant des amibes [2]. Cela a entraîné une modification de la définition, qui est recentrée sur la différence entre les organismes cellulaires, possédant une machinerie moléculaire de réplication, et les virus, qui possèdent une capsidite [3].

Les virus existent sous forme de particule virale ou sous forme de provirus. Une particule virale ou virion est un acide nucléique entouré d'une capsidite protéique, et capable d'infecter un nouvel hôte. La particule virale de certains virus possède également une enveloppe entourant la capsidite. Un provirus est le génome viral sous forme d'acide nucléique, intégré ou non au génome de l'hôte.

Les virus peuvent également être définis comme des entités ou organismes qui suivent un cycle de désintégration, par la perte de l'intégrité de leur capsidite, et de reconstitution, par la synthèse de nouvelles parties constituant une nouvelle capsidite [4]. Cette définition prend en compte le fait que le maintien de l'intégrité de l'information génétique d'un virus peut être suffisant pour la continuité de ce cycle. Cette définition fait particulièrement sens si l'on considère les phases de latence virale.

1.2. Classification des virus

La classification des virus peut être basée sur la nature des acides nucléiques du génome. La classification comprend les virus à ARN, dont le génome est un ARN, les virus à ADN, dont le génome est un ADN et les virus à transcriptase inverse, quelle que soit la nature du génome. Ces trois groupes sont subdivisés en fonction du mode de réplication du génome. On considère pour cette classification la polarité du brin d'acide nucléique (positif ou négatif) et s'il s'agit d'un simple ou d'un double brin. Cette classification basée sur le génome est également appelée « classification de Baltimore » [5]. Selon la classification de Baltimore, les virus animaux sont donc subdivisés en sept groupes : les virus à ADN double brin (groupe I), les virus à ADN simple brin (groupe II), les virus à ARN double brin (groupe III), les virus à ARN simple brin positif (groupe IV), les virus à ARN simple brin négatif (groupe V), les virus à transcriptase inverse à ARN (groupe VI) et les virus à transcriptase

inverse à ADN (groupe VII) (Figure 1-1). La classification des virus peut également inclure les caractéristiques morphologiques. La présence d'une enveloppe virale classe le virus dans le groupe des virus enveloppés, et son absence dans le groupe des virus non-enveloppés ou virus nus.

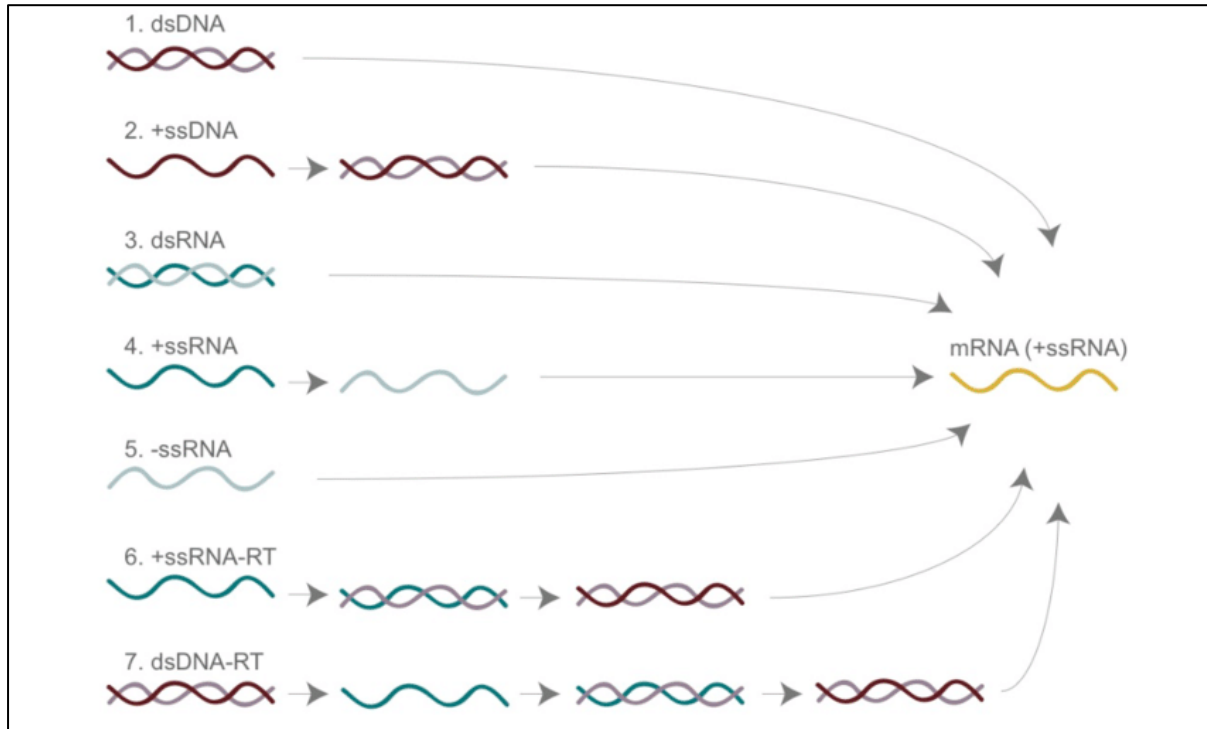


Figure 1-1 : Classification de Baltimore des virus animaux, tiré de Mahmoudabadi et Philips, 2018 [6]. Les virus sont classés selon le type d'acide nucléique.

Les virus font également l'objet d'une classification phylogénétique, suivant une taxonomie similaire à celle des organismes cellulaires, à savoir divisée en Ordres, Familles, Sous-familles, Genres et Espèces.

1.3. Emergence des zoonoses virales

Les zoonoses sont des maladies infectieuses provoquées par la transmission d'un pathogène d'un animal vers l'homme. Bien que ces virus ne soient pas adaptés pour se répliquer chez l'homme, quelques-uns parviennent à compléter leur cycle de réplication, franchissant ainsi la barrière des espèces [7]. Parmi ces virus ayant réussi cette première étape, seul un petit nombre peuvent se transmettre entre humains, devenant ainsi des virus humains.

Parmi ces zoonoses, certaines sont responsables d'épidémies, comme le virus de la grippe aviaire A, la grippe porcine ou le virus de la fièvre jaune. Certains virus dont l'homme est l'hôte principal ont pour réservoir épidémique une autre espèce animale, comme le virus de la dengue, ou le virus de la rage.

L'émergence de ces zoonoses peut conduire à l'apparition d'un événement épidémique, en particulier lorsque la transmission inter-humaine est importante. Une augmentation du nombre

d'épisodes épidémiques liés à des zoonoses virales a été constaté récemment, avec des conséquences importantes sur les systèmes de santé et sur les populations affectées [8,9].

1.4. Une zoonose pandémique : le VIH.

Un virus issu du franchissement de la barrière des espèces est le virus de l'immunodéficience humaine (VIH). Il s'agit d'une zoonose ayant pour origine les virus de l'immunodéficience simienne [10]. Son passage chez l'homme s'est accompagné d'une propagation pandémique.

Actuellement, la pandémie de VIH reste une menace importante pour la santé humaine : d'après le rapport de l'UNAIDS publié pour l'année 2020, plus de 37 millions de personnes sont infectées par le VIH, dont la majorité en Afrique sub-saharienne [11]. Depuis les premiers cas décrits en 1981, 36 millions de personnes sont mortes du syndrome d'immunodéficience acquise (SIDA), de par les caractéristiques uniques qui font du VIH un pathogène difficile à éradiquer.

En effet, le VIH utilise et trompe durablement le système immunitaire, se répliquant et détruisant les cellules immunitaires durant sa phase de répllication active. De plus, il persiste dans les cellules mémoire à longue durée de vie, sous la forme d'un provirus latent caché du système immunitaire. Aujourd'hui, les thérapies antivirales combinées (cART) préviennent efficacement le développement du SIDA et ce traitement, malgré sa toxicité et les problèmes métaboliques associés est nécessaire toute la vie pour les patients infectés [12]. En conséquence, l'infection par le VIH est passée d'une maladie mortelle à une maladie chronique. Cependant, les traitements actuels échouent toujours à éradiquer complètement le VIH, permettant à sa forme provirale de persister dans des réservoirs cellulaires conduisant à un rebond viral en cas d'arrêt du traitement. Une connaissance approfondie des déterminants cellulaires de la latence du VIH est donc cruciale pour permettre son éradication.

1.5. Nouveaux virus émergents

De nouveaux virus émergents, principalement des zoonoses, entraînent des épidémies, plus ou moins localisées. Pour la plupart des virus concernés, il n'existe pas de traitement curatif, ni de vaccin préventif qui pourraient réduire la propagation et l'impact sur les populations.

Le virus Zika, bien qu'identifié en 1947, a été responsable d'une épidémie importante en Amérique du Sud en 2015. Cette réémergence faisait suite à deux précédents épisodes épidémiques, en 2007 en Micronésie et en 2013 en Polynésie Française. [13]

Le virus Ebola a été responsable d'une épidémie en Afrique de l'Ouest, entre 2014 et 2016, résultant en plus de 10 mille morts [13]. Des épisodes épidémiques moins importants continuent de se produire régulièrement, notamment en République Démocratique du Congo en 2017, 2018, 2020 et 2021 et en Guinée en 2021 également.

Ces virus émergents représentent un risque sanitaire important par l'absence de traitements efficaces. Ils constituent un ensemble de virus peu étudiés et peu connus, ce qui limite considérablement la possibilité de développer des stratégies thérapeutiques efficaces.

1.6. Emergence des coronavirus

Un coronavirus à l'origine d'un syndrome respiratoire aigu sévère (SARS-CoV) a émergé en Asie du Sud-Est en 2002 avec une mortalité de 9.6% et un coronavirus du syndrome respiratoire du Moyen-Orient (MERS-CoV) a émergé au Moyen-Orient en 2012 avec une mortalité de 35.5%. Ces deux virus sont à l'origine de syndromes de détresse respiratoire aigüe chez l'homme. Leur origine se trouve dans des coronavirus infectant d'autres mammifères et ayant passé la barrière des espèces. Ces deux zoonoses ont été responsables d'épidémies largement mortelles.

L'émergence en décembre 2019, dans la ville de Wuhan en Chine, d'un nouveau coronavirus, nommé SARS-CoV-2 a conduit à une crise sanitaire mondiale [14]. Ce virus, responsable de la maladie à coronavirus 2019 (Covid-19), a entraîné la mise en place de mesures drastiques de limitation de la propagation à l'échelle mondiale. Bien que des vaccins préventifs aient été développés [15], il n'y a pour l'instant toujours pas de traitement curatif spécifique et efficace contre le SARS-CoV-2 [16]. L'approche initiale, guidée par l'urgence sanitaire, a consisté à réutiliser des traitements déjà validés pour d'autres pathologies et de tester leur efficacité antivirale sur le SARS-CoV-2. Etant donné les résultats intéressants mais néanmoins limités, la recherche de nouvelles molécules, plus spécifiques et plus efficaces, a logiquement suivi. Malgré les efforts actuels, la pandémie de Covid-19 continue d'impacter la population mondiale. D'après l'Organisation Mondiale de la Santé, au 5 Septembre 2021, plus de 220 millions de cas de Covid-19 ont été rapportés, entraînant plus de 4,5 million de morts.

2. Virus de l'immunodéficience humaine

Le virus de l'immunodéficience humaine (VIH) est un virus appartenant à la famille des *Retroviridae* et au genre des *Lentivirus*. Il est responsable lors de l'infection chez l'homme du syndrome d'immunodéficience acquise (SIDA).

2.1. Caractéristiques du VIH

Le virus de l'immunodéficience humaine (VIH) est un virus appartenant à la famille des *Retroviridae* et au genre des *Lentivirus* [17]. Le virus de l'immunodéficience humaine de type 1 (VIH-1) a été découvert en 1983 [18] et défini comme responsable du syndrome d'immunodéficience acquise (SIDA) en 1984 [19]. Le VIH est classé en types 1 et 2 (VIH-1, VIH-2), selon des spécificités génétiques et antigéniques. Le VIH-1 et le VIH-2 sont les seuls lentivirus humains connus. Les virus de l'immunodéficience simienne sont également regroupés dans le genre *Lentivirus*. Tous ces rétrovirus possèdent un génome d'ARN simple brin diploïde et se répliquent via un intermédiaire d'ADN (un « provirus ») au moyen d'une ADN polymérase virale dirigée par l'ARN, plus communément appelée transcriptase inverse (RT). C'est ce transfert « en sens inverse » de l'information génétique de l'ARN vers l'ADN qui classe ces virus parmi les rétrovirus. Les données obtenues par les analyses phylogénétiques semblent impliquer que le VIH ait été introduit dans la population humaine avant 1915, date extrapolée pour le dernier ancêtre commun pour le VIH-1 [20]. Le VIH-1 aurait évolué à partir de virus d'immunodéficience de primates non humains provenant de chimpanzés d'Afrique centrale (SIVcpz) et le VIH-2 de mangabeys fuligineux d'Afrique de l'Ouest (SIVsm) [10,21,22].

2.2. Structure du génome viral

Le génome du VIH est constitué de deux molécules d'ARN monocaténares identiques qui sont enfermées dans une capsidie protéique. Le génome du VIH intégré au génome de la cellule hôte, également appelé ADN proviral, est généré par la transcription inverse du génome de l'ARN viral en ADN, la dégradation de l'ARN par la RNase H et l'intégration de l'ADN double brin du VIH dans le génome humain. Le génome ADN est flanqué aux deux extrémités par des séquences LTR (long terminal repeat). La région 5' LTR code pour le promoteur de la transcription des gènes viraux (Figure 2-1).

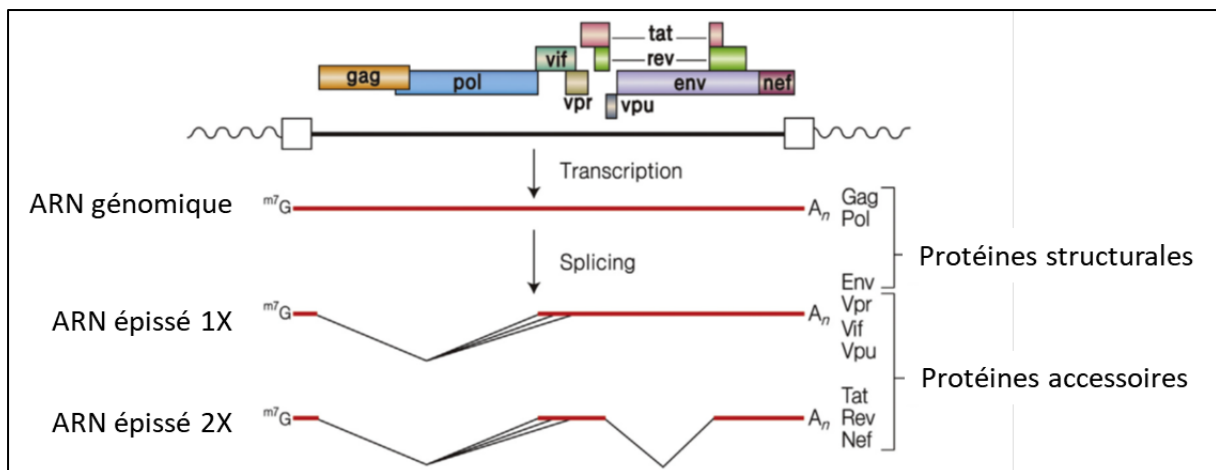


Figure 2-1 : Structure de l'ARN génomique du VIH, d'après Ryu, 2017 [23].

Les trois principaux gènes de structure du VIH-1 et du VIH-2 sont les gènes gag, env et pol, qui donnent initialement naissance à des polyprotéines (respectivement Pr55gag, Pr160gag-pol et gp160) qui sont ensuite transformées pour donner les protéines structurelles du virus et des enzymes. Premier gène dans le sens 5' à 3', le gène gag code pour les protéines de la matrice (MA, p17), la protéine de capsid (CA, p24), la protéine de nucléocapsid (NC, p7) et la protéine p6, impliquée dans la stabilisation des acides nucléiques. Le cadre de lecture gag est suivi du cadre de lecture pol, donnant la polyprotéine Gag-Pol. Le clivage de cette polyprotéine permet l'obtention de plusieurs enzymes catalysant le cycle viral. Cela inclut la forme mature de la protéase PR (p12), de la transcriptase inverse (RT, p51) incluant une activité RNase (p15 seule ou ensemble p66) et l'intégrase virale (IN, p32). Adjacent au gène pol, le cadre de lecture env permet la production de la protéine précurseur Env (gp160) à partir de laquelle les deux glycoprotéines d'enveloppe gp120 (protéine de surface, SU) et gp41 (protéine transmembranaire, TM) sont dérivées par clivage.

En plus des protéines structurelles, le génome du VIH code pour plusieurs protéines régulatrices. Le gène bipartite tat, dont l'un des exons chevauche d'autres cadres de traduction, code pour la protéine Tat (protéine transactivatrice). Tat est impliquée dans la régulation de la transcription virale, par interaction avec les protéines cellulaires, l'ADN proviral et l'ARN polymérase II. Le produit du gène rev (régulateur de l'épissage de l'ARN) permet l'export des ARN ayant subi un épissage incomplet ou sans épissage, nécessaires à la production des protéines structurales. Les autres protéines régulatrices Nef (facteur de régulation négative), Vif (facteur d'infectivité virale), Vpr (protéine virale r) et Vpu (protéine virale unique) ont un impact sur la réplication virale, le bourgeonnement du virus et la pathogénèse [24,25]. Nef augmente la pathogénicité et favorise l'échappement immunitaire par des interactions avec de nombreuses voies de signalisation cellulaires. Le génome du VIH-2 code pour Vpx (protéine virale x) au lieu de Vpu, ce qui est partiellement responsable de la pathogénicité réduite du VIH-2 [26]. La structure du génome des virus d'immunodéficience des chimpanzés (SIVcpz) et des gorilles (SIVgor) est identique à celle du VIH-1 [27].

2.3. Groupes et sous-types

Le VIH-1 est subdivisé en groupes M, N, O et P [28–30]. Les virus VIH-1 du groupe M, qui constituent la majorité des infections, sont subdivisés en sous-types A à D, F à H, J et K (

Figure 2-2). Les VIH recombinants sont dérivés de divers sous-types et sont appelés CRF (circulating recombinant form). Environ 20 % des virus du groupe M appartiennent à ces virus [31]. Jusqu'à présent, plus de 70 différents CRF épidémiologiquement stables ont été décrits [27,32–34]. Des quasi-espèces, qui sont des variants possédant des mutations similaires, se développent chez un patient et présentent des variations modifiant l'infectivité et possiblement la réponse aux traitements. L'apparition de ces quasi-espèces est liée à l'échappement immunitaire, et tend à augmenter dans la phase précoce de l'infection [35]. Sur la base d'une analyse phylogénétique, divers SIV de chimpanzés peuvent être répartis entre les groupes N et O des humains et le SIV des gorilles (SIVgor) dans le groupe P [10,21,36].

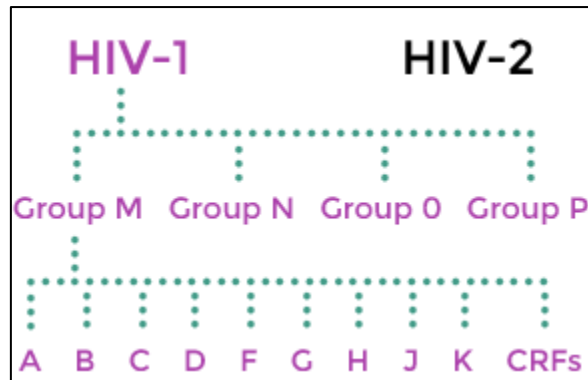


Figure 2-2 : Groupes et sous-groupes du VIH

2.4. Structure et protéines de la particule virale

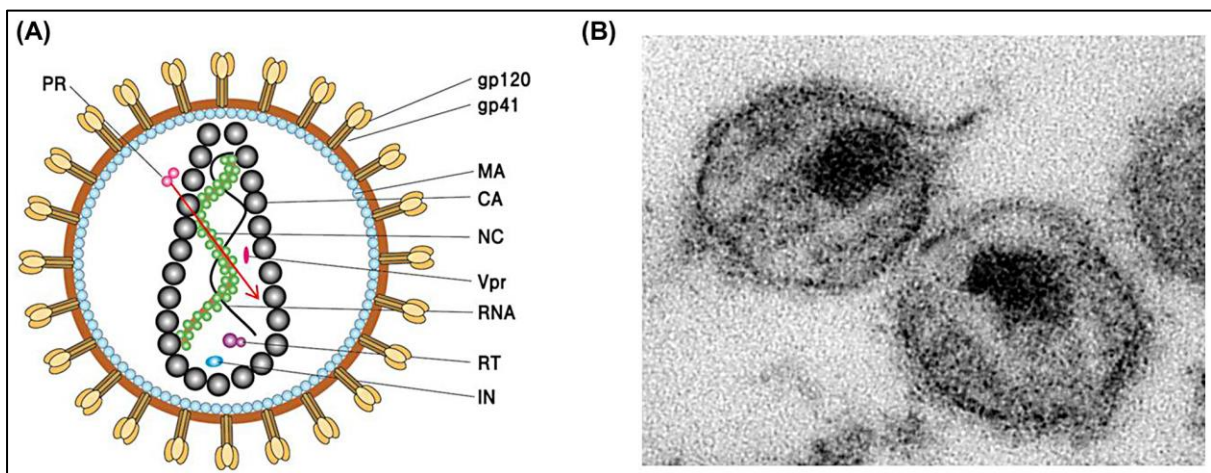


Figure 2-3 : Structure du virion du VIH. Diagramme schématisé (A) et imagerie en microscopie électronique (B). [23]

La particule mature du VIH est ronde, mesure environ 120 nm de diamètre et possède une enveloppe constituée d'une bicouche lipidique (Figure 2-3). L'enveloppe contient 72 spicules, composés de trimères des protéines Env. Les trimères de la protéine de surface gp120 (SU) sont associés de manière non covalente à la surface du virion par les trimères de la protéine transmembranaire gp41 (TM) [37]. Des épitopes neutralisants dépendant de la conformation sont présents sur la protéine gp120. Ils sont présents sur la protéine native mais ne sont que partiellement exprimés sur la protéine dénaturée dépliée [38]. L'enveloppe recouvre une capside conique tronquée, renfermant l'ARN viral. La capside icosaédrique est assemblée à partir de la protéine de capside interne p24 (CA) [39]. Le noyau viral est un complexe composé de la RT (p55/66), de l'intégrase (IN ; p32), de la protéase (PR ; p10, p12 ou p151), et des protéines de la nucléocapside (NC ; p6 et p7) qui sont liés à de deux copies de l'ARN viral à brin positif. [17]. Entre l'enveloppe et la capside se trouve la protéine de matrice (MA, p17). Les particules virales contiennent également des oligopeptides qui sont générés après leur libération de la cellule au cours de la maturation des virions par clivage des protéines précurseurs (p55, p160).

La maturation du virion a lieu par clivage des protéines précurseurs Gag et Gag-Pol (p55, p160) en protéines individuelles à la fin du processus de bourgeonnement et pendant la libération des virions de la cellule. Dans les études au microscope électronique, la perte des projections de surface (trimères SU) sous l'effet des forces de cisaillement, également appelée « shedding », peut être observée après la libération [40]. Les particules dépourvues de gp120 ne sont plus infectieuses [41].

2.5. Enzymes virales

2.5.1. Transcriptase inverse

La transcriptase inverse (RT) du VIH permet la synthèse de l'ADN proviral à partir du brin d'ARN du génome viral compris dans la particule virale (Figure 2-4) [42]. Cette étape de transcription inverse nécessite deux activités enzymatiques : une activité ADN polymérase et une activité RNase H, toutes deux apportées par la RT [43]. L'activité ADN polymérase de la RT nécessite une amorce et une matrice. L'amorce est un ARNt cellulaire complémentaire de la séquence du site de liaison d'amorce (primer binding site, pbs) situé à l'extrémité 5' de l'ARN viral, qui constitue la matrice [44]. La néo-synthèse du brin d'ADN crée un complexe ADN-ARN, qui sert de substrat pour la RNase H. Cette fonction enzymatique dégrade l'ARN pour libérer le brin d'ADN négatif néo-synthétisé. La RNase H est stoppée par un motif résistant au clivage, le tractus polypurine (PPT), situé à l'extrémité 3' [45]. Ce motif sert d'amorce pour la synthèse du brin positif d'ADN. Le produit ADN de la transcription inverse possède à ses extrémités des séquences provenant de la matrice d'ARN, et formant les séquences LTR.

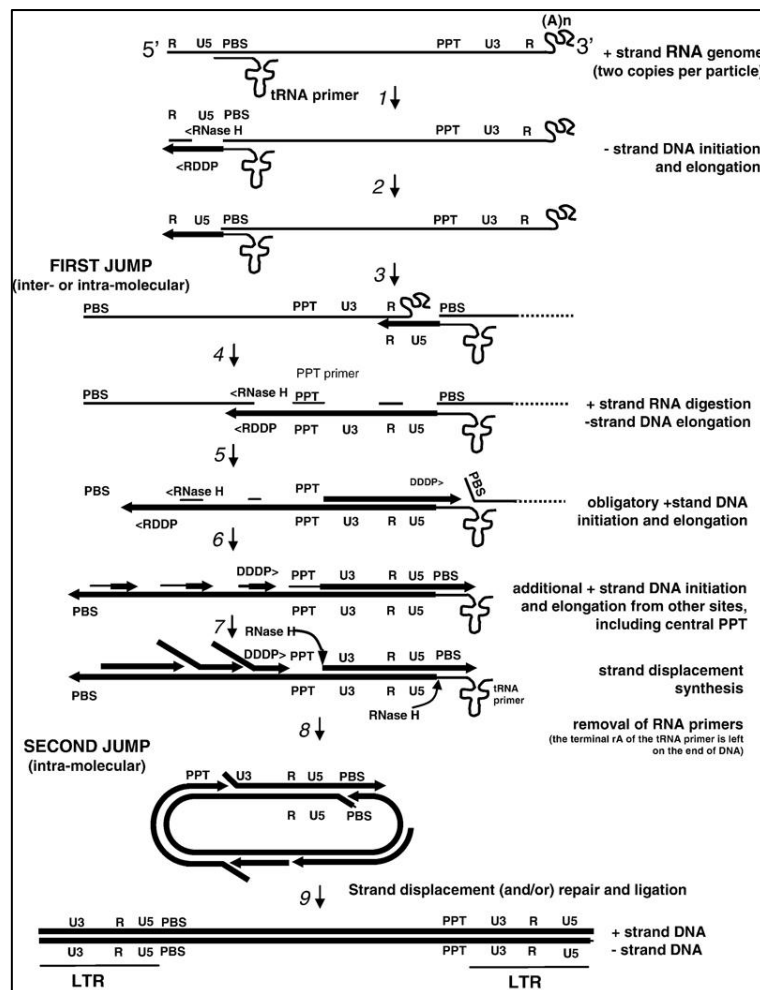


Figure 2-4 : Mécanisme moléculaire d'action de la transcriptase inverse, d'après Sarafianos et al., 2009 [46].

2.5.2. Intégrase

L'intégrase est une protéine de 32 kDa catalysant l'intégration de l'ADN viral dans l'ADN de l'hôte. L'intégrase se fixe, après la transcription inverse, sur le brin d'ADN viral néo-synthétisé au niveau de l'extrémité 3' (Figure 2-5). L'intégrase catalyse l'excision de deux bases GT à chaque extrémité 3' (3' end processing) [47]. Après le transport de l'ADN viral dans le noyau, au sein du complexe de pré-intégration (PIC), l'intégrase catalyse la jonction covalente des extrémités 3' de l'ADN ainsi préparées avec les brins opposés de l'ADN de l'hôte [47]. L'intégration complète de l'ADN viral est réalisée par des enzymes cellulaires, ayant des fonctions de réparation de l'ADN [48].

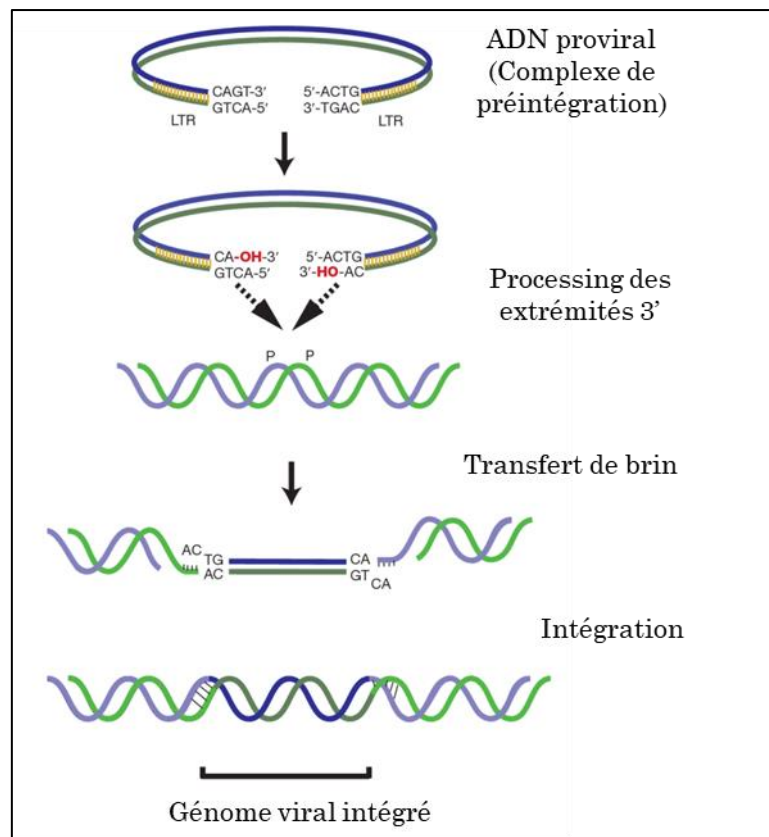


Figure 2-5 : Mécanisme moléculaire d'action de l'intégrase virale, d'après Ingale et Manish, 2011 [49].

2.5.3. Protéase

La protéase du VIH-1 est protéine de 12 kDa, dont la fonction est la protéolyse de la polyprotéine Gag-Pol, pour la production des différentes enzymes virales et protéines structurales (Figure 2-6). Elle est liée à la maturation de la particule virale. Cette protéine forme un dimère, qui est stabilisé par l'interaction des extrémités N et C terminales [50]. Le clivage de la protéine Gag est réalisé au niveau de 5 sites, donnant lieu à la libération des protéines MA, CA, NS, du peptide d'espacement 2 (spacer peptide 2 SP2) et de p6. Cela conduit à la maturation de l'ARN génomique, l'assemblage du pré-complexe de transcription inverse et l'encapsidation du noyau viral [51]. Le clivage de la polyprotéine Gag-Pro-Pol permet la libération de la RT, de l'intégrase et de la protéase. Ce clivage est pour la libération de la protéase est le résultat d'une autoprotéolyse [52].

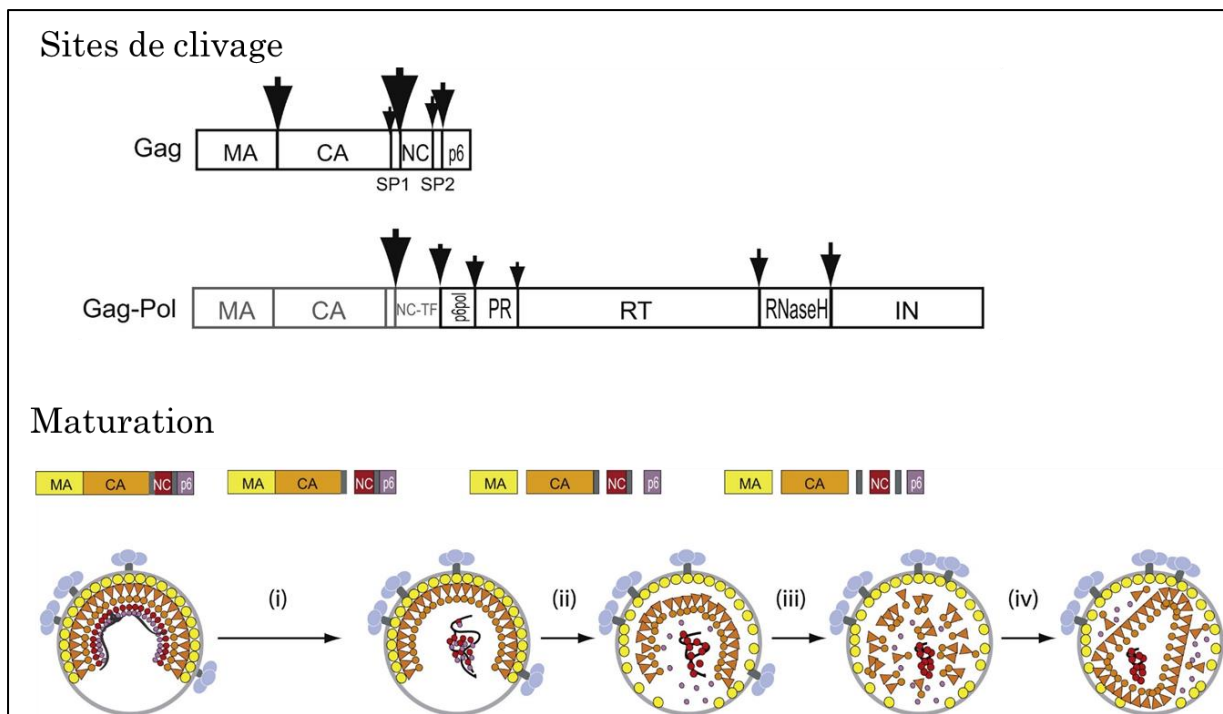


Figure 2-6 : Mécanismes moléculaires d'action de la protease, d'après Konvalinka et al., 2015 [53].

2.6. Protéines régulatrices

2.6.1. Tat

Tat est la protéine virale transactivatrice (trans-activator of transcription), responsable de l'élongation lors de la transcription virale. Bien que l'initiation de la transcription soit réalisée de manière indépendante par NFκB, NFAT, Sp1 et TBP, associés au sein du complexe de pré-initiation de la transcription, cette phase dite précoce est considérée inefficace [54]. La présence de Tat, qui participe à la phase tardive d'expression virale, permet l'élongation et ainsi l'expression des gènes viraux. Par son rôle essentiel, la régulation de l'expression de Tat est un facteur important de la régulation de la réplication et de l'établissement de la latence virale [55].

La protéine Tat de 14 kDa est localisée dans le noyau au moyen d'un signal de localisation nucléaire riche en arginine dans son domaine de base. Dans le noyau, Tat interagit avec une structure d'ARN viral en boucle dans le LTR, appelée élément de réponse de trans-activation (TAR), mais pas avec l'ARN cellulaire (Figure 2-7). Elle permet alors le recrutement de la kinase cycline-dépendante 9 (CDK9) et de la cycline T1, qui forment le complexe P-TEFb, ce qui permet le passage en phase d'élongation active [56]. P-TEFb fait partie du complexe de super élongation (SEC), qui contient également des facteurs d'élongations ELL et ELL2, les protéines d'échafaudage AFF1 et 4, ainsi que EAF1, ENL et AF9. Ces protéines forment un complexe qui entraîne la levée de l'état de pause de la polymérase et permet le remplacement des complexes de transcription non efficaces par des complexes Tat/P-TEFb/ARN Pol II [57].

Tat participe également au remodelage de la chromatine via la disruption du nucléosome 1, par le recrutement d'histones acétyltransférases (HAT) et de PBAF pour favoriser la formation d'euchromatine, ce qui permet l'élongation [58]. Ce remodelage et cette disruption de nuc-1 sont consécutifs à l'acétylation de Tat sur la lysine 50, facilitant le relargage de P-TEFb et le recrutement de PBAF.

Tat est également impliquée dans l'activation de NFκB. Tat interagit directement avec IκB et avec la sous-unité p65 de NFκB [59]. Cela entraîne l'activation de la voie de signalisation NFκB, ainsi que l'activation de la transcription du génome viral, dont l'initiation est consécutive au recrutement du facteur de transcription II H et du facteur d'élongation P-TEFb par NFκB au niveau du promoteur du VIH [60].

Tat est essentielle à la réplication virale et agit pour augmenter les niveaux d'équilibre des transcrits viraux (pour les protéines virales structurales et régulatrices) initiés dans la LTR. La protéine Tat augmente d'environ 100 fois la disponibilité de l'ARN viral pour la production du virus. L'expression de Tat est induite par Tat mais aussi par des facteurs de transcription notamment le complexe transcriptionnel NF-κB [61].

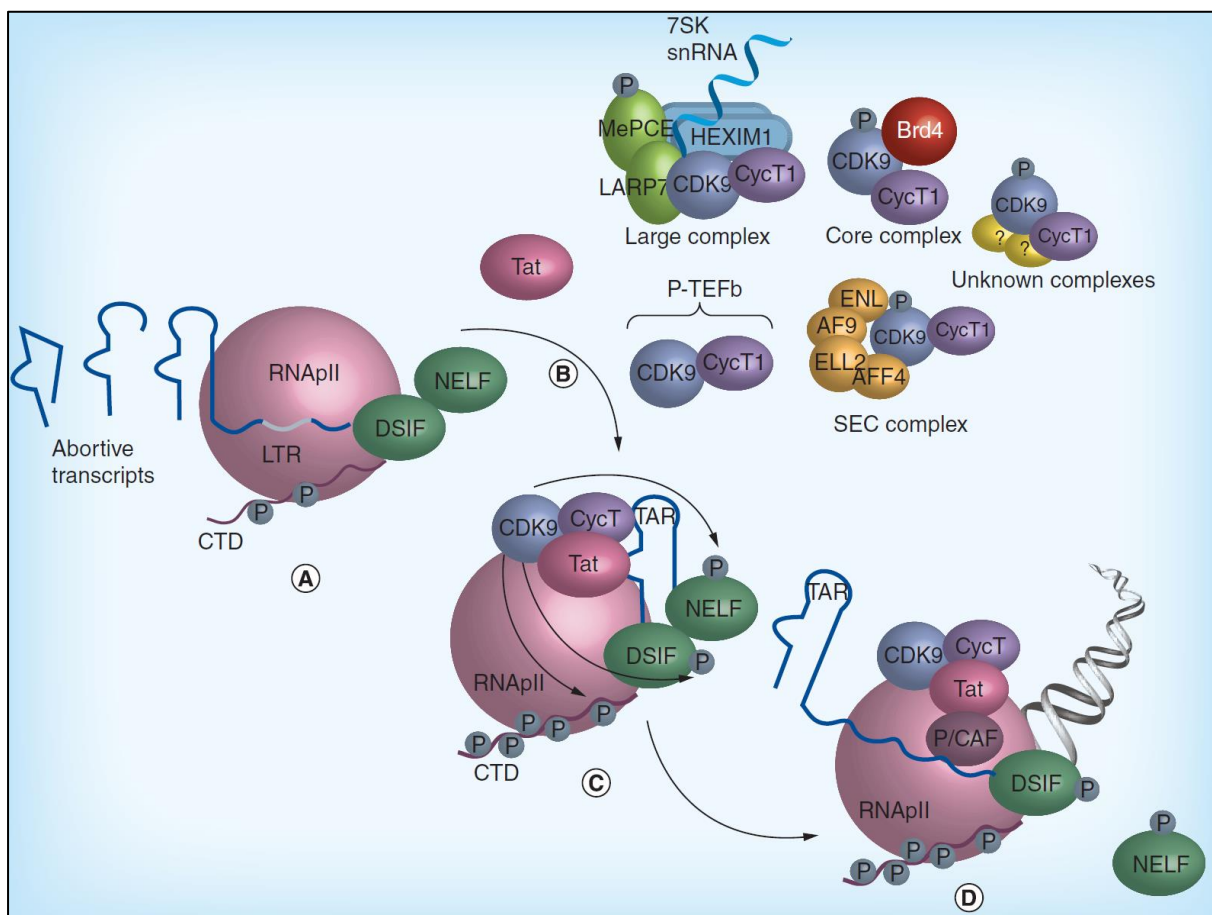


Figure 2-7 : Mécanismes moléculaires de la transcription virale, sous le contrôle de Tat, d'après Tabarrini et al., 2016 [62].

2.6.2. Rev

Rev est la protéine régulatrice de l'expression des protéines virales (regulator of expression of viral proteins). Il s'agit d'une protéine essentielle de 13 kDa située dans le nucléole qui facilite l'épissage alternatif des transcrits dans le cytoplasme. Rev est responsable de l'export nucléaire des transcrits ARN partiellement épissés ou non-épissés du VIH, en se liant à l'ARN viral au niveau de l'élément de réponse Rev (RRE) (Figure 2-8) [63]. Ce motif RRE n'est pas présent dans les ARN complètement épissés, qui sont exportés directement dans le cytoplasme via les voies d'export nucléaire classiques. Ces ARN épissés codent pour les protéines régulatrices, incluant Rev. Après la liaison de Rev avec le motif RRE, l'exportine cellulaire CRM1 et RanGTP sont recrutées au niveau de Rev [64]. Ces exportines forment un complexe d'export incluant d'autres protéines, notamment des phosphoprotéines et nucléoporines [65,66]. Rev permet donc l'export des ARN non épissés par la voie d'export CRM1.

En parallèle de sa fonction dans l'export d'ARN, Rev participe à la stabilisation des ARN viraux. L'interaction de Rev avec le motif RRE réduit l'instabilité de l'ARN liée aux motifs riches en nucléotides A et U, regroupés sous le terme séquences d'instabilité (INS) [67]. Rev participe également à la régulation de l'épissage, en inhibant l'épissage cellulaire. Rev lié au RRE semble interagir avec des facteurs d'épissage, tels que SF2/ASF. La liaison de SF2/ASF à Rev entraîne la séquestration de ce facteur, permettant l'existence des différents motifs d'épissage de l'ARN viral [68].

Cela permet la production de protéines régulatrices au début de la réplication, suivie de la traduction des protéines structurales à la fin du cycle de réplication. En l'absence de Rev, seuls les transcrits épissés à plusieurs reprises sont traduits, de sorte qu'aucune protéine de structure, aucune enzyme ni aucun ARN génomique ne peut être emballé dans la particule virale [69].

2.6.3. Nef

Nef, initialement considérée comme un facteur négatif (Negative Factor) de la réplication virale, est la première protéine virale à être exprimée. C'est une protéine de 25-30 kDa qui est principalement localisée dans le cytoplasme et la surface interne de la membrane des cellules infectées [70]. Nef est une protéine multifonctionnelle : elle joue un rôle important dans la pathogenèse en favorisant la réplication et dissémination virale, et en permettant l'échappement immunitaire [71].

Nef a un effet négatif sur la présentation des molécules CD4 à la surface des cellules [72]. Son effet sur l'échappement immunitaire est lié à son altération de la machinerie de transport intracellulaire, en particulier des protéines de la membrane. Nef se lie à la partie cytosolique des récepteurs CD4 et à la protéine AP-2 (Figure 2-8). Cette protéine est responsable de l'endocytose des récepteurs CD4, et l'action de Nef accélère ce processus [73]. Par conséquent, la molécule CD4 est sous-exprimée au niveau de la membrane de la cellule infectée suite à l'endocytose et ne peut pas être surinfectée par d'autres VIH. La perte de CD4 réduit la réaction immunitaire cellulaire contre la cellule infectée.

Nef a également un effet négatif sur la présentation des antigènes par le complexe majeur d'histocompatibilité de classe I, passant par une interaction avec AP-1. Cet antagonisme du MHC-I

fonctionne selon deux modèles, pour les MHC-I présents dans la membrane et pour les MHC-I nouvellement synthétisés. Dans le mode d'action par « signallement », Nef entraîne l'activation des kinases de la famille Src, démarrant ainsi une cascade de signalisation résultant en l'augmentation des niveaux de PI3K. Cela induit l'activation des GTPases Arf1 et 6, qui entraînent l'endocytose du MHC-I. Le MHC-I ainsi internalisé forme, au sein des vésicules, un complexe avec Nef et AP-1, ce qui empêche son recyclage vers la membrane [74]. Dans le mode d'action « stoichiométrique », l'interaction entre Nef et AP-1 permet le blocage des MHC-I nouvellement synthétisés au niveau du réseau trans-Golgi, par l'action d'Arf1 [75]. Ces deux modèles font intervenir une interaction de Nef avec AP-1 et avec la partie cytoplasmique du MHC-I.

De plus, Nef augmente la pathogénicité *in vivo* et est nécessaire à la progression de l'infection [24]. Cette augmentation de l'infectivité est liée à l'interaction de Nef avec le facteur de restriction SERINC5. Cette protéine transmembranaire, exprimée par les cellules en réponse à l'infection, est incorporée aux virions néo-formés et entraîne la disruption de la fusion des membranes lors de l'infection d'une nouvelle cellule [76]. La liaison de Nef avec SERINC5 au niveau de la membrane cellulaire entraîne son endocytose par l'action d'AP-2, empêchant ainsi son incorporation aux nouveaux virions [77].

Les actions de Nef sur les protéines cellulaires sont réalisées par le recrutement et l'activation de kinases cellulaires. Pour les kinases de la famille Src, Nef induit une activation par la modification de la conformation du domaine SH3 lors de sa liaison avec les kinases [78]. Les kinases de la famille Tec sont également activées par Nef. Cette activation est également liée à l'interaction avec le domaine SH3, mais requiert également un recrutement des kinases vers la membrane cellulaire, pour permettre une autophosphorylation [79]. Ce recrutement semble impliquer une dimérisation de Nef [71]

La protéine Nef du VIH-1 est constituée d'un plus petit nombre d'acides aminés et a une plus grande capacité à augmenter la pathogénicité du VIH-1 par rapport au VIH-2. Les virus mutés pour Nef présentent une diminution de leur réplication et de leur infectivité [80]. Les patients infectés avec un virus mutant pour Nef présentent un ralentissement de l'évolution de la maladie vers le stade SIDA [81]. Ces virus mutants se répliquent plus difficilement et semblent induire une diminution limitée de la concentration en lymphocytes T CD4+ [82].

2.6.4. Vpr

Le produit du gène *vpr* est une protéine oligomérique de 14 kDa [83–85]. Le VIH-2 et la plupart des souches de SIV portent un gène supplémentaire, le *vpx*, qui partage une homologie de séquence avec le *vpr*. Vpr et Vpx sont toutes deux emballées dans les virions [86]. Vpr a de multiples interactions avec les protéines cellulaires, et entraîne une augmentation de la pathogénèse virale, en particulier dans les macrophages [87]. L'utilisation d'un virus défectif pour Vpr a montré un niveau de réplication identique à un virus non muté dans des lymphocytes, mais une diminution dans des macrophages. Il semble que les signaux de localisation nucléaires d'autres protéines virales, notamment MA, soient suffisants dans les lymphocytes mais ne permettent pas une translocation efficace dans les macrophages [88].

Vpr augmente la réplication virale, en particulier dans les macrophages. Vpr induit une dégradation du facteur de transcription hélicase-like (HLTF), responsable de la réparation de l'ADN post-

réplication (Figure 2-8) [89]. Vpr semble également augmenter l'importation nucléaire du complexe de pré-intégration, mais n'est pas nécessaire à ce transport [90]. *In vitro*, l'utilisation d'un virus déficient pour Vpr entraîne une réplication virale complète, et la production d'ADNc proviral, favorisée par Vpr, peut être obtenue avec un virus déficient, en supplémentant les cellules avec un extrait de cytosol [90–92].

Vpr contribue également à réduire la réponse interféron suite à l'infection par le VIH. Vpr active le complexe SLX, ce qui réduit la transcription des interférons α et β [90]. Vpr entraîne également une dégradation du facteur régulateur de l'interféron 3 (IRF3), ce qui réduit l'induction d'IFN β [93].

Vpr est également responsable de l'arrêt du cycle cellulaire à la transition G2/M [94]. Cet arrêt est causé par l'hyperphosphorylation de la protéine cellulaire cdc2, qui régule le checkpoint de dommages à l'ADN [95]. Cette phosphorylation, sous le contrôle de la phosphatase cdc25 et de la kinase Wee1, inactive cdc2. Vpr interagit avec cdc25 et Wee1 [96,97].

Vpr est également impliquée dans la dégradation de plusieurs protéines cellulaires. Certaines protéines sont des cibles directes de Vpr, qui entraîne l'ubiquitinylation par le cofacteur cellulaire DCAF1 [98]. Les cibles de Vpr incluent notamment UNG2, TET2 et REAF [99,100]. UNG2 est une ADN glycosylase, qui amorce la réparation de l'ADN. REAF est un facteur antiviral associé à l'ARN, qui inhibe la réplication du VIH-1 durant la transcription inverse. TET2 est une protéine impliquée dans la régulation de la différenciation Th1 et Th17, et dans la réparation de l'ADN.

Vpr semble également augmenter l'expression de la protéine virale Env, dans les macrophages, en limitant sa dégradation et en augmentant son incorporation dans les nouvelles particules virales. Cette augmentation semble être réalisée par l'interaction de Vpr avec TET2, ce qui réduit l'expression du facteur de restriction antiviral IFITM3, et une inhibition de l'expression des récepteurs au mannose, par l'inhibition de la transcription de MRC1 [91,101].

2.6.5. Vif

Vif, une protéine cytoplasmique de 23 kDa, est également essentielle à la réplication virale [102]. En l'absence de Vif, les virions du VIH-1 ont une morphologie anormale [103] et ont une capacité très réduite à synthétiser l'ADN proviral après l'infection de nouvelles cellules cibles [104].

La principale fonction connue de Vif est la dégradation des désaminases de la famille APOBEC3, qui inhibent le cycle de réplication viral par modification de l'ADN proviral (Figure 2-8) [105]. Cette modification est accompagnée d'une réduction de la transcription inverse et de l'intégration [106,107]. L'échappement du VIH à cette réponse cellulaire est obtenu par l'action de Vif, qui est associée à l'ubiquitin ligase E3. Cette interaction induit l'ubiquitinylation d'APOBEC3G et sa dégradation par le protéasome [108].

Une deuxième fonction importante de Vif est son rôle dans l'arrêt du cycle cellulaire en phase G2/M [109]. Cet arrêt est consécutif à la dégradation par Vif de facteurs cellulaires. Ces facteurs cellulaires ne sont pas définitivement identifiés. L'arrêt du cycle cellulaire par Vif est dépendant du remodelage du phosphoprotéome de la cellule hôte [110]. Parmi les potentielles cibles de Vif, on trouve les protéines phosphatase 2A, notamment PP2A1, dont les fonctions régulatrices incluent la régulation de la transition G2/M [111–113].

2.6.6. Vpu

Le VIH-1 et le SIVcpz apparenté contiennent un gène *vpu* dont le produit est une protéine phosphorylée de 16 kDa, localisée au niveau des membranes cellulaires internes, notamment dans la région trans-golgi et les endosomes des cellules infectées (Figure 2-8) [114].

La protéine Vpu modifie la concentration et la distribution des protéines membranaires de la cellule hôte, avec pour conséquence une augmentation du relargage des virions néo-formés, via son association au système réticulum endoplasmique/Golgi [115]. La protéine Vpu interagit avec la tetherin, protéine cellulaire qui interfère avec le relargage des virions néoformés. L'interaction de Vpu et tetherin entraîne sa séquestration dans l'appareil de golgi [114].

Vpu cible également les peroxisomes, en réduisant l'expression des facteurs impliqués dans la biogenèse, la division et la translocation de la machinerie peroxisomale [116]. Cette inhibition de l'activité peroxisomale des cellules semble induire une réduction de la production d'interférons [117]. Vpu entraîne, comme Vif et Vpr, une réduction de l'expression de l'IFN β . Cette inhibition par Vpu est consécutive à l'inhibition de NF κ B. Vpu stabilise I κ B, et empêche la translocation nucléaire de NF κ B [118]. Par son action inhibitrice de l'expression de NF κ B et de l'activité peroxisomale, Vpu induit une réduction de la réponse antivirale cellulaire [119].

Les mutants du VIH-1 déficients en Vpu continuent à se répliquer dans les lignées de cellules T CD4+, les lymphocytes T primaires et les macrophages, mais à un titre réduit en raison de l'accumulation des virions dans les vésicules intracytoplasmiques [120]. Le VIH-2 et d'autres SIV que SIVcpz ne possèdent pas de gène *vpu*, mais sa fonction est probablement codée ailleurs dans le génome.

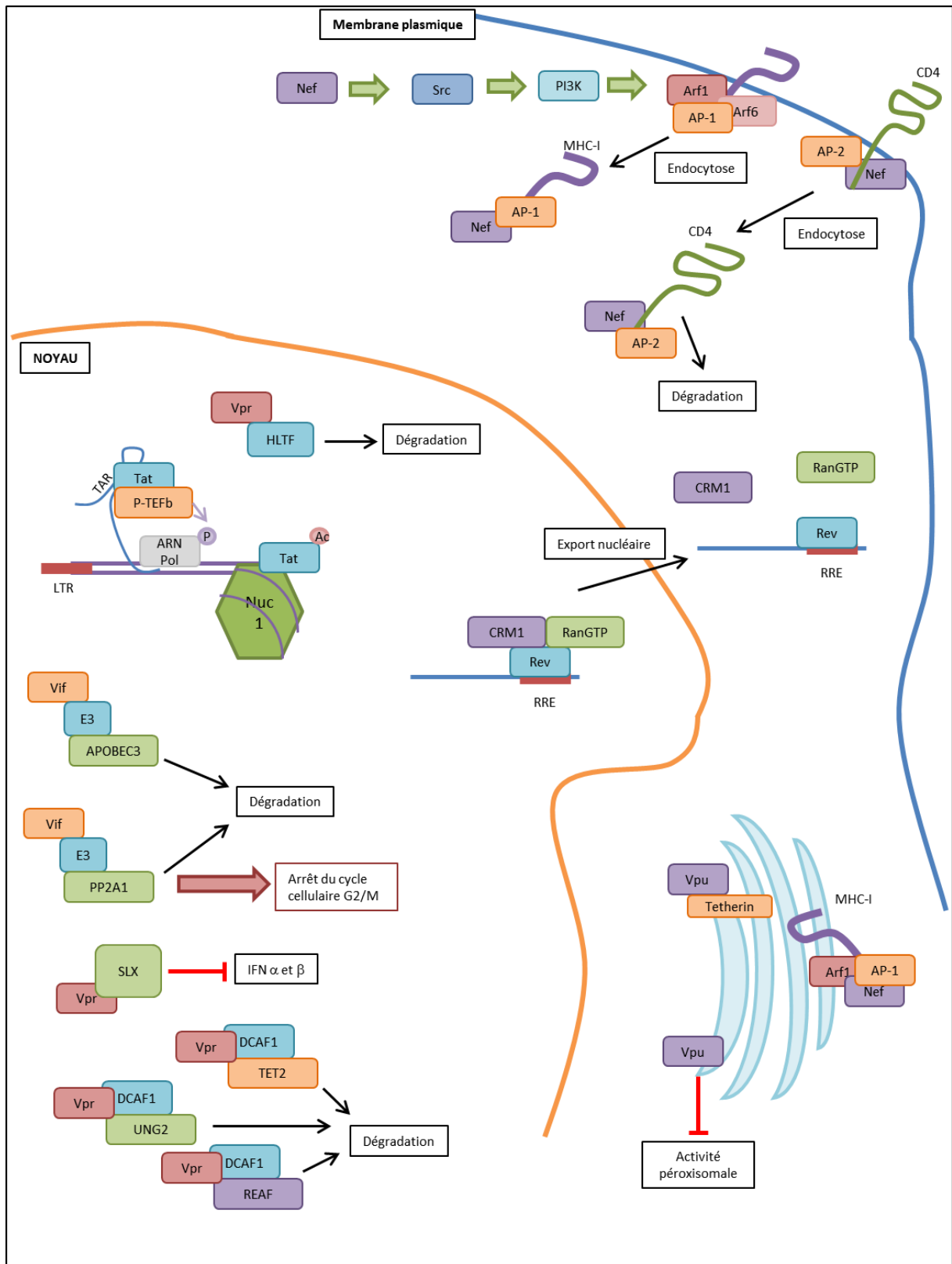


Figure 2-8 : Interactions moléculaires des protéines régulatrices du VIH avec les protéines cellulaires.

2.7. Cycle de vie du virus

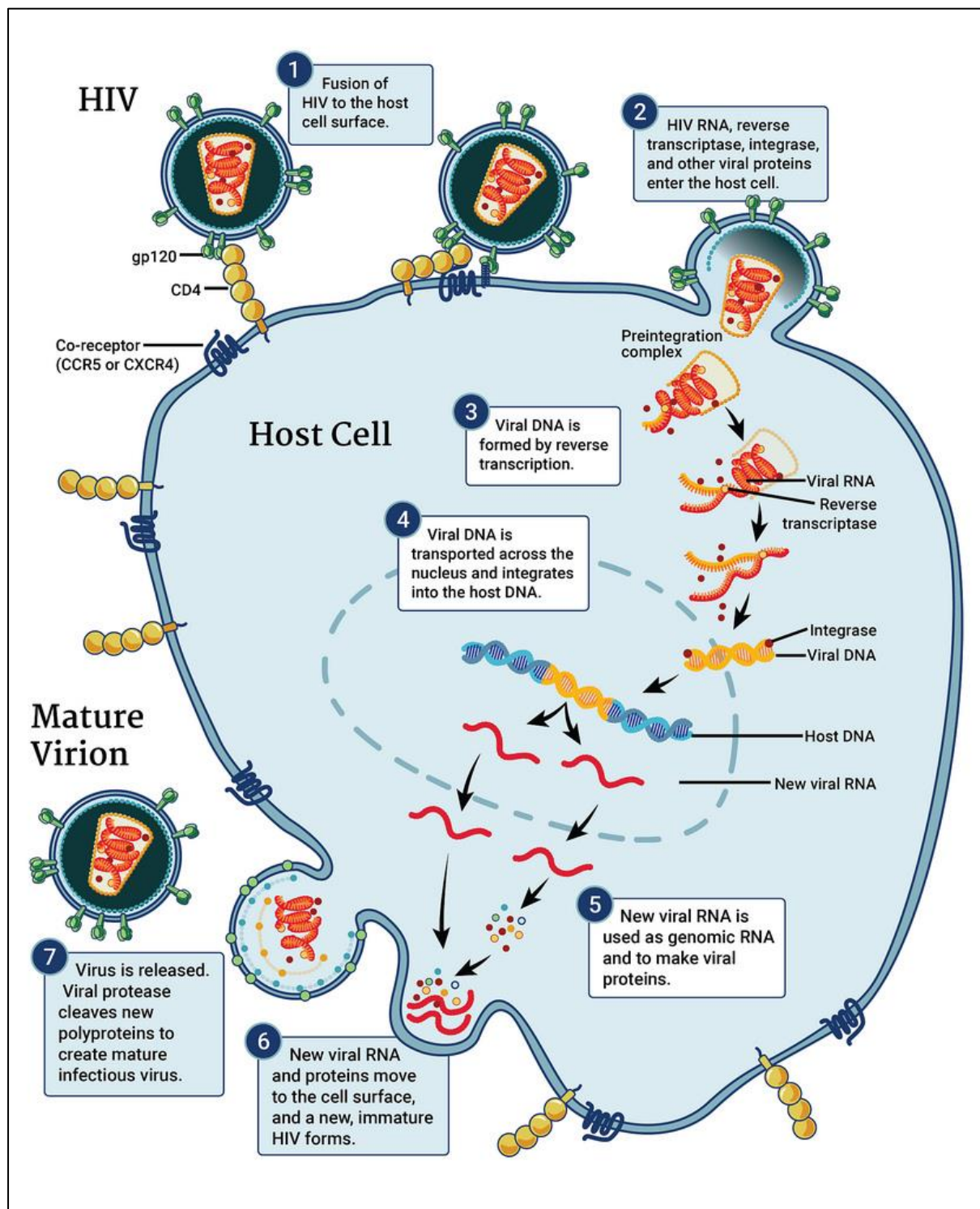


Figure 2-9 : Cycle de réplication du VIH. National Institute of Allergy and Infectious Diseases (NIAID)

L'infection par le VIH est initiée lorsque le virus se lie à une cellule cible par l'intermédiaire de la glycoprotéine d'enveloppe virale, gp120, de la particule mature du VIH qui se lie au récepteur CD4 de la cellule hôte (Figure 2-9). Toutes les cellules CD4-positives telles que les cellules T helper, les macrophages, les cellules dendritiques et les cellules souches hématopoïétiques sont permises au

VIH. Les astrocytes sont également permissifs au VIH, de manière indépendante du CD4 [121]. Pour les cellules CD4+, après l'attachement à la molécule CD4 via le domaine C4 de la gp120, un changement de conformation du CD4 et de la gp120 se produit, ouvrant un site supplémentaire pour la gp120 afin de permettre la liaison au corécepteur, c'est-à-dire le récepteur de chimiokine 5 (CCR5) ou le récepteur de chimiokine 4 (CXCR4 ou fusine) à la surface de la cellule [122,123]. Il existe également d'autres corécepteurs, parmi lesquels d'autres récepteurs couplés aux protéines G, ainsi que le récepteur au mannose [124–126]. Cette liaison déclenche un changement de conformation de la glycoprotéine Env pour exposer la protéine TM, gp41, ce qui entraîne une fusion, éventuellement médiée par le corécepteur, entre le virus et la membrane de la cellule hôte [17,127].

La fusion de l'enveloppe virale et de la membrane cellulaire entraîne la translocation de la capsid virale dans le cytoplasme. La capsid est prise en charge par un endosome, permettant la libération du contenu de la capsid dans le cytoplasme [128]. La transcriptase inverse (RT) du VIH transcrit le génome ARN simple brin du VIH en ADN (ADN complémentaire ou ADNc) qui est converti en ADN double brin (ADN proviral) [129]. Cet ADN proviral est transporté par des nucléopores dans le noyau de la cellule sous la forme d'un complexe de pré-intégration composé de l'intégrase (IN) et de l'ADN proviral. L'intégrase insère ensuite de façon aléatoire le génome proviral dans le génome de la cellule hôte humaine. Cet ADN proviral sert de matrice pour la production de la descendance de l'ARN viral et entraîne l'établissement d'une infection persistante.

La transcription du génome viral est dirigée par un promoteur situé dans le LTR 5' du provirus intégré, ce qui entraîne la production de molécules d'ARNm. Celles-ci permettent la synthèse de nouvelles protéines virales et d'ARN génomique. L'ARN génomique est ensuite emballé dans des virions qui bourgeonnent à la surface de la membrane cellulaire. Au cours de la maturation du virion, les polyprotéines Gag et Gag-Pol sont clivées par la protéase virale en protéines sous-unitaires, ce qui donne le virion mature qui est dirigé vers la surface cellulaire par la myristoylation amino-terminale de Gag [130]. Le virion est ensuite libéré de la surface cellulaire, ce qui complète le cycle de vie.

Le génome proviral peut également se répliquer en même temps que le génome de la cellule hôte et en faire partie pendant la division cellulaire mais cela reste un événement rare, qui participe à la latence virale. Cependant, après activation des cellules infectées, le promoteur LTR du génome proviral peut servir de site d'attachement pour les ARN polymérase ADN-dépendantes cellulaires et une variété de facteurs de transcription initiant la synthèse de l'ARNm viral et de l'ARN génomique, entraînant la réactivation du virus latent.

2.8. Tropisme cellulaire et réservoirs

2.8.1. Réservoirs de latence virale

Une caractéristique distinctive du VIH-1 et du VIH-2 est leur capacité à infecter les lymphocytes T CD4+ et les macrophages. En effet, c'est cette observation précoce qui a conduit à l'identification de l'antigène de différenciation cellulaire CD4 comme récepteur pour l'entrée du VIH-1 dans les cellules [131,132]. Le tropisme cellulaire est étroitement lié, mais probablement pas exclusivement, à la capacité des différentes enveloppes du VIH-1 à se lier aux CD4 et aux récepteurs de chimiokines CC ou CXC et à initier la fusion virale avec la cellule cible.

La plupart des souches primaires de VIH-1 sont macrophages-tropiques (M-tropiques), infectent les cultures primaires de macrophages et de cellules T CD4+ et utilisent le CCR5 comme corécepteur privilégié. Chez environ 40-50% des malades, il y a un glissement de l'utilisation du corécepteur CCR5 vers le corécepteur CXCR4, ce qui s'accompagne habituellement d'une déplétion rapide en lymphocytes T CD4+ [133]. Les virus T-tropiques (T-tropiques) peuvent infecter des cultures primaires de cellules T CD4+ et des lignées de cellules T établies, mais pas les macrophages primaires. Les virus T-tropiques utilisent le CXCR4 comme corécepteur pour l'entrée virale. Les cellules présentatrices d'antigènes, telles que les cellules dendritiques et les cellules de Langerhans, peuvent jouer un rôle important dans la transmission muqueuse et sexuelle du VIH-1 [134]. Toutes les souches de VIH-1 et de VIH-2 peuvent infecter les lymphocytes CD4+ du sang périphérique (cellules T auxiliaires), mais le degré d'infection des lignées de cellules T immortalisées ou leucémiques varie d'une souche à l'autre [135].

Les données montrent que le pool de VIH proviral, latent mais compétent pour la réplication, est principalement situé dans les lymphocytes T CD4+ mémoire centraux quiescents à longue durée de vie [136]. Ces cellules non activées hébergent le virus sous forme latente, permettant la persistance à long terme de l'infection [137,138]. Cette infection latente repose sur une restriction de la production de protéines virales et sur l'intégration de l'ADN proviral à l'ADN génomique cellulaire [139,140].

Les patients infectés par le VIH voient la maladie évoluer de différentes manières et à différents rythmes. La progression de la maladie est évaluée principalement par la quantification des lymphocytes T CD4+ et par la virémie plasmatique. Des sous-groupes de patients, ayant des caractéristiques de progressions différentes, ont été identifiés. Les patients ayant un taux de lymphocytes T CD4+ stable pendant un minimum de 7 à 10 ans sont considérés comme des progresseurs lents [141]. Ils représentent entre 5 et 15% des patients infectés. Les patients montrant une progression vers le stade SIDA en moins de 3 ans sont considérés progresseurs rapides [142]. Ils représentent environ 5% des patients. Les patients présentant une réduction de la virémie à des niveaux faibles sans traitement sont considérés comme contrôleurs. Les patients présentant une virémie indétectable sans traitement sont considérés comme contrôleurs élite et représentent entre 0,2 et 0,5% des patients [143].

2.8.2. Lymphocytes T CD4+

L'établissement de la latence dans les lymphocytes T CD4+ intervient dans les différents types de T CD4+, à une fréquence variable selon le type. Bien que la fréquence des cellules infectées soit plus faible pour les T naïfs que pour les T mémoire centraux, un large réservoir compétent pour la réplication a été trouvé dans les T naïfs [144]. Parmi les T mémoire, la plus grande proportion de génomes intacts est présent dans les T effecteurs mémoire [145]. La catégorisation des lymphocytes T selon leurs propriétés fonctionnelles permet également de définir des sous-types ayant un rôle de réservoir important. Les lymphocytes Th1 infectés par le VIH participent, par leur prolifération, à la persistance virale en maintenant le nombre de cellules contenant un provirus compétent pour la réplication [146]. Les lymphocytes Th17 jouent également un rôle important dans la persistance virale, notamment par leur longue durée de vie [147,148].

Le profil des lymphocytes T CD4+ impliqués dans l'établissement de la latence virale ne correspond pas à une population homogène. La recherche d'un ou plusieurs marqueurs spécifiques des cellules constituant le réservoir de latence virale a conduit à l'identification de plusieurs sous-populations participant à ce réservoir [149].

Le CD2 est un marqueur qui a été identifié comme présent à la surface des cellules T CD4+ infectées de manière latente. Les T mémoire quiescents CD4+ CD2^{high} semblent héberger un nombre plus élevé de copies du génome viral que la population T CD4+ générale [150]. Ces lymphocytes T CD4+ CD2^{high} peuvent, sous l'effet d'agents de réactivation, présenter une réactivation de la réplication virale [150]. Le CD30 est un marqueur jouant un rôle dans la réplication du VIH et dans la mort cellulaire des lymphocytes T CD4+ infectés [151]. Les cellules T CD4+ CD30+ présentent une augmentation de la quantité d'ARN viral. L'activation du CD30 a été montrée comme associée à l'activité transcriptionnelle du VIH [152].

Le CD32a, récepteur Fc gamma IIa, a été identifié comme surexprimé dans les cellules T CD4 infectées de manière latente [150]. Il s'agit d'un marqueur associé à l'activation des cellules T. L'étude de cette sous-population CD32+ CD4+ a montré un enrichissement en ADN proviral par rapport à la population générale de T CD4+ [153]. Cela semble indiquer que les lymphocytes T CD32+ CD4+ constituent un réservoir de latence important [154]. La considération du CD32 comme marqueur de réservoir du VIH reste cependant débattue [155,156].

2.8.3. Monocytes/Macrophages

Un second réservoir cellulaire de latence virale est situé dans les cellules de la lignée monocyttaire, les monocytes et les macrophages pouvant être infectés par le VIH, puisque porteurs des récepteurs CD4 et CCR5 ou CXCR4. L'établissement de la latence dans ces cellules, lié aux facteurs de restriction cellulaires APOBEC3, SAMHD1 et MX2 (pré-intégration) et à des mécanismes de régulation épigénétique (post-intégration), contribue au maintien d'un réservoir de latence qui est disséminé, en raison de la présence des macrophages dans l'ensemble des tissus et de l'extravasation des monocytes sanguins vers les tissus [157,158].

L'importance de l'infection des macrophages dans l'infection est liée à la progression vers un tropisme macrophagique du virus au cours de l'évolution de la maladie chez certains patients. Les patients présentant une progression vers le stade SIDA sont infectés en majorité par des souches macrophage trophiques [133]. Ces cellules étant plus résistantes que les lymphocytes aux effets cytopathiques de l'infection par le VIH, elles permettent la survie du virus pendant une plus longue période [159]. Les phénomènes de latence dans les lymphocytes CD4+ sont largement étudiés, y compris concernant la réactivation du VIH latent, mais le sont moins concernant la latence dans les monocytes/macrophages [160]. L'étude de la latence dans les monocytes/macrophage est rendue plus difficile par les taux d'infection et d'intégration plus faibles dans ces cellules [161]. Cependant, bien que les monocytes infectés de manière latente ne produisent pas de particules virales infectieuses, leur différenciation en macrophage s'accompagne d'une stimulation de la production de protéines virales, indiquant un rôle important dans les phases de réactivation [162]. Le réservoir de latence établi dans les monocytes et dans les macrophages persiste malgré les traitements cART [163,164].

2.8.4. Cellules gliales

Depuis l'identification du CD4 comme récepteur du VIH-1 et du VIH-2, il est apparu que le virus est également capable d'une infection limitée de certaines cellules CD4+, notamment les cellules gliales [165]. L'infection des cellules gliales semble se faire par le franchissement de la barrière hémato-encéphalique par des monocytes CD14+ CD16+ infectés [166]. L'infection des cellules gliales est favorisée par la phosphorylation de SAMDH1, levant l'effet de ce facteur de restriction [167]. Le génome viral a été détecté dans les cellules microgliales de patients avirémiques sous thérapie cART, confirmant le rôle de réservoir de latence cérébral [168]. L'infection des cellules microgliales, qui présentent une longue durée de vie, est associée au développement des maladies neurocognitives associées au VIH (HAND) [169,170]. Les cellules gliales semblent composer le réservoir principal du VIH dans le cerveau [171].

La latence dans les cellules gliales est consécutive à l'intégration du génome viral dans le génome cellulaire. La régulation de la latence dans les cellules gliales implique la répression de la réplication, par des mécanismes épigénétiques d'interférence transcriptionnelle et de remodelage de la chromatine [171]. Dans les cellules gliales, le facteur de transcription NFκB et la protéine Sp1 sont suffisants pour la transcription [172]. La formation d'hétérochromatine, qui réduit la transcription en réduisant la disponibilité du promoteur, est sous le contrôle de CTIP2. CTIP2 recrute au niveau du promoteur viral les HDACs1 et 2 et la HMT SUV39H1, ce qui induit la formation d'hétérochromatine [173].

2.9. Mécanismes moléculaires de la latence

La latence virale est le résultat d'un silence transcriptionnel réversible, sous le contrôle des enzymes de modification de la chromatine de l'hôte. La modification des histones impacte l'accessibilité des LTR. Les HDACs 1 et 2 sont recrutées par NFκB p50 au niveau du promoteur viral, ce qui induit la formation d'hétérochromatine [174] (Figure 2-10). Les facteurs de transcription YY1 et LSF recrutent également HDAC1 pour maintenir ce silence épigénétique [175]. Indépendamment du recrutement des HDACs, la HMT EZH2 est recruté et induit la méthylation du promoteur viral [176]. Ces modifications des histones et de l'ADN induisent l'état de latence.

En plus des modifications de chromatine, des modifications de la méthylation de l'ADN au niveau des îlots CpG du promoteur viral participent à l'établissement de la latence. Cette hyperméthylation du promoteur entraîne le recrutement de MBD2, qui est associée au complexe NuRD contenant HDAC2 [177].

La régulation de la latence est également influencée par le site d'intégration. L'intégration du provirus implique des cofacteurs cellulaires, notamment LEDGF/p75 qui reconnaît les marques de méthylation de l'ADN, favorisant l'intégration dans des sites transcriptionnellement actifs [178,179]. Des modifications de la méthylation post-intégration modifient l'environnement de manière à induire l'hétérochromatinisation.

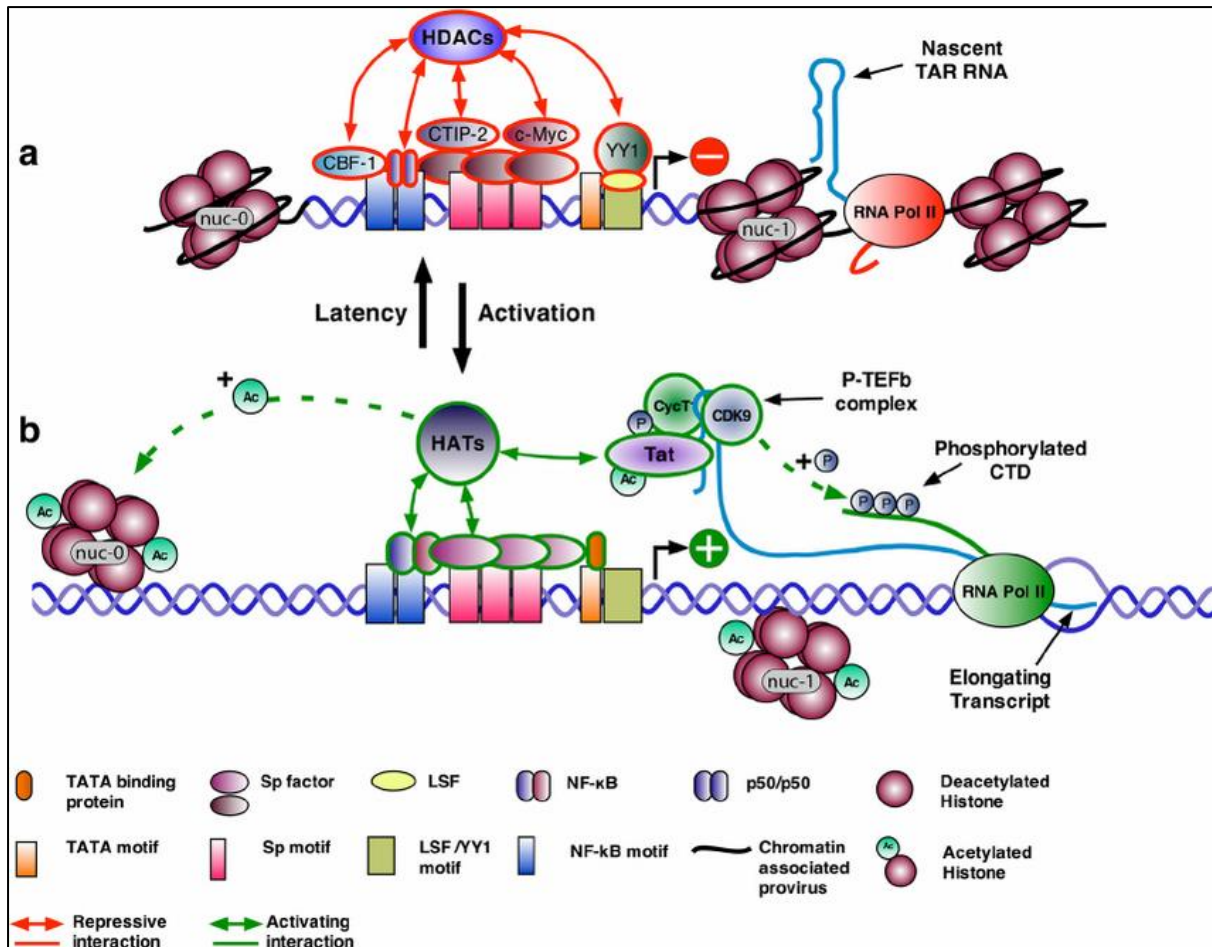


Figure 2-10 : Mécanismes moléculaires de la latence virale. L'ADN proviral passe d'un état de transcription active (b) à un état de latence (a) réversible. D'après Churchill et al., 2014 [180].

2.10. Rôle d'Akt dans l'infection

La voie PI3K/AKT/mTOR est un acteur clé de la pathogenèse du VIH-1 et est ciblée par plusieurs protéines virales pour moduler l'entrée, la transcription/réactivation, la maturation et la propagation virale, et pour favoriser l'échappement immunitaire.

La liaison de la protéine gp120 du VIH-1 à CXCR4 induit une activité PI3K, ce qui entraîne l'activation de la cofiline. L'actine corticale statique limite l'entrée du VIH dans les cellules T, et cette restriction est levée par l'activation de la cofiline.[181] La liaison de la glycoprotéine Env aux récepteurs cellulaires de surface induit l'activation d'Akt. Cela réduit l'activation des caspases, permettant l'inhibition de l'apoptose induite par gp120 [182]. Suite à l'activation d'Akt, couplée à l'induction de miR-718, le schéma de phosphorylation des protéines riches en sérine impliquées dans la régulation de la traduction est modifié. Un modèle d'épissage alternatif des ARNm du VIH-1 est favorisé suite à cette dérégulation, conduisant à une réduction de la réplication virale [183]. Ainsi, la modulation de l'épissage par la voie PI3K/Akt semble essentielle pour l'établissement de la latence.

La transcription et la réactivation du VIH-1 sont médiées par P-TEFb, qui est sous le contrôle de complexes protéiques régulateurs, dont un complexe snRNA HEXIM1/7SK. La phosphorylation de HEXIM1 induit la levée de l'inhibition de la transcription et est consécutive à l'activation de la voie

PI3K/Akt [184]. La transactivation des LTR du génome viral est sous le contrôle de Tat. Cette activation est médiée par la voie PI3K/Akt, parallèlement à la phosphorylation de p65 et IKK [185]. Tat interagit avec d'autres protéines régulatrices de l'activité de la LTR, notamment EZH2. L'inhibition de la transcription par EZH2 est levée par sa phosphorylation consécutive à l'activation d'Akt [186].

2.11. Traitements et stratégies thérapeutiques

2.11.1. Traitements

La réplication virale conduit à une dysfonction immunitaire, par la perte des lymphocytes T CD4+. Cette évolution de la maladie peut être interrompue par l'utilisation de traitements antirétroviraux, utilisés de façon combinés. Les traitements peuvent être classés dans différentes catégories, selon le mécanisme moléculaire ciblé (Tableau 2-1). Les inhibiteurs de la transcriptase inverse, analogues nucléosidique (NRTI) ou non-analogues nucléosidiques (NNRTI) ciblent l'étape de transcription inverse. Les inhibiteurs de l'intégrase ciblent l'étape d'intégration génomique. Les inhibiteurs de protéase ciblent la production virale, les inhibiteurs de fusion ciblent l'entrée virale, comme les antagonistes des corécepteurs [187]. Les traitements combinés appartiennent principalement aux NRTI, NNRTI, inhibiteurs de l'intégrase et inhibiteurs de protéase. Ces traitements entraîne une diminution de la charge virale chez les patients, jusqu'à une virémie indétectable [188]. Depuis l'introduction de la thérapie antirétrovirale combinée (cART), la survie et la qualité de vie des patients infectés par le VIH se sont considérablement améliorées [189], avec un résultat plus favorable lorsque la thérapie est initiée dans le cadre d'une infection asymptomatique précoce [190]. Cependant, l'infection par le VIH-1 reste une maladie incurable, en raison de la présence de réservoirs de latence virale, cellulaires et tissulaires, dans lesquels un provirus est réduit au silence par régulation épigénétique. L'importance du poids physique et psychologique des traitements [191,192], conduit parfois à une moindre observance ou l'abandon du traitement [193] ce qui entraîne un rebond viral et une persistance de l'infection [194].

Parallèlement aux traitements curatifs, le développement des traitements a permis la mise en place récente de la prophylaxie pré-exposition (PrEP) [195]. Ce traitement s'est avéré efficace, bien que le niveau d'efficacité soit variable selon les populations et l'adhésion au traitement [196]. Ce traitement nécessite, en particulier chez les femmes, une dose orale journalière [197].

Tableau 2-1 : Liste des traitements anti-VIH

Type de traitement	Classe de molécule	Cible
Traitement antiviral	Inhibiteur non-nucléosidique de la transcriptase inverse	Trascriptase inverse
	Inhibiteur nucléosidique de la transcriptase inverse	Trascriptase inverse
	Inhibiteur de protéase	Protéase virale
	Inhibiteur de l'intégrase	Intégrase virale
Prophylaxie pré-exposition	Inhibiteurs de la transcriptase inverse	Transcriptase inverse
	Inhibiteurs de l'intégrase	Intégrase virale
Vaccins	Vaccins ARNm	Lymphocytes B
Traitement épigénétique	Inhibiteurs des HDACs	HDACs
	Inhibiteurs des HMT	HMT
	Inhibiteurs de Tat	Tat

2.11.2. Limites des thérapies

Le phénomène de rebond viral est dû à la présence de réservoirs du VIH, principalement dans les cellules T CD4+ mémoire au repos infectées de manière latente et dans les cellules myéloïdes telles que les macrophages et la microglie, qui sont difficiles à cibler par les traitements antirétroviraux ou les mécanismes effecteurs du système immunitaire [198–200].

Il est intéressant de noter que le provirus intégré dans ces cellules est soumis à une extinction transcriptionnelle et réversible et sous le contrôle des enzymes modificatrices de la chromatine de l'hôte, notamment les désacétylases et les méthyltransférases [201]. Cela a ouvert la voie à l'émergence de deux nouvelles approches thérapeutiques épigénétiques, à savoir les stratégies « shock and kill » et « block and lock » [54,202–204] (Figure 2-11).

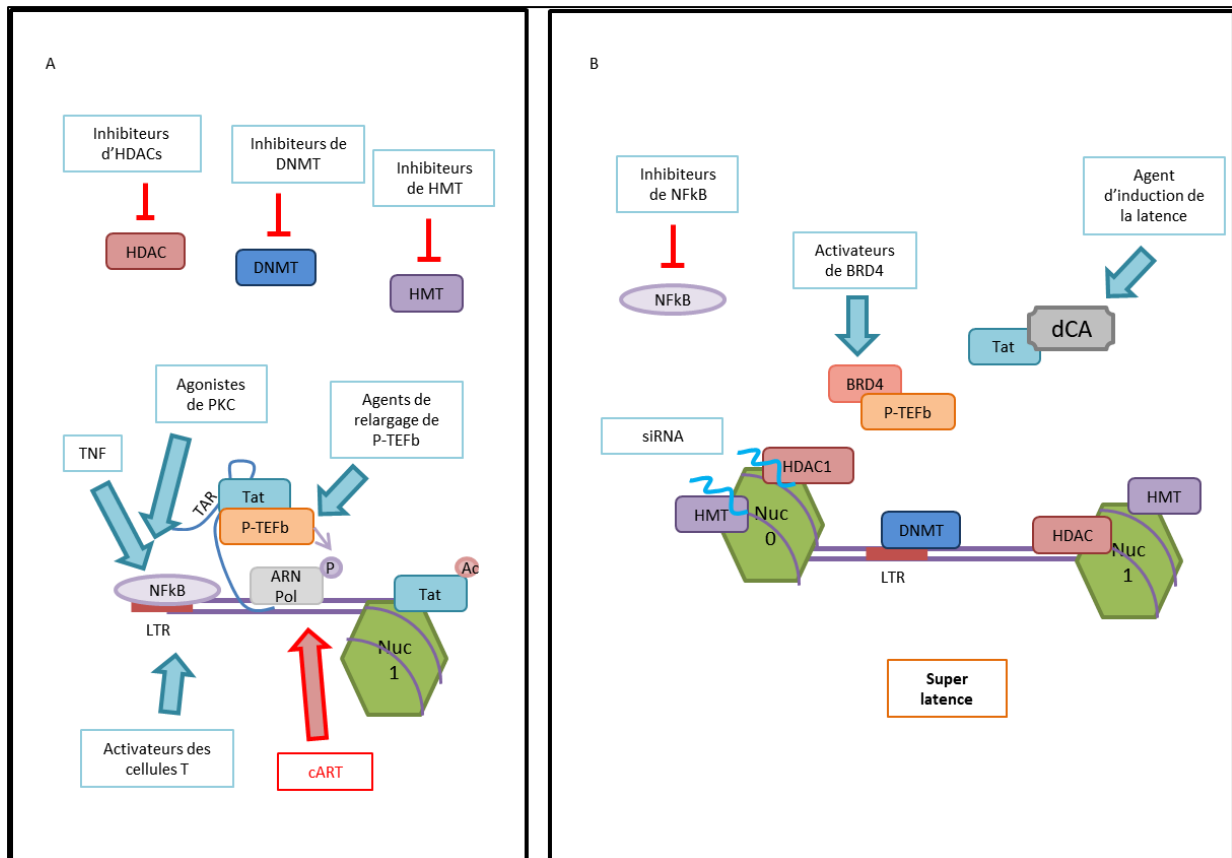


Figure 2-11 : Stratégies épigénétiques de thérapies anti-VIH, selon les modèles Shock and Kill (A) ou Block and Lock (B). [205]

Stratégie « shock and kill »

Cette stratégie repose sur la réactivation du provirus latent (choc) pour pouvoir éradiquer les cellules infectées (destruction) par le biais de la réponse immunitaire humorale, de la lyse médiée par les cellules T CD8+, de l'apoptose induite par le virus ou de la mort cellulaire induite par l'activation [127]. Dans cette stratégie, la thérapie antirétrovirale combinée (cART), chargée de tuer, est associée à des agents d'inversion de latence (LRA). Plusieurs LRA ou inducteurs de « choc » ont été proposés [206,207] pour cibler les régulateurs épigénétiques de la latence virale (Tableau 2-2).

Ces traitements incluent notamment des inhibiteurs d'histone désacétylase (HDAC), permettant la levée de la répression de Nuc-1, comme le SAHA, le TPA, le VPA ou le parabinostat [207]. Des activateurs de NFκB ont également été utilisés en combinaison avec les inhibiteurs d'HDAC, pour favoriser la réactivation [208].

Les inhibiteurs d'histone méthyltransférase (HMT), comme la Chaetocin, et d'ADN méthyltransférase (DNMT), comme le 5-AzadC, peuvent également être utilisés pour favoriser la réactivation, en levant le silence épigénétique au niveau de Nuc-1 et du promoteur du VIH. Leur association avec le TNF permet d'activer la transcription via NFκB et d'empêcher la formation d'hétérochromatine, ce qui améliore la transcription de la longue répétition terminale (LTR) du VIH-1 et la purge virale [209].

Le fait de cibler plusieurs protéines cellulaires impliquées dans le contrôle épigénétique de l'expression des gènes viraux amplifie généralement la réactivation du VIH-1. Bien que cette approche se heurte à plusieurs obstacles, dont celui de ne réactiver et éventuellement d'éliminer qu'un petit sous-ensemble du génome latent du VIH, elle constitue cependant une approche qui pourrait être utilisée en parallèle à d'autres pour parvenir à une guérison pleinement efficace [210].

Tableau 2-2 : Liste des LRA ayant été testés *in vitro* et *in vivo*. Adapté de Ait-Ammar et al. 2020 [211].

Classe de LRA	Molécules	Cibles
Atagonistes de PKC	Prostratin Bryostatine-1 Ingenols: Ingenol-B, Ingenol 3,20-dibenzoate (Ingenol-db), ingenol-3-angelate (ingenol mebutate, PEP005)	Activation de NFκB
Agonistes de MAPK	Procyanidin trimer C1	Activation des MAP Kinase
Antagonistes de CCR5	Maraviroc	Activation de NFκB
Vaccin Tat	Tat Oyi vaccine Tat-R5M4 protein	Activation du LTR
Mimétisme de SMAC	SBI-0637142 Birinapant	Induction des voies non-canoniques de NFκB
Inducteurs du relargage de P-TEFb	BETis: JQ1, I-BET, I-BET151, OTX015, UMB-136, MMQO, CPI-203, RVX-208, PFI-1, BI-2536 and BI-6727 HMBA	Relargage de P-TEFb
Activateurs de la voie Akt	Disulfiram	Activation de la voie Akt
Dérivés de benzotriazole	1-hydroxybenzotriazol (HOBt)	Activation de STAT5
Modificateurs épigénétiques	Inhibiteurs d'HDAC: TSA, trapoxin, SAHA, romidepsin, panobinostat, entinostat, givinostat, valproic acid, MRK-1/11, AR-42, fimepinostat, chidamide	Inhibition des HDAC
	Inhibiteurs de HMT: chaetocin, EPZ-6438, GSK-343, DZNEP, BIX-01294, UNC-0638	Suv39H1, G9a, SMYD2
	Inhibiteurs de DNMT: 5-AzaC, 5-Azac	DNMT1, 3a, 3b
LRA immunomodulateurs	Agonistes du TLR: Agonistes de TLR2 (Pam3CSK4), TLR7 (GS-9620), TLR8, TLR9 (MGN 1703) Agoniste de IL-15 (ALT-803) Inhibiteurs des checkpoints immunitaires: anti-PD-1 (nivolumab, pembrolizumab), anti-CTLA-4 (ipilimumab)	

Stratégie « block and lock »

Contrairement aux LRA, des agents chimiques pourraient "bloquer" la virémie en cours pendant la cART, en "verrouillant" le promoteur du VIH dans un état de super latence résistant aux stimuli de réactivation. Les modifications des histones associées à la latence virale sont stabilisées par l'action de régulateurs épigénétiques, associés à un blocage des activateurs du LTR. L'utilisation d'un inhibiteur de Tat, la didehydro-cortistatine A (dCA) bloque la réactivation virale, en empêchant la liaison de Tat avec l'ARN viral. Cela induit un blocage du recrutement des facteurs de transcription

comme le P-TEFb [212]. Cette approche entraîne l'établissement d'un état de latence persistant [213].

Une approche complémentaire est le ciblage de la voie de signalisation Akt. La protéine Akt est une protéine centrale du métabolisme cellulaire, impliquée dans la régulation de nombreuses fonctions. L'activation d'Akt favorise la réactivation du VIH par une augmentation globale de l'activation des cellules, incluant une activation des voies de signalisation NFkB, et des voies de survie cellulaire [214]. Des inhibiteurs d'Akt pourraient favoriser l'état de latence et réduire également la viabilité cellulaire, permettant la clairance cellulaire.

L'ajout d'agents de blocage aux traitements antirétroviraux pourrait être particulièrement bénéfique en cas d'inobservation du traitement ou d'interruption de courte durée, car ils limitent la reconstitution du réservoir de cellules T CD4+ [215].

3. Les Coronavirus

3.1. Caractéristiques des Coronavirus

Les coronavirus (CoV) constituent une famille très diversifiée de virus à ARN simple brin positifs enveloppés, appartenant au groupe IV de la classification de Baltimore. Ils infectent l'homme, d'autres mammifères et des espèces aviaires, y compris le bétail et les animaux de compagnie. Les coronavirus ont été classés comme membres de l'ordre des Nidovirales. Ils appartiennent à la sous-famille des Orthocoronavirinae, qui comprend quatre genres : alphacoronavirus, betacoronavirus, deltacoronavirus et gammacoronavirus. Les alphacoronavirus comprennent les Coronavirus humains (HCoV) 229E et NL63. Les bêtacoronavirus sont subdivisés en groupes A à D, le HCoV HKU1 étant inclus dans le groupe A, le SARS-CoV et SARS-CoV-2 dans le groupe B et le MERS-CoV dans le groupe C. Alors que les alphacoronavirus et les betacoronavirus infectent exclusivement les espèces mammifères, les gammacoronavirus et les deltacoronavirus ont une gamme d'hôtes plus large qui inclut les espèces aviaires. Les infections à coronavirus humaines et animales entraînent principalement des maladies respiratoires et entériques [216,217].

Les coronavirus humains, tels que les alphacoronavirus HCoV-229E et HCoV-NL63 et le bêtacoronavirus du groupe A HCoV-HKU1 identifié plus récemment, circulent dans la population humaine et n'induisent que des maladies légères des voies respiratoires supérieures chez les hôtes immunocompétents, bien que certains d'entre eux puissent provoquer des infections graves chez les nourrissons, les jeunes enfants et les personnes âgées [218,219]. En revanche, les bêtacoronavirus des groupes B et C qui sont apparus dans la population humaine au cours des 20 dernières années, sont hautement pathogènes. Cela inclut le coronavirus du syndrome respiratoire aigu sévère (SARS-CoV), le coronavirus du syndrome respiratoire du Moyen-Orient (MERS-CoV) et le nouveau SARS-CoV-2 responsable de la maladie à coronavirus 2019 (COVID-19). En infectant les cellules épithéliales bronchiques, les pneumocytes et les cellules des voies respiratoires supérieures chez l'homme, les infections à SARS-CoV, MERS-CoV et SARS-CoV-2 provoquent un syndrome respiratoire grave de détresse respiratoire aigue chez l'homme, potentiellement mortel.

3.2. Structure du génome viral

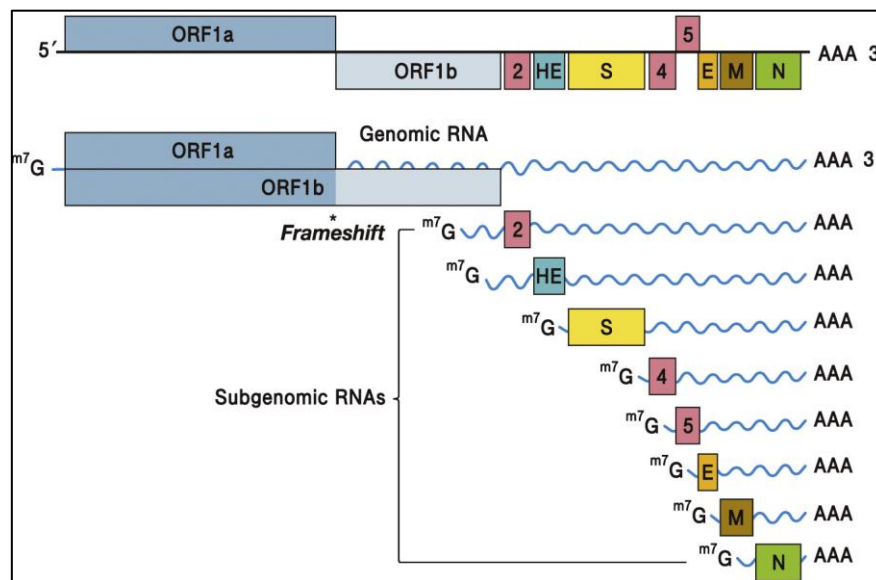


Figure 3-1 : Structure de l'ARN génomique des Coronavirus. [23]

Les coronavirus possèdent les plus grands génomes d'ARN connus, d'une taille de 25,4 à 31,8 kb et flanqués de régions non traduites 5' et 3' qui contiennent des structures d'ARN secondaires essentielles à la synthèse de l'ARN. Le génome est un simple brin positif, coiffé et adénylé. À l'extrémité 5', l'ARN génomique présente deux grands cadres de lecture ouverts (ORF ; ORF1a et ORF1b) qui occupent les deux tiers du génome (Figure 3-1). Les ORF1a et ORF1b codent pour 15-16 protéines non structurales (nsp), dont 15 composent le complexe viral de réplication et de transcription (RTC) qui comprend, entre autres, des enzymes de traitement et de modification de l'ARN et une fonction de relecture de l'ARN nécessaire au maintien de l'intégrité du génome [220]. L'ordre des gènes dans les ORF qui codent pour des protéines structurales est à peu près identique chez toutes les espèces de coronavirus, à savoir : 5'-réplicase - spicule (S) - enveloppe (E) - protéine membranaire (M) - nucléocapside (N)-3'. Chez les espèces qui contiennent le gène HE, celui-ci se trouve entre le gène de la réplicase et le gène de la protéine S. L'hémagglutinine-estérase (HE) contribue à l'attachement du virion à la cellule [221]. Les protéines accessoires des coronavirus, codées par des ORFs intercalés, sont un ensemble de protéines présentant une grande variété de fonctions et dont la présence peut varier selon les espèces et d'un virus à l'autre. On pense principalement qu'elles contribuent à moduler les réponses de l'hôte à l'infection et qu'elles sont des déterminants de la pathogénicité virale [222,223]. Néanmoins, les fonctions moléculaires de nombreuses protéines accessoires restent largement inconnues en raison de l'absence d'homologie avec les protéines accessoires d'autres coronavirus ou avec d'autres protéines connues [224].

3.3. Structure et protéines de la particule virale

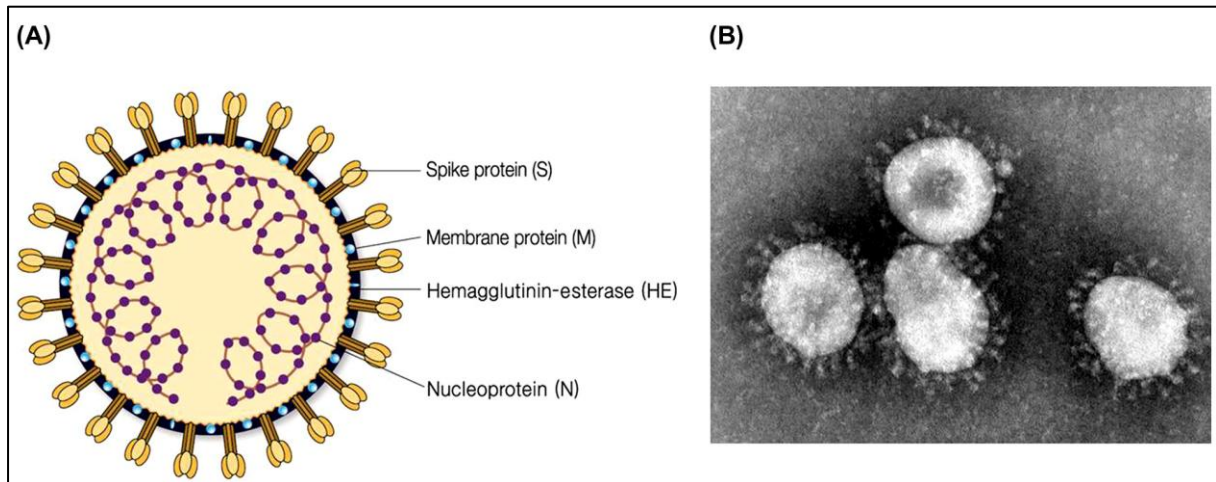


Figure 3-2 : Structure du virion des coronavirus. Diagramme schématisique (A) et imagerie en microscopie électronique (B). [23]

Les virions des coronavirus sont des particules rondes, enveloppées, modérément pléomorphes, de taille moyenne, mesurant 100 à 150 nm de diamètre et recouvertes d'une frange distinctive de spicules en forme de massue, largement espacées [225] (Figure 3-2). Les spicules mesurent environ 20 nm de long et sont constituées par la protéine spike (S), qui s'agrège en trimères pour former les peplomères caractéristiques du virus, en forme de couronne. Certains membres des bêta-coronavirus, dont l'OC43, contiennent également une protéine d'enveloppe plus courte, appelée hémagglutinine estérase (HE). L'enveloppe virale est constellée d'un trimère de glycoprotéine spike (S), d'un dimère d'hémagglutinine-estérase (HE), d'une protéine membranaire (M) et d'une protéine d'enveloppe (E). Les coronavirus contiennent une grande nucléocapside hélicoïdale. On a observé que ces particules bourgeonnaient des membranes de l'appareil de Golgi ou du réticulum endoplasmique (RE) d'une cellule infectée et s'accumulaient dans des vésicules cytoplasmiques [226]. Les cellules infectées présentent souvent des particules à la surface de la cellule qui représente le virus excrété dans des vésicules cytoplasmiques, plutôt qu'un bourgeonnement du virus à la membrane plasmique

3.3.1. Protéine Spike (S)

La protéine S est une grande glycoprotéine de surface de 150 kDa, assemblée en trimères et formant les projections caractéristiques des coronavirus. Son extrémité amino est tournée vers l'extérieur et elle est N-glycosylée. Il s'agit d'une protéine de fusion prototypique de classe 1 qui est impliquée dans les fonctions de liaison et de fusion des récepteurs [227]. Les protéases de l'hôte clivent la protéine spike par voie protéolytique, y compris, mais sans s'y limiter, les cathepsines endosomales, les protéases transmembranaires protéase/sérine (TMPRSS) de surface cellulaire, la furine et la trypsine [228,229]. Son clivage par les protéases cellulaires en deux sites possibles de clivage est nécessaire à l'entrée virale [230,231]. Le clivage résulte en deux sous-unités. La sous-unité S1 est impliquée dans l'interaction avec les récepteurs et la sous-unité S2 est impliquée dans la fusion des membranes virale et cellulaire. Cette protéine participe au tropisme cellulaire et tissulaire, et à la pathogenèse de l'infection. La protéine spike suscite également des réponses anticorps

neutralisantes. Les mutations de cette protéine impactent fortement le tropisme et l'infectivité des virus, donnant lieu à l'apparition de variants pouvant avoir des capacités de transmission et une létalité très différentes.

3.3.2. Glycoprotéine hémagglutinine estérase HE

La glycoprotéine HE n'est présente qu'à la surface de certains bêta-coronavirus. Elle est présente sous forme de dimères à la surface de l'enveloppe virale. Sa fonction estérase semble jouer un rôle dans la libération du virus des cellules infectées. Elle interagit avec les acides sialiques, via son domaine estérase [232]. Son domaine R est impliqué dans la reconnaissance des récepteurs.

3.3.3. Protéine membranaire M

La protéine M est incorporée dans la membrane de la particule virale. Il s'agit d'une protéine glycosylée de 20 à 35 kDa à trois domaines transmembranaires de l'enveloppe virale [233]. Elle joue un rôle clé dans l'assemblage du virion, interagissant avec toutes les autres protéines structurales majeures [223]. Sa liaison à la protéine N stabilise la nucléocapside et son interaction avec la protéine E constitue l'enveloppe virale, définissant ainsi sa forme. Elle joue également un rôle dans la réponse de l'hôte au virus, en activant les voies de signalisation NF κ B et IFN β [234].

3.3.4. Protéine de l'enveloppe E

La protéine E est une protéine faiblement représentée également présente dans l'enveloppe virale. Il s'agit de la plus petite des protéines structurales d'une taille comprise entre 8 et 12 kDa. Elle est abondamment exprimée à l'intérieur de la cellule infectée, mais seule une petite partie est incorporée dans l'enveloppe du virion [235]. Elle possède un domaine hydrophobe et une queue cytoplasmique chargée [235]. Son absence dans les coronavirus recombinants réduit significativement leur capacité de réplication. Elle joue un rôle important dans la production et la maturation des virions [236,237].

3.3.5. Protéine de la nucléocapside N

La protéine de la nucléocapside est une phosphoprotéine de 50 à 60 kDa, qui se lie à l'ARN à brin positif du virus et le stabilise. Elle est hautement conservée entre les espèces de coronavirus [238]. Par la formation du complexe ARN-nucléoprotéine, elle forme la nucléocapside [239]. Elle régule également la réplication et la transcription de l'ARN viral. Elle inhibe plusieurs fonctions cellulaires, notamment le métabolisme et le cycle cellulaire, par l'inhibition de CDK4 [240]. Elle est nécessaire à la formation de virions complets infectieux [241,242].

3.3.6. Protéines non structurales nsp

Le cadre de lecture ouvert 1a/b (ORF1a/b) du génome du coronavirus code pour une énorme polyprotéine qui est clivée par les protéases cellulaires et virales en quelque 16 protéines non structurales (nsp). Les diverses fonctions de ces protéines sont globalement conservées entre les coronavirus, et remplissent des rôles primordiaux dans la réplication virale. Cela inclut une ARN polymérase ARN-dépendante, plusieurs RNases, plusieurs protéases et plusieurs autres protéines essentielles. Les principales fonctions des 16 nsp sont listées dans le Tableau 3-1.

Tableau 3-1 : Protéines non structurales (nsp) et leur fonctions, adapté de Rohaim et al., 2021 [243]

Protéine non structurale (nsp)	Fonction
nsp 1	Favorise la dégradation de l'ARNm cellulaire et bloque la traduction de la cellule hôte, ce qui entraîne le blocage de la réponse immunitaire innée.
nsp 2	Aucune fonction connue, se lie aux protéines prohibitives.
nsp 3	Grosse protéine transmembranaire à plusieurs domaines, ses activités incluent : <ul style="list-style-type: none"> - Domaines Ubl1 et Ac, interagissent avec la protéine N - Activité ADRP, favorise l'expression des cytokines - Domaine PLPro/Deubiquitineuse, clive la polyprotéine virale et bloque la réponse immunitaire innée de l'hôte. - Domaines Ubl2, NAB, G2M, SUD, Y, fonctions inconnues
nsp 4	Protéine d'échafaudage transmembranaire potentielle, importante pour la structure correcte des DMVs
nsp 5	Mpro, clive la polyprotéine virale
nsp 6	Protéine d'échafaudage transmembranaire potentielle
nsp 7	Forme un complexe hexadécamérique avec nsp8, peut agir comme une pince de processivité pour l'ARN polymérase.
nsp 8	Forme un complexe hexadécamérique avec nsp7, peut agir comme pince de processivité pour l'ARN polymérase ; peut agir comme primase
nsp 9	Protéine phosphatase de liaison à l'ARN
nsp 10	Cofacteur pour nsp16 et nsp14, forme un hétérodimère avec les deux et stimule l'activité d'ExoN et de 2'-O-MT
nsp 12	Enzyme de réplication (ARN polymérase ARN-dépendante)
nsp 13	ARN hélicase, 5' triphosphatase
nsp 14	N7 MTase et 3'-5' exoribonucléase, ExoN ; la N7 MTase ajoute la coiffe 5' aux ARN viraux, l'activité d'ExoN est importante pour la relecture du génome viral
nsp 15	Endoribonucléase virale, NendoU
nsp 16	2'-O-MT ; protège l'ARN viral de la reconnaissance de MDA5

Contrairement aux autres virus à ARN, qui ne disposent pas de mécanismes de relecture permettant de corriger les mutations survenant au cours de la réplication de l'ARN, la protéine nsp14 des coronavirus assure une activité de relecture [244].

3.3.7. Protéines accessoires

Les coronavirus possèdent un certain nombre de protéines accessoires, dont certaines ne sont pas nécessaires à la réplication virale *in vitro* mais sont importantes *in vivo*. Certaines d'entre elles, par exemple, l'ORF6, l'ORF3b et l'ORF4a, sont des antagonistes de l'interféron et favorisent l'échappement aux réponses immunitaires innées de l'hôte [222,245].

3.4. Cycle de vie des coronavirus

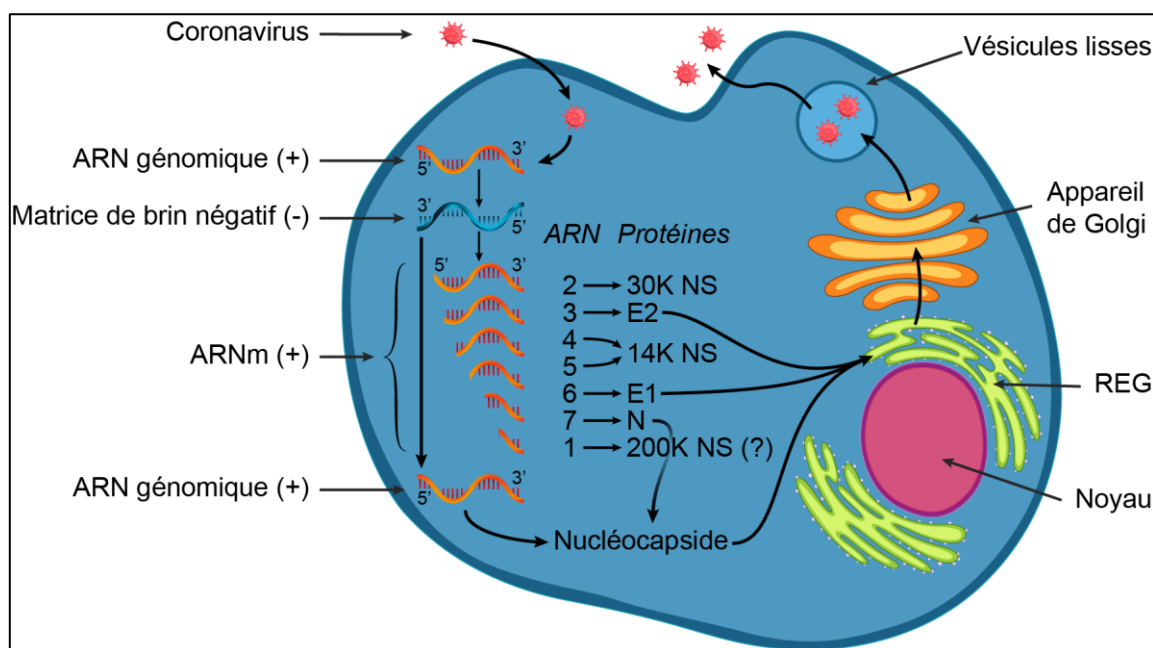


Figure 3-3 : Cycle de réplication des coronavirus, adapté de Ryu 2017 [23]

Les premières étapes de l'infection par les coronavirus impliquent la liaison spécifique de la protéine S aux récepteurs d'entrée cellulaires. Ceux-ci varient en fonction du virus. Les récepteurs ont été identifiés pour plusieurs coronavirus : l'aminopeptidase N humaine (APN) est ciblée par le HCoV-229E; l'enzyme de conversion de l'angiotensine 2 (ACE2) est la cible de HCoV-NL63, et des SARS-CoV et SARS-CoV-2 ; et la dipeptidyl peptidase 4 (DPP4) est le récepteur associé au MERS-CoV [246–249]. L'expression et la distribution tissulaire des récepteurs d'entrée influencent par conséquent le tropisme et la pathogénicité du virus. La protéine HE, lorsqu'elle est présente, sert également à la liaison de co-récepteurs [221,232]. L'entrée virale est sous le contrôle du clivage de la protéine S en sous-unités S1 et S2 par les protéases cellulaires, notamment TMPRSS2, la furine et la trypsine. La sous-unité S1 assure la liaison au récepteur cellulaire, ce qui induit un changement conformationnel résultant en la transition de la sous-unité S2 vers une conformation stable. Cette sous-unité S2 stabilisée entraîne la fusion des membranes virales et cellulaires, éventuellement associée à la protéine HE [250]. Cette fusion permet la libération du génome dans le cytoplasme.

La libération du génome du coronavirus dans le cytoplasme de la cellule hôte lors de l'entrée marque le début d'un programme complexe d'expression des gènes viraux, qui est hautement régulé dans l'espace et dans le temps (Figure 3-3). Le génome ARN positif des coronavirus permet la transcription immédiate, sans nécessiter de protéines accessoires présentes dans le virion. La

traduction des ORF1a et ORF1b à partir de l'ARN génomique produit deux polyprotéines, pp1a et pp1ab, respectivement. Seize protéines non structurales sont libérées de manière co-traductionnelle et post-traductionnelle lors du clivage protéolytique par deux protéases à cystéine qui sont situées dans nsp3 (protéase de type papaïne ; PLpro) et nsp5 (protéase de type chymotrypsine, 3CLpro ou Mpro).

Nsp1, dont la libération est rapide, cible la machinerie de traduction de la cellule hôte [251]. Les nsp2 à 16 composent le complexe de réplication et de transcription viral (RTC) (Figure 3-4). Les nsp12 à 16 contiennent les fonctions enzymatiques essentielles à la synthèse, la correction et la modification de l'ARN [222,252]. La nsp 12 possède une fonction ARN polymérase ARN dépendante et, à l'aide des cofacteurs nsp7 et nsp8, est responsable de la synthèse d'ARN [222,252,253]. La nsp14 possède une fonction unique dans les virus à ARN de relecture, par son activité 3'-5' exonucléase [254]. Les nsp13, 14 et 16 participent à la machinerie de coiffage pour la modification de l'ARN [252,255–257].

Les protéines structurales (S, E, M, N et le cas échéant HE), sont codées par des ORFs situés dans la partie 3' du génome. Globalement, l'assemblage des protéines structurales est réalisé au niveau du réticulum endoplasmique, avec un bourgeonnement de nouveaux virions vers le Golgi. La nucléocapside est formée par l'interaction des protéines N avec l'ARN génomique. Cette nucléocapside est incorporée au virion par l'interaction avec les protéines M, dont l'interaction avec les protéines S et E permet la production et la maturation des virions. Les détails du processus d'assemblage des virions restent méconnus. Les virions néo-produits sortent des cellules infectées par exocytose, ou dans le cas des bêtacoronavirus, via le trafic lysosomal.

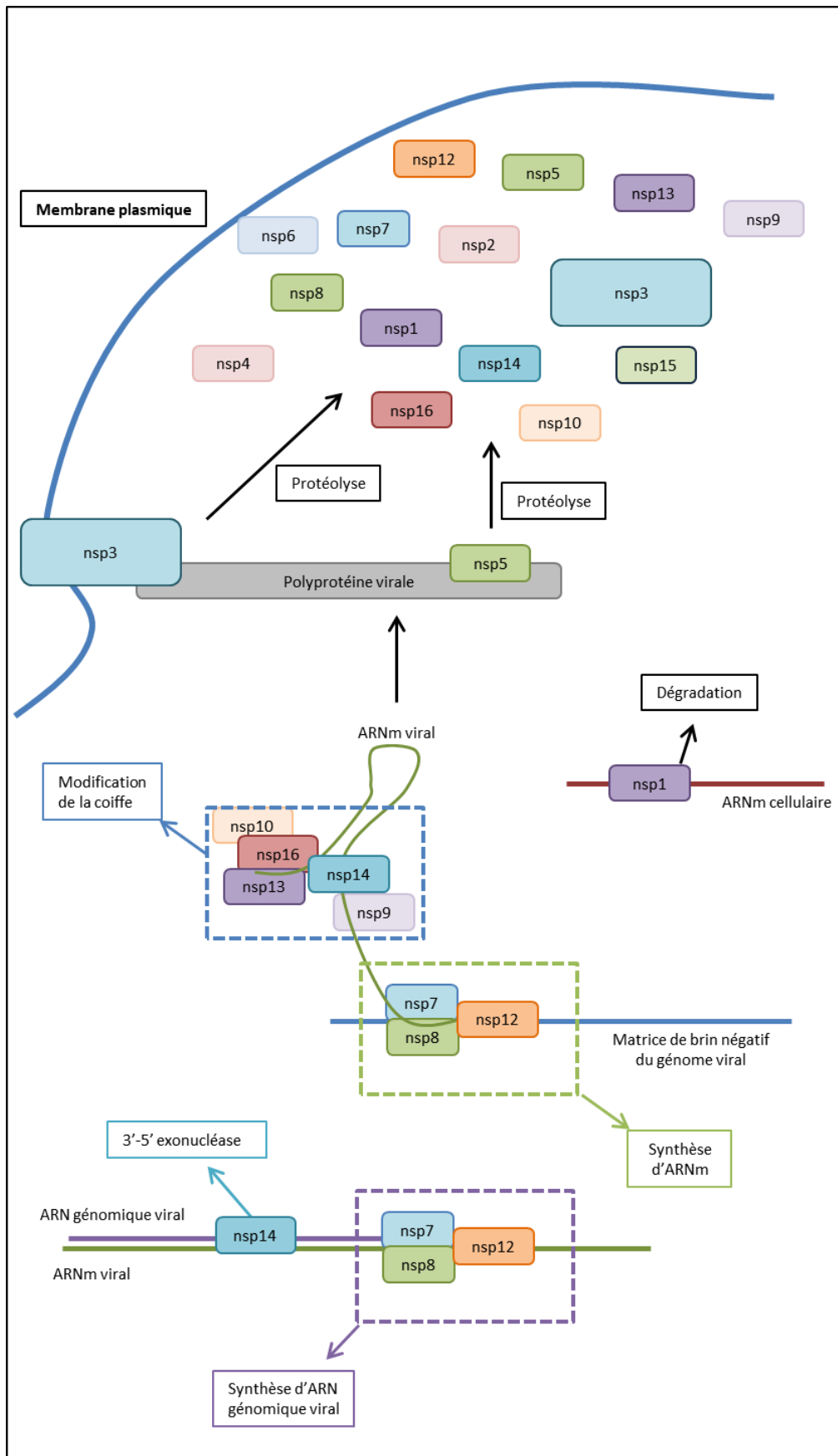


Figure 3-4 : Fonctions connues des protéines non structurales (nsp) des coronavirus.

3.5. Pathogénicité et interaction avec l'hôte.

Le cycle de vie d'un coronavirus intracellulaire repose invariablement sur des interactions moléculaires critiques avec les protéines de l'hôte, qui sont modifiées pour répondre aux besoins du virus. Il s'agit notamment des facteurs de l'hôte nécessaires à l'entrée du virus (comme le récepteur d'entrée et les protéases de la cellule hôte), des facteurs nécessaires à la synthèse de l'ARN viral et à l'assemblage du virus (comme les composants du RE et du Golgi et les voies de trafic vésiculaire associées) et des facteurs nécessaires à la traduction des ARNm viraux (comme les facteurs critiques d'initiation de la traduction) [258–260].

Les coronavirus échappent efficacement aux réponses immunitaires innées. Dans ce contexte, les interactions virus-hôte sont multifformes et comprennent des stratégies visant à cacher les motifs moléculaires associés aux pathogènes viraux [261,262]. Le complexe viral de réplication et de transcription des coronavirus contribue également à l'évasion immunitaire innée par le biais de plusieurs fonctions codées par les nsp. Il s'agit notamment de l'activité de déubiquitylation médiée par PLpro [263,264], de la dé-ADP-ribosylation par les macro-domaines codés par nsp3 [265], des activités enzymatiques de modification de l'ARN codées par nsp14, 15 et 16 [266–268].

Selon nos connaissances actuelles, le SRAS et le COVID-19 sont une conséquence des fonctions codées par le virus et des réponses retardées de l'interféron et, dans les cas graves, ils sont associés à des réponses immunitaires dérégulées et à des immunopathologies [269,270]. Par conséquent, l'augmentation des réponses pro-inflammatoires aberrantes et l'infiltration de cellules immunitaires dans les poumons provoquent des lésions tissulaires et contribuent à la manifestation clinique du SRAS [270].

De même, les réponses de l'hôte, telles que l'expression des cytokines, qui sont connues pour être à l'origine de l'inflammation et des immunopathologies, sont considérablement affectées par l'infection par les coronavirus.

L'ensemble de ces effets peut se traduire par des réponses pro-inflammatoires fortes et dérégulées, tandis que les cellules présentent une faible activation des défenses antivirales innées [271,272]. Dans les cas graves de COVID-19, par opposition aux cas légers, un recrutement aberrant de macrophages inflammatoires et une infiltration de lymphocytes T, y compris de lymphocytes T cytotoxiques, ainsi que de neutrophiles ont été mesurés dans les poumons [272]. L'accumulation de preuves de la dérégulation des réponses pro-inflammatoires au cours des infections par le SRAS-CoV-2 a conduit à l'utilisation de modulateurs immunitaires pour inhiber les réponses immunitaires pathogènes hyperactivées [269,273–275].

3.6. Traitements

Actuellement, les stratégies de traitement curatif des pathologies associées aux coronavirus sont des approches symptomatiques. La phase pulmonaire de l'infection par le SARS-CoV-2 entraîne l'apparition de fièvre et d'hypoxémie, nécessitant l'hospitalisation. Un syndrome hyperinflammatoire est également observé dans les formes graves avec une hypercoagulabilité.

La phase inflammatoire nécessite des traitements pouvant inhiber les réponses inflammatoires. Le dexaméthasone, un glucocorticoïde, a montré, dans plusieurs essais cliniques, une augmentation de

la survie des patients traités, et constitue un traitement préconisé pour cette phase [276,277]. L'utilisation d'IFN β semble également réduire les symptômes chez les patients présentant une infection modérée [278]. Au cours de cette phase inflammatoire, une forte expression d'IL-6 entraîne une réponse hyper-inflammatoire. L'utilisation d'inhibiteurs d'IL-6, tels que le Tocilizumab et le Sarilumab, est testé dans différents essais cliniques (NCT04315298 ; NCT04317092). Ces deux traitements sont utilisés en clinique pour réduire la réponse hyper-inflammatoire observée dans les formes graves de la Covid-19 [279–281].

Associée à la phase inflammatoire, une coagulopathie, consécutive à un trouble systémique de la coagulation, semble impliquée dans les troubles pulmonaires [282]. Cette coagulopathie est caractérisée par une augmentation du taux plasmatique de l'inhibiteur d'activateur de plasminogène 1 (PAI-1) [283]. L'utilisation des traitements anticoagulants, tel l'héparine, a montré une réduction de la mortalité [284].

L'absence de traitement spécifiquement antiviral a entraîné la réutilisation de traitements existants (Tableau 3-2). Parmi les traitements envisagés, l'hydroxychloroquine et le lopinavir ont fait l'objet de plusieurs essais cliniques, qui ont montré l'absence d'effet significatif [285,286]. Le favipiravir, un inhibiteur de l'ARN polymérase, et l'umifenovir, un inhibiteur de la liaison Spike/ACE2, ont fait l'objet d'essais cliniques ayant montré un effet positif dans les formes modérées de Covid-19 [16]. Le remdesivir, un inhibiteur des ARN polymérases virales, fait également l'objet de plusieurs essais cliniques [287,288]. Un essai clinique, testant le remdesivir et trois autres traitements, a montré l'absence d'efficacité *in vivo* [289]. L'utilisation de statines et d'inhibiteurs du récepteur de l'angiotensine font également l'objet de tests. Bien que ces traitements entraînent l'augmentation de l'expression d'ACE2, l'inhibition de l'inflammation suite au traitement serait globalement bénéfique [290].

Tableau 3-2 : Liste des composés testés contre le SARS-CoV-2. Adapté de Valle et al. 2020 [291]

Composé	Cible/Mode d'action	Résultats des essais cliniques
Arbidol hydrochloride	Endocytose/inhibition de la fusion des membranes	Amélioration insuffisante dans l'infection modérée
Ribavirin	ARN polymérase/inhibition	Faible effet sur la mortalité
Remdesivir	ARN polymérase/inhibition	
Favipiravir	ARN polymérase/inhibition	Faible amélioration dans l'infection modérée
Sofosbuvir	ARN polymérase/inhibition	Faible effet sur la mortalité
Ritonavir	Proteases/inhibition	Pas d'effet sur les formes sévères
Lopinavir	Proteases/inhibition	Pas d'effet sur les formes sévères
Nelfinavir	Proteases/inhibition	Essai clinique en cours
Dolutegravir	Proteases/inhibition	Pas d'effet sur la mortalité
Nafamostat mesylate	TMPRSS2/inhibition	Pas d'effets significatifs
Camostat mesylate	TMPRSS2/inhibition	Pas d'effets significatifs
Chloroquine Hydroxychloroquine	Augmentation du pH dans le compartiment endosomal	Pas d'effets significatifs
Azithromycin	Augmentation du pH dans le compartiment endosomal /immunomodulation	Pas d'amélioration significative
Omeprazole	Inhibiteur de pompe protonique	Augmentation du risque de complication
Vonoprazan	Inhibiteur de pompe protonique	Augmentation du risque de complication
Ivermectin	Limite l'infection virale	Réduction de la mortalité
Oseltamivir	Inhibiteur de neuraminidase	Pas d'effets significatifs

Des traitements préventifs, sous la forme de vaccins, ont été développés très rapidement suite à la pandémie de Covid-19. Parmi les vaccins développés contre le SARS-CoV-2, les vaccins Oxford Astrazeneca, Janssen et Spoutnik V sont des vaccins utilisant un adénovirus comme vecteur viral modifié [292]. Les vaccins Pfizer et Moderna sont des vaccins à ARNm, premiers vaccins de ce type à être déployés à grande échelle [293].

Malgré le déploiement de stratégies vaccinales contre le SARS-CoV-2, l'apparition de variants du virus, en raison de la forte propagation dans la population, ne permet pas d'envisager une baisse immédiate des infections par le SARS-CoV-2 [294]. Les traitements curatifs, limités à une approche symptomatique, sont limités. La nécessité de trouver de nouveaux traitements antiviraux, spécifiques des coronavirus, requiert une meilleure connaissance des mécanismes moléculaires, afin de pouvoir déterminer des cibles efficaces.

II. Etude de l'impact des traitements antirétroviraux sur les réservoirs de latence virale du VIH

4. Ciblage du TNF et des récepteurs TNF dans l'infection par le VIH

4.1. Voies de signalisation du TNF et des récepteurs TNF dans l'infection par le VIH

Le TNF est produit par de nombreuses cellules en réponse notamment à l'inflammation ou à l'infection. Le TNF se lie à deux récepteurs membranaires TNFR1 et TNFR2. La forme liée à la membrane du TNF se lie préférentiellement au TNFR2 et la forme soluble du TNF se lie préférentiellement au TNFR1. L'activation du TNFR1 entraîne une augmentation de l'expression des facteurs de croissance et de réponse au stress, ainsi que l'augmentation de la prolifération cellulaire, par une cascade de kinases cellulaires. L'activation du TNFR2 entraîne une augmentation de l'expression des cytokines pro-inflammatoires, transmise par la phosphorylation d'I κ B. Le TNF induit l'activation de plusieurs voies de signalisation moléculaires, incluant les voies du NF κ B, de JNK et des MAP Kinases p38 [295,296].

Le TNF entraîne une inhibition de l'entrée du VIH dans les cellules permissives. Cette inhibition est principalement liée à la baisse de CCR5 à la surface cellulaire, suite à la stimulation des cellules par le TNF [297,298]. Le TNF entraîne également une activation de NF κ B et favorise la sécrétion de facteur de suppression du VIH, en particulier les protéines pro-inflammatoires des macrophages MIP-1 α , MIP-1 β et RANTES, qui inhibent l'entrée virale par inhibition de l'expression de CCR5 à la surface cellulaire [299–301]. L'activation des voies du TNF entraîne une augmentation de l'activation de NF κ B, et de sa liaison avec le promoteur du HIV. Cela induit une augmentation de la transcription virale.

4.2. Interaction des protéines du VIH avec les récepteurs TNF et les voies de signalisation en aval

La protéine virale Vpr entraîne une activation de NF κ B, AP-1 et JNK, de manière similaire au TNF [302]. Cela permet une augmentation de la réplication virale. Vpr entraîne également une augmentation de la production de TNF dans les cellules infectées. Cela entraîne une augmentation de la réactivation virale à partir des réservoirs de latence [303,304].

La protéine virale Nef entraîne une activation de NF κ B et la production de cytokines pro-inflammatoires IL-6, MIP-1 α et MIP-1 β . Nef entraîne également une augmentation de la production de TNF [305]. Elle induit aussi, par modification du transport endosomal de l'enzyme de conversion du TNF, l'augmentation de la forme soluble du TNF [306].

La protéine virale Tat entraîne l'activation des voies de signalisation NF κ B, JNK, p38 et ERK1/2, d'une manière similaire au TNF. L'activation de la voie de signalisation TRF4, consécutive à la stimulation par Tat, entraîne une augmentation de la production de TNF [307]. Tat entraîne également l'activation de PKC, des MAP kinases et de NF κ B, par l'interaction avec MyD88 et TRIF [308].

La liaison du récepteur CD4 avec la protéine virale gp120 entraîne l'activation des voies de signalisation PI3K et MAPK, ce qui conduit à une augmentation de la production de TNF, en plus de la production d'IL-6, IL-10 et interféron γ .

4.3. Thérapies anti-TNF et impact sur les réservoirs de latence du VIH

L'augmentation de la quantité plasmatique ou tissulaire du TNF est observable à tous les stades de l'infection par le VIH, en parallèle de l'activation immunitaire. L'utilisation d'inhibiteurs du TNF, en plus des thérapies antivirales, pourrait permettre de réduire l'activation immunitaire chronique. Plusieurs inhibiteurs du TNF (thalidomide, etanercept, infliximab, adalimumab) ont montré une réduction des niveaux de TNF plasmatique et une diminution des symptômes de l'infection. Bien qu'une augmentation du nombre d'infections opportunistes soit habituellement observée sous traitement anti-TNF, l'utilisation de ces inhibiteurs ne semble pas augmenter le taux d'infections opportunistes [309–312].

Le TNF a été utilisé pour réactiver la réplication du VIH à partir de cellules infectées de manière latente. Cependant, le niveau de réactivation est limité. L'utilisation du TNF en combinaison avec des agents d'inversion de la latence (LRA) entraîne une forte réactivation, notamment lors de l'utilisation d'inhibiteurs des HDACs et des HMTs comme prostratin . et pourrait permettre de purger les réservoirs de latence dans le cadre d'une stratégie « shock and kill ».

A l'opposé, l'inhibition du TNF pourrait permettre de réduire la réactivation du VIH, en bloquant notamment les voies de signalisation NF κ B, MAPK et p38. Associés à des inhibiteurs d'autres voies de signalisation impliquées, cela pourrait participer à une stratégie « block and lock ».

4.4. Article paru dans la revue Viruses en 2017 « Targeting TNF and TNF Receptor Pathway in HIV-1 Infection: from Immune Activation to Viral Reservoirs »



Review

Targeting TNF and TNF Receptor Pathway in HIV-1 Infection: from Immune Activation to Viral Reservoirs

Sébastien Pasquereau, Amit Kumar and Georges Herbein *

Department of Virology, University of Franche-Comte, University of Bourgogne-Franche-Comté (UBFC), CHRU Besançon, UPRES EA4266 Pathogens & Inflammation/EPILAB, SFR FED 4234, F-25030 Besançon, France; sebastien.pasquereau@univ-fcomte.fr (S.P.); amit.aiims2005@gmail.com (A.K.)

* Correspondence: georges.herbein@univ-fcomte.fr; Tel.: +33-370-632-512

Academic Editor: Jens H. Kuhn

Received: 31 January 2017; Accepted: 23 March 2017; Published: 30 March 2017

Abstract: Several cellular functions such as apoptosis, cellular proliferation, inflammation, and immune regulation involve the tumor necrosis factor- α (TNF)/TNF receptor (TNFR) pathway. Human immunodeficiency virus 1 (HIV-1) interacts with the TNF/TNFR pathway. The activation of the TNF/TNFR pathway impacts HIV-1 replication, and the TNF/TNFR pathway is the target of HIV-1 proteins. A hallmark of HIV-1 infection is immune activation and inflammation with increased levels of TNF in the plasma and the tissues. Therefore, the control of the TNF/TNFR pathway by new therapeutic approaches could participate in the control of immune activation and impact both viral replication and viral persistence. In this review, we will describe the intricate interplay between HIV-1 proteins and TNF/TNFR signaling and how TNF/TNFR activation modulates HIV-1 replication and discuss new therapeutic approaches, especially anti-TNF therapy, that could control this pathway and ultimately favor the clearance of infected cells to cure HIV-infected patients.

Keywords: TNF; TNF receptor; HIV-1; reservoirs; immune activation; anti-TNF therapy

1. Introduction

Tumor necrosis factor- α (TNF) was first described as a glycoprotein induced in response to endotoxin (the old name of which was cachectin) and has the capacity to kill tumor cells [1]. Then, TNF was shown to be a T helper type 1 (Th1) cytokine produced by several cell types, including monocytes/macrophages and T-cells, and to be involved in proinflammatory responses like interleukin (IL)-1 β and IL-6 [2]. TNF exerts its effects by binding as a trimer to two cell membrane TNF receptors (TNFRs), TNFR1 (55-kDa) and TNFR2 (75 kDa) [2]. In concert with chemokines, TNF and TNFRs are involved in the regulation of inflammatory processes like arthritis, of infectious diseases including HIV infection, and of malignancies, since chronic inflammation has been recognized to play a major role in carcinogenesis [3–5]. TNF was first recognized at the protein level, followed by identification of its cDNA sequence [4,6,7]. TNFRs are composed of an extracellular domain common to all TNFR superfamily members, a typical cysteine rich motif, and intracellular domains with distinct motifs, allowing the accomplishment of various intracellular functions [8,9]. Death receptors are members of the TNFR superfamily characterized by a cytoplasmic region, the death domain, that enables the receptors to trigger pro-death signals within the cell [4]. TNFR1 possesses a death domain, and TNFR2 lacks one. This allows TNF to deliver distinct intracellular signals after binding to its receptors. We will discuss in this review how the binding of TNF to its receptors modulates the cellular activation, the interaction between human immunodeficiency virus 1 (HIV-1) proteins, and TNF/TNFR signaling, and the role of TNF/TNFRs in the modulation of the viral life cycle. In addition, we will address the

impact of the TNF/TNFR pathway on the immune activation and the formation of viral reservoirs during the course of HIV-1 infection. The opportunity to modulate the TNF effect and the TNFR pathway by anti-TNF therapy and/or to stimulate viral reactivation by TNF or TNF inducers to purge the HIV-1 reservoir will be discussed.

2. TNF/TNFR Signaling

Many cell types, especially immune cells, produce TNF in response to pathological conditions such as inflammation and infection, including: activated macrophages and T lymphocytes, natural killer (NK) cells, mast cells, and fibroblasts [4,8]. A membrane protein belonging to the ADAM (a disintegrin and metalloproteinase) family, the TNF- α converting enzyme (TACE) cleaves various membrane proteins including the TNF precursor, a 25 kDa plasma bound protein, into a 17 kDa soluble TNF form [10]. In their trimeric configuration, both membrane-bound and soluble TNF forms are functional when binding to their receptors. Although both TNF forms can bind to each of the two receptors, they display a dichotomic effect. The plasma membrane associated with 25 kDa TNF binds preferentially to the TNFR2, whereas the soluble 17 kDa TNF interacts with TNFR1 with high specificity [11,12]. Several intracellular pathways are triggered by TNF binding to its receptors, including the modulation of the apoptotic pathway, NF- κ B stimulation, and the activation of p38 mitogen-activated protein kinases (MAPK), extracellular signal-regulated kinase (ERK), and c-Jun N-terminal kinase (JNK) [4,8] (Figure 1).

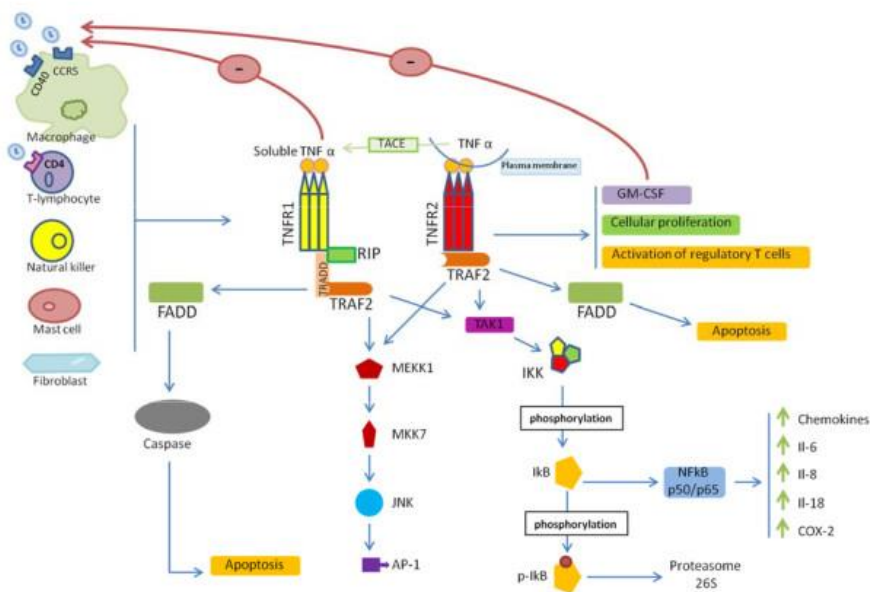


Figure 1. Tumor necrosis factor- α (TNF)/TNF receptor (TNFR)-mediated cell signaling. The binding of TNF to TNFRs leads to the recruitment of adaptor proteins—TNFR-associated death domain (TRADD), Fas-Associated death domain (FADD), TNF receptor associated factor (TRAF), and receptor interacting protein (RIP)—that activates several signaling cascades leading to the activation of transcription factors (NF- κ B, AP-1 among others) and/or caspase cascades. COX-2: cyclooxygenase-2; FADD: Fas-associated death domain; GM-CSF: Granulocyte-macrophage colony-stimulating factor; I κ B: NF- κ B inhibitor; IKK: I κ B kinase; IL: Interleukin; NF κ B: Nuclear factor- κ B; TACE: TNF- α converting enzyme; TRAF2: TNFR-associated factor 2; TRADD: TNFR-associated death domain.

A 34 kDa adapter protein called TNFR-associated death domain (TRADD) interacts with the cytoplasmic death domain of TNFR1 through its own death domain in response to the binding of TNF to TNFR1 [13] (Figure 1). Further, a Fas-Associated death domain protein (FADD) binds to TRADD and activates apoptosis via the caspase cascade [14] (Figure 1). On the other hand, the sequential recruitment of receptor interacting protein (RIP), transforming growth factor (TGF)- β -activated kinase 1 (TAK1), and I κ B kinase (IKK) by the TNF receptor associated factor 2 (TRAF2) forms a complex that interacts with TRADD [8,15,16]. The phosphorylation of I κ B by IKK and its subsequent degradation in the proteasome leads to NF- κ B translocation into the nucleus, where it binds to the promoters of genes involved in inflammation, such as TNF, IL-6, IL-8, IL-18, chemokines, and cyclooxygenase-2 (COX-2) genes [4,8,15,17] (Figure 1). In addition to stress and growth factors, cellular proliferation can be triggered by TNF binding to TNFR1, followed by activation of the following axis; TRADD, TRAF2-RIP, mitogen-activated protein kinase kinase kinase 1 (MEKK1), mitogen-activated protein kinase kinase 7 (MKK7), and JNK, ultimately leading to the activation of the AP-1 transcription factor [4,8,18] (Figure 1). Although TNFR2 lacks a death domain, it interacts with TRAF2 and can activate the transcription factors NF- κ B and AP-1 (Figure 1). Cell proliferation and survival, activation of regulatory T-cells, and the induction of granulocyte-macrophage colony-stimulating factor (GM-CSF) secretion can be elicited through TNF/TNFR2 signaling [19–21].

3. TNF/TNFRs and HIV-1 Entry

The role of TNF in HIV-1 entry has been extensively described, especially in primary human macrophages (Figure 1) [22]. In vitro, the primary macrophages infected by HIV-1 or treated with HIV-1 envelope protein gp120 release TNF in culture supernatants [23]. A delayed detection of HIV DNA long terminal repeat (LTR) up to 72 h has been observed in macrophages pre-treated by TNF parallel to the downregulation of the CD4 receptor [22–26]. Therefore, one explanation for the inhibition of HIV entry into permissive cells by TNF might be the down regulation of the expression of the HIV CD4 receptor and the C-C chemokine receptor type 5 (CCR5) coreceptor on the cell surface [24]. In fact, CCR5 downregulation on the cell surface results from the secretion of GM-CSF following the stimulation of macrophages with TNF, which will ultimately result in the inhibition of the entry of CCR5-dependent HIV-1 strains [25]. In addition, the binding of TNF to TNFR2 activates NF- κ B and favors enhanced secretion of HIV suppressive factors, such as macrophage inflammatory protein-1 α (MIP-1 α), MIP-1 β , and regulated on activation, normal T-cell expressed and secreted (RANTES). These factors also downregulate CCR5 on the cell surface [27–31], indicating that the TNF inhibition of HIV entry is mediated through TNFR2 but not TNFR1. However, the inhibition of viral entry is not related to the CD4 downregulation in macrophages. Both surface and total CD4 are downregulated in macrophages at the level of transcription after TNF pretreatment for one to five days [23,24]. However, after TNF pretreatment for 2 h, a 75% decrease in virus entry is observed, although the levels of surface CD4 expression are not yet modified. In addition, the surface CD4 downregulation in TNF-treated macrophages is mediated through TNFR1 but not through TNFR2 [22–24,26]. Therefore, the inhibition of HIV-1 entry into macrophages by TNF is mediated through the TNF-R2 but not through TNF-R1 and is independent of the modulation of surface CD4 expression. Altogether, the downregulation of the CCR5 co-receptor is the main mechanism that inhibits HIV entry into macrophages treated with TNF and is not related to the downregulation of the CD4 receptor. However, we cannot exclude that the inhibition of the HIV-1 entry into macrophages could also result from post-binding steps, which have still to be identified.

4. TNF/TNFR Signaling in HIV-1 Infection

Several intracellular signaling pathways, including NF- κ B, JNK, and p38 MAP kinase pathways, are triggered by TNF (Figure 1) [32]. Diverse cellular functions are regulated by the members of the p38 family, consisting of p38 α , p38 β , p38 γ , and p38 δ , through the phosphorylation of transcription factors such as activator protein 1 (AP-1), activating transcription factor 2 (ATF-2), and cAMP response

element binding protein(CREB) [33]. The first isoform discovered for its role in endotoxin-induced inflammatory response and osmotic shock is p38 α [33,34]. Independent or synergistic activation of NF- κ B and p38 by TNF can be observed [35,36]. Specific signaling pathways leading to the activation of either pro-survival mediators or effectors of cell death can be observed in response to TNF stimulation. By binding to TNFR, TNF activates NF- κ B, which promotes cell survival, JNKs, and caspases, which favor cell death. Therefore, an exquisite crosstalk between the two pathways will decide the cellular fate [37]. In addition, in HIV-1 infected cells, TNF stimulation will favor the binding of p65/p50 NF- κ B and AP-1 complexes to their respective transcription factor binding sites present in the HIV-1 long terminal repeat (LTR) promoter. Thus, TNF-mediated translocation of NF- κ B to the nucleus, followed by LTR stimulation, fuels HIV-1 production in chronically infected T-cell lines and promonocytic cell lines, such as the ACH2 and U1 cell lines, respectively [38–42]. Although TNF could be a major driver of HIV-1 transcription early in the disease, later on, during the progression of the disease, a T helper Th1/Th2 shift has been described [43]. Since Th2 cytokines are mostly immunosuppressive with decreased levels of TNF production at advanced stages of the disease, HIV-1 replication could be sustained by the production of some HIV proteins that mimic TNF activity. We and others reported previously that several HIV-1 proteins, especially viral protein R (Vpr), trans-activator of transcription (Tat), negative regulatory factor (Nef), and envelope glycoprotein gp120, activate signaling pathways that are also triggered by TNF, especially in macrophages (Reviewed in [44]) (Figure 2).

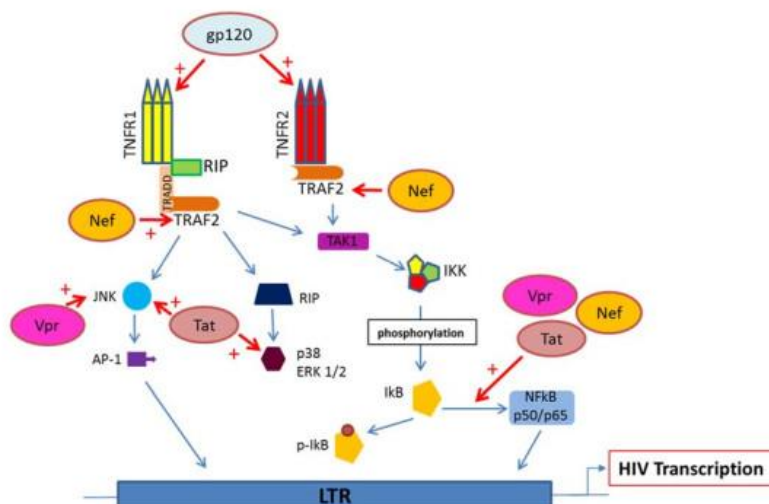


Figure 2. The TNF/TNFR pathway is targeted by human immunodeficiency virus 1 (HIV-1) proteins. HIV-1 proteins viral protein R (Vpr), trans-activator of transcription (Tat), negative regulatory factor (Nef), and envelope glycoprotein gp120 interfere with the TNF/TNFR pathway to enhance HIV transcription in infected cells.

5. HIV Proteins Interaction with TNFR and Downstream Signaling Pathways

Vpr encodes a 96-amino acid, 14 kDa protein, which is conserved among HIV-1, HIV-2, and simian immunodeficiency virus. Multiple cellular functions are targeted by Vpr, including the induction of cell cycle arrest in the G2 phase, transactivation of the LTR promoter, nuclear import of preintegration complexes, and modulation of apoptosis. Both pro- and anti-apoptotic roles of Vpr have been described. Vpr triggers apoptosis in infected T-cells through activation of caspases 3/7, 8, and 9 [45,46] and by blocking NF- κ B activation in myeloid cells [47]. In contrast, Vpr expression in the Jurkat cell line has

been reported to enhance resistance to TNF-induced apoptosis [48]. In addition to these functions, Vpr triggers the mitochondrial dysfunction [49,50]. Vpr is critical for HIV replication in non-dividing cells, e.g., macrophages [44], but is not required for replication in T-cells. Like TNF, recombinant Vpr activates NF- κ B, AP-1, and JNK in primary macrophages and monocytoid U937 cells, resulting in the stimulation of HIV-1 LTR and the subsequent enhancement of viral replication [44,51]. Vpr-dependent activation of NF- κ B has also been reported in primary T-cells [52]. In addition, HIV production is increased by Vpr through toll-like receptor 4 (TLR4) and IL-6 secretion [50]. Recently, Vpr has been reported to enhance TNF production by HIV-1-infected T-cells, implicating the cellular proteins TAK1 and damage-specific DNA binding protein 1 (DSB1) in this phenomenon [53]. TNF produced by infected cells activates NF- κ B in bystander cells and favors viral reactivation from the HIV-1 reservoir [53]. Also, enhanced stimulation of HIV-1 LTR results from a synergistic effect of Vpr and Tat [54]. A few reports indicate that in some cases Vpr can inhibit NF- κ B activation. For example, the inhibition of NF- κ B mediated gene expression results from a synergistic action of Vpr and glucocorticoid receptors via a pathway involving the suppressor of poly (ADP-ribose) polymerase (PARP)-1 nuclear trafficking in response to TNF [47,55]. Thus, the respective roles of Vpr and TNF on cellular functions and HIV-1 replication are complex and depend on the cell type involved.

A close interplay between TNF and HIV-1 Nef is present throughout the progression of the disease [56]. The multifunctional 27kDa myristoylated Nef protein triggers NF- κ B activation in macrophages and monocytoid U937 cells, favoring the HIV LTR activation in vitro [57,58], and the transcription of several genes coding for proinflammatory cytokines and chemokines such as TNF, IL-6, MIP-1 α , and MIP-1 β [59–61]. The phosphorylation of several key-signaling molecules, including α / β subunits of I κ B kinase, ERK1/2, JNK, and p38, is triggered in macrophages treated with recombinant Nef in vitro [60]. Moreover, Nef and TNF synergistically stimulate the HIV-1 replication in monocytic cells and primary macrophages [62], and Nef directly interacts with several TRAF proteins (TRAF2, TRAF5, TRAF6) to enhance the HIV-1 replication in macrophages [63]. In addition to a plasma membrane (PM)-associated TNF shedding, the secretion of vesicular TNF endosomes has been recently described as an alternative TNF secretion mechanism, where Nef-mediated routing of the TNF alpha-converting enzyme (TACE) ADAM17 into Rab4+ early endosomes and the Rab27+ secretory pathway leads to intracellular pro-TNF cleavage and secretion of vesicular TNF endosomes [64]. For the vesicular TNF endosomal trafficking of disintegrin and metalloproteinase domain-containing protein 17 (ADAM17), neurogenic locus notch homolog protein 1 (Notch1) is required [64]. In fact, in exosomes released from cells infected with viruses other than HIV, the presence of viral components has been reported including, among others, components of Epstein-Barr virus, cytomegalovirus, herpes simplex virus, and hepatitis C virus [65]. Although the role of vesicular TNF has been less studied so far than its soluble TNF counterpart, T-cell activation, viral replication, and viral reactivation from HIV reservoir are enhanced by exosomes of HIV-1-infected cells through a Nef- and TNF-dependent mechanism [66,67]. In addition, exosomes from HIV-1-infected cells containing trans-activation response (TAR) RNA stimulate the production of TNF in primary macrophages [68]. Finally, exposure to Nef-containing exosomes released from transfected T-cell lines increases CD4+ T-cell apoptosis [57].

The HIV-1 Tat protein is critical for the elongation process during viral transcription, but also activates NF- κ B, JNK, p38, and ERK1/2 pathways and thereby could mimic the effect of TNF on cellular gene expression [44,69] (Figure 2). Both MyD88 and TIR-domain-containing adapter-inducing interferon- β (TRIF) axes recruit the TLR4 pathway, following stimulation by Tat, and lead to the activation of protein kinase C beta2 isoform, MAP kinase, and NF- κ B, thus resulting in enhanced TNF production in monocytes [70].

The production of TNF, but also of IL-6, IL-10, and interferon γ , has been reported after the interaction of gp120 with CD4 (Reviewed in [71]). The production of TNF is triggered by gp120 in peripheral blood mononuclear cells isolated from HIV-1 infected patients [72–74], in macrophages treated with gp120 in vitro [75], and in animal models [76]. The PI3K and MAPK pathways are involved in the increase of TNF production, which is triggered by HIV gp120 [75,77]. In HIV-infected patients,

a positive correlation between the levels of gp120 and the expression profiles of proinflammatory cytokines, including TNF, has been reported [78].

The interaction between macrophage-membrane bound TNF and TNFR2 present on CD8+ T-cells triggers CD8+ T-cell apoptosis [79]. Chronic inflammation in HIV-1 infection is characterized by the detection of soluble markers of immune activation, including, among others, neopterin, β 2 microglobulin, soluble CD30, TNF, and the soluble TNFR2 (sTNFR2) [80]. In symptomatic HIV-infected patients, increased TNF levels are detected in the serum, and elevated levels of sTNFR2 are predictive of disease progression [81].

6. Anti-TNF Medications

In several chronic inflammatory diseases, such as rheumatoid arthritis, ankylosing spondylitis, juvenile arthritis, psoriatic arthritis, Crohn's disease, and ulcerative colitis, the blockade of TNF signaling is commonly used. Thus, medications such as antibodies or receptors, which target the TNF/TNFR pathway for treating various inflammatory diseases, are now in preclinical or clinical phases of development [82,83]. In 1998, the human/murine chimeric antibody, infliximab, was approved. It binds with high affinity to both the soluble and the membrane-bound TNF. It was then followed by etanercept, a TNFR2-fragment crystallizable region (Fc) fusion protein, that acts like a decoy to block TNF [84]. In 2002 and 2009, the fully human antibodies adalimumab and golimumab, which bind to TNF, were approved. Moreover, certolizumab pegol was approved in 2008. This anti-TNF monoclonal antibody contains a PEGylated Fab fragment, which targets TNF, with the advantage of being administered to pregnant women. Thus, pregnant women with autoimmune diseases and in need of anti-TNF therapy can be treated with certolizumab pegol, since polyethylene glycol does not cross the placenta [85]. Additionally, the cells involved in inflammation could be lysed by adalimumab and infliximab, since both medications trigger apoptosis and cell cycle arrest in transmembrane TNF-expressing Jurkat T-cells. This could result in immune suppression, thus improving the course of inflammatory diseases, such as Crohn's disease, but with a risk of reactivation of latent tuberculosis in infected patients [82]. This negative effect can be prevented by appropriate screening, and HIV-infected patients should be adequately vaccinated when possible and closely monitored for early signs of infection. When serious infections occur, withdrawal of anti-TNF therapy may be necessary until the infection has been identified and properly treated.

7. Anti-TNF Therapy and Control of Immune Activation in HIV-1 Infection

At all stages of HIV-1 infection, increased amounts of TNF can be detected either in the plasma or in the tissues [38,86,87]. Even in combination antiretroviral therapy (cART) treated patients with undetectable viremia, immune activation is still present along with increased levels of plasma TNF [88–91]. In HIV-infected patients, the increased viral replication and depletion of CD4+ T-cells could result from enhanced TNF expression [92,93]. In addition, TNF impairs CD4+ T-cell-mediated immunological control in chronic viral infection [94]. Therefore, in addition to the inhibition of viral replication by cART, new therapeutic approaches, which control TNF production, could be helpful to curtail immune activation in HIV-infected patients. Thus, increased levels of TNF in HIV infection could be counteracted by anti-TNF therapy (Figure 3A).

Moreover, the release of soluble TNF receptors (sTNFR1 and sTNFR2), following the membrane-bound TNFR shedding by proteolytic cleavage, can compete with the membrane-bound TNFRs for binding to TNF, thus inhibiting its activity. In addition, the cells could be transiently desensitized to TNF action, due to the decrease in the number of membrane receptor molecules as a result of receptor shedding [95–97]. Nevertheless, to modulate HIV disease, TNF blocking agents and/or TNF inhibitor therapy could be useful, since HIV infection is characterized by immune activation and inflammation [98]. Several years ago the use of thalidomide, a TNF inhibitor, was suggested to reduce the serum TNF level and to control the viral load [99,100]. The transcription and biosynthesis of TNF is blocked by LMP-420—(2-amino-6-chloro-9-[5(dihydroxyboryl)-pentyl] purine—,

a purine nucleoside analog, which inhibits the replication of HIV-1 [101]. A considerable argument in favor of the use of anti-TNF therapy in HIV-1 infected patients is the safety of its use, since no increase in the mortality rate has been observed [102]. Furthermore, reports showed that HIV-1 symptoms are improved in treated patients, even if anti-TNF therapy could favor the immunosuppressive status of the patients and thereby increase the risk of opportunistic infections [103]. Anti-TNF therapy (etanercept, infliximab, adalimumab) has been shown to be well-tolerated in HIV-infected patients, with no enhancement of the rate of opportunistic infections, unless they had uncontrolled HIV replication [104–108]. Anti-TNF therapy may be helpful for the treatment of autoimmune diseases, without enhancing the plasma viremia in patients whose HIV disease is under control by cART [103,109,110].

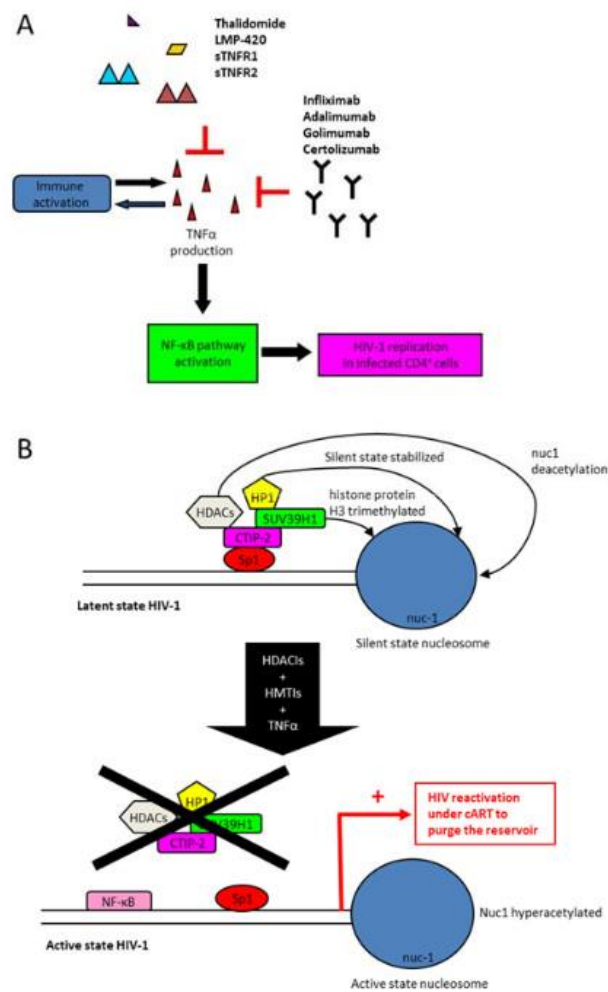


Figure 3. Cont.

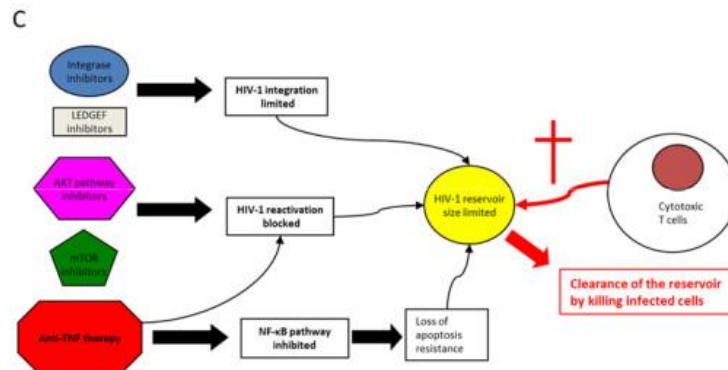


Figure 3. Potential TNF-based therapies to control HIV-1 infection. (A) Anti-TNF therapy against immune activation. Anti-TNF therapy limits immune activation triggered by HIV-1 infection. (B) TNF stimulation to enhance viral reactivation: the ‘shock and kill’ strategy. During HIV-1 latency, nuc-1 nucleosome is hypoacetylated. Coup-TFI interacting protein 2 (CTIP-2) interacts with Sp1, switching nuc-1 from transcriptionally active to a repressive state. Furthermore, CTIP-2 recruits histone deacetylase inhibitors (HDACs) that deacetylate the nuc-1 nucleosome. Suppressor of variegation 3-9 homolog 1 (SUV39H1) adds a trimethylation mark onto the histone protein H3. Furthermore, the heterochromatin protein 1 (HP1) protein stabilizes the nuc-1 in a transcriptionally silent state. The combination of TNF with HDAC inhibitors (HDACIs) or histone methyltransferase (HMT) inhibitors (HMTIs) can disrupt the HIV-1 latency. TNF and HDACIs can trigger the activation of transcriptional activators such as NF-κB (p50/p65 heterodimer). HDACIs prevent the formation of heterochromatin, resulting in nuc-1 hyperacetylation and remodeling, thereby alleviating the HIV transcriptional block. The use of TNF, HDACIs, and HMTIs enhances HIV-1 LTR transcription and could participate in the purge of the HIV-1 reservoir under combination antiretroviral therapy (cART). (C) Anti-TNF therapy to limit the size of the HIV-1 reservoir; the ‘block and lock’ strategy. Integrase inhibitors, lens epithelium-derived growth factor (LEDGF) inhibitors, mammalian target of rapamycin (mTOR) inhibitors and protein kinase B (AKT) inhibitors limit both HIV-1 integration and reactivation, thereby blocking and locking a limited amount of provirus, which then could be cleared by anti-HIV cytotoxic T-cells (CTLs). nuc-1: single nucleosome.

8. Modulation of TNF Activity to Clear HIV-1 Reservoirs

For a complete viral eradication in HIV-1 infected patients, the persistence of HIV-1 reservoirs is a major challenge [111–113]. Even in non-viremic patients that are under cART, the formation of an HIV-1 reservoir could result from the persistent immune activation observed especially in the presence of TNF [114]. TNF is a well-known activator of NF-κB, which is required to favor HIV-1 integration and the establishment of latency in CCL19-treated resting CD4+ T-cells [115]. Among HIV-1 reservoirs, resting or memory CD4+ T-cells are the main target [113]. Macrophages are another important HIV-1 reservoir, due to their resistance to apoptosis with sustained viral growth at low levels [116,117]. To eradicate the latent HIV-1 pool, an activation induced purging of HIV-1 in patients receiving cART has long been a proposed mechanism as a ‘shock and kill’ approach (Figure 3B) [118]. TNF has been used to reactivate HIV-1 from latently infected cells, although with limited levels of viral reactivation [119]. Although TNF consistently activates latent HIV-1 provirus in J-Lat cells, it is much less effective in primary CD4+ T-cells isolated from aviremic HIV-1 infected patients [120,121]. Moreover, toxicity associated with TNF treatment has been observed in some cases [122]. Therefore, to purge cellular reservoirs, several new drugs that target the epigenetic regulation of HIV-1 gene expression have been tested (Figure 3B). HIV-1 is synergistically

reactivated from latency by a combination therapy, using both TNF and histone deacetylase inhibitors (HDACis) [123,124]. HIV-1 transcription is activated by the HDACis such as trichostatin A (TSA), trapoxin (TPX), valproic acid (VPA), and sodium butyrate (NaBut), which favor the remodeling of the single nucleosome (nuc-1) present in the HIV-1 promoter [125,126]. In view of the 'shock and kill' paradigm, forced HIV-1 gene expression elicited by HDACis and TNF in the presence of an effective cART regimen may lead to clearance of the pool of latently infected cells [119,125,127]. In addition to the direct effect of HDACis on HIV-1 transcription, the enhancement of TNF mediated NF- κ B activation by HDACis could result in a sustained delay in I κ B alpha cytoplasmic reappearance [128]. To reactivate HIV-1 from latency in resting CD4+ T-cells of HIV-infected cART-treated patients, inhibitors of histone methyltransferase (HMTIs) have also been recently shown to be efficient [129]. Synergistic activation of HIV-1 expression by HDACi and prostratin, a NF- κ B inducer that stimulates TNF production, favors viral reactivation [119,130]. Disulfiram also reactivates HIV-1 from latency in parallel to increasing the production of TNF in CD4+ T-cells [131,132]. The reactivation potential of prostratin is potentiated when used in combination with P-TEFb-releasing agents such as JQ1 [133]. Combining TNF treatment with other HIV-1 reactivating drugs such as HDACis, HMTIs, and prostratin enhances HIV-1 replication and ultimately could lead to the clearance of HIV reservoirs in infected patients under cART [134].

The 'shock and kill' paradigm has been challenged due to significant concerns about the feasibility and possible problems of this approach [135,136]. A novel paradigm, 'block and lock', has emerged recently and is based on the blockade of HIV reactivation from the latency reservoir to allow the clearance of actively infected cells by the immune system [137]. The 'block and lock' paradigm relies on the blockade of HIV reactivation, which could be based on several approaches (Figure 3C). First, treatments that block the signaling pathways involved in HIV-1 reactivation could be useful, including, among others, inhibitors of the mammalian target of rapamycin (mTOR) complex [138] and of the AKT pathway [139,140], potentially as well as anti-TNF treatments. Anti-TNF therapy could be useful for limiting HIV-1 reactivation from latency through the inhibition of NF- κ B, MAPK, and p38 pathways. Second, treatments that limit new HIV-1 integration could also, in time, diminish the size of the reservoir. In addition to inhibitors of integrase and of lens epithelium-derived growth factor (LEDGF)/p75, which limit viral integration [141,142], anti-TNF therapy could curtail the size of the HIV-1 reservoir by controlling the cellular activation. Potential reactivation of the remaining reservoir could be subsequently cleared by HIV-specific cytotoxic T-cells (CTLs) (Figure 3C). During the initial differentiation of effector cells into memory CD4+ T-cells, NF- κ B activation enhances T-cell survival [143]. TNF/TNFR signaling may contribute, through its activation of NF- κ B, to the establishment and maintenance of latent HIV-1 reservoirs in memory CD4+ T-cells [115]. Additionally, TNF stimulation of Nef-expressing HIV-infected T-cells inhibits CD4+ T-cell apoptosis and thereby could increase the size of the T-cell reservoir [144]. Thus, TNF-based therapy could be used to clear HIV-1 reservoirs in HIV-infected patients under cART and treated with latency reversing agents (LRA), which will reactivate a replication-competent provirus, resulting in the expression of viral proteins, such as Nef, at increased levels. In cART-treated patients, anti-TNF therapy could be an interesting approach to favor the clearance of latently infected cells by promoting their apoptosis after stimulation by LRA, in parallel to the action of HIV-specific CTLs.

9. Conclusions

Both immune activation and viral reservoirs are hallmarks of HIV-1 infection and involve the TNF/TNFR pathway. Thus, modulating TNF/TNFR pathway by new therapeutic approaches could limit immune activation and curtail the size of the HIV-1 reservoir in cART-treated patients with undetectable viremia, with the ultimate goal being to cure HIV-infected patients.

Acknowledgments: This work was supported by grants from the University of Franche-Comté, the Région Franche-Comté (RECH-FON12-000013), the Agence Nationale de Recherche sur le SIDA (ANRS, n° 13543 and 13544), and HIVERA 2013 (EURECA project) to G.H. A.K. is a recipient of a postdoctoral fellowship of the Region Franche-Comté.

Author Contributions: S.P., A.K., and G.H. wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Carswell, E.A.; Old, L.J.; Kassel, R.L.; Green, S.; Fiore, N.; Williamson, B. An endotoxin-induced serum factor that causes necrosis of tumors. *Proc. Natl. Acad. Sci. USA* **1975**, *72*, 3666–3670. [[CrossRef](#)] [[PubMed](#)]
2. Beutler, B.; Cerami, A. Cachectin and tumour necrosis factor as two sides of the same biological coin. *Nature* **1986**, *320*, 584–588. [[CrossRef](#)] [[PubMed](#)]
3. Bradley, J.R. TNF-mediated inflammatory disease. *J. Pathol.* **2008**, *214*, 149–160. [[CrossRef](#)] [[PubMed](#)]
4. Aggarwal, B.B.; Gupta, S.C.; Kim, J.H. Historical perspectives on tumor necrosis factor and its superfamily: 25 years later, a golden journey. *Blood* **2012**, *119*, 651–665. [[CrossRef](#)] [[PubMed](#)]
5. Moelants, E.A.V.; Mortier, A.; Van Damme, J.; Proost, P. Regulation of TNF- α with a focus on rheumatoid arthritis. *Immunol. Cell Biol.* **2013**, *91*, 393–401. [[CrossRef](#)] [[PubMed](#)]
6. Pennica, D.; Nedwin, G.E.; Hayflick, J.S.; Seeburg, P.H.; Derynck, R.; Palladino, M.A.; Kohr, W.J.; Aggarwal, B.B.; Goeddel, D.V. Human tumour necrosis factor: Precursor structure, expression and homology to lymphotoxin. *Nature* **1984**, *312*, 724–729. [[CrossRef](#)] [[PubMed](#)]
7. Kelker, H.C.; Oppenheim, J.D.; Stone-Wolf, D.; Henriksen-DeStefano, D.; Aggarwal, B.B.; Stevenson, H.C.; Vitek, J. Characterization of human tumor necrosis factor produced by peripheral blood monocytes and its separation from lymphotoxin. *Int. J. Cancer* **1985**, *36*, 69–73. [[CrossRef](#)] [[PubMed](#)]
8. Herbein, G.; O'Brien, W.A. Tumor necrosis factor (TNF)- α and TNF receptors in viral pathogenesis. *Proc. Soc. Exp. Biol. Med.* **2000**, *223*, 241–257. [[CrossRef](#)] [[PubMed](#)]
9. Beutler, B.; van Huffel, C. Unraveling function in the TNF ligand and receptor families. *Science* **1994**, *264*, 667–668. [[CrossRef](#)] [[PubMed](#)]
10. Black, R.A.; Rauch, C.T.; Kozlosky, C.J.; Peschon, J.J.; Slack, J.L.; Wolfson, M.F.; Castner, B.J.; Stocking, K.L.; Reddy, P.; Srinivasan, S.; et al. A metalloproteinase disintegrin that releases tumour-necrosis factor- α from cells. *Nature* **1997**, *385*, 729–733. [[CrossRef](#)] [[PubMed](#)]
11. Grell, M.; Wajant, H.; Zimmermann, G.; Scheurich, P. The type 1 receptor (CD120a) is the high-affinity receptor for soluble tumor necrosis factor. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 570–575. [[CrossRef](#)] [[PubMed](#)]
12. Grell, M.; Douni, E.; Wajant, H.; Löhden, M.; Clauss, M.; Maxeiner, B.; Georgopoulos, S.; Lesslauer, W.; Kollias, G.; Pfizenmaier, K.; et al. The transmembrane form of tumor necrosis factor is the prime activating ligand of the 80 kDa tumor necrosis factor receptor. *Cell* **1995**, *83*, 793–802. [[CrossRef](#)]
13. Hsu, H.; Xiong, J.; Goeddel, D.V. The TNF receptor 1-associated protein TRADD signals cell death and NF- κ B activation. *Cell* **1995**, *81*, 495–504. [[CrossRef](#)]
14. Hsu, H.; Shu, H.B.; Pan, M.G.; Goeddel, D.V. TRADD-TRAF2 and TRADD-FADD interactions define two distinct TNF receptor 1 signal transduction pathways. *Cell* **1996**, *84*, 299–308. [[CrossRef](#)]
15. DiDonato, J.A.; Hayakawa, M.; Rothwarf, D.M.; Zandi, E.; Karin, M. A cytokine-responsive I κ B kinase that activates the transcription factor NF- κ B. *Nature* **1997**, *388*, 548–554. [[PubMed](#)]
16. Mercurio, F.; Zhu, H.; Murray, B.W.; Shevchenko, A.; Bennett, B.L.; Li, J.; Young, D.B.; Barbosa, M.; Mann, M.; Manning, A.; et al. IKK-1 and IKK-2: Cytokine-activated I κ B kinases essential for NF- κ B activation. *Science* **1997**, *278*, 860–866. [[CrossRef](#)] [[PubMed](#)]
17. Devin, A.; Cook, A.; Lin, Y.; Rodriguez, Y.; Kelliher, M.; Liu, Z. The distinct roles of TRAF2 and RIP in IKK activation by TNF-R1: TRAF2 recruits IKK to TNF-R1 while RIP mediates IKK activation. *Immunity* **2000**, *12*, 419–429. [[CrossRef](#)]
18. Natoli, G.; Costanzo, A.; Moretti, F.; Fulco, M.; Balsano, C.; Levrero, M. Tumor necrosis factor (TNF) receptor 1 signaling downstream of TNF receptor-associated factor 2. NUCLEAR FACTOR KB (NFKB)-INDUCING KINASE REQUIREMENT FOR ACTIVATION OF ACTIVATING PROTEIN 1 AND NFKB BUT NOT OF C-JUN N-TERMINAL KINASE/STRESS-ACTIVATED PROTEIN KINASE. *J. Biol. Chem.* **1997**, *272*, 26079–26082. [[PubMed](#)]
19. Grell, M.; Becke, F.M.; Wajant, H.; Männel, D.N.; Scheurich, P. TNF receptor type 2 mediates thymocyte proliferation independently of TNF receptor type 1. *Eur. J. Immunol.* **1998**, *28*, 257–263. [[CrossRef](#)]

20. Gehr, G.; Gentz, R.; Brockhaus, M.; Loetscher, H.; Lesslauer, W. Both tumor necrosis factor receptor types mediate proliferative signals in human mononuclear cell activation. *J. Immunol.* **1992**, *149*, 911–917. [[PubMed](#)]
21. Zheng, L.; Fisher, G.; Miller, R.E.; Peschon, J.; Lynch, D.H.; Lenardo, M.J. Induction of apoptosis in mature T cells by tumour necrosis factor. *Nature* **1995**, *377*, 348–351. [[CrossRef](#)] [[PubMed](#)]
22. Herbein, G.; Montaner, L.J.; Gordon, S. Tumor necrosis factor alpha inhibits entry of human immunodeficiency virus type 1 into primary human macrophages: A selective role for the 75-kilodalton receptor. *J. Virol.* **1996**, *70*, 7388–7397. [[PubMed](#)]
23. Karsten, V.; Gordon, S.; Kim, A.; Herbein, G. HIV-1 envelope glycoprotein gp120 down-regulates CD4 expression in primary human macrophages through induction of endogenous tumour necrosis factor- α . *Immunology* **1996**, *88*, 55–60. [[CrossRef](#)] [[PubMed](#)]
24. Herbein, G.; Doyle, A.G.; Montaner, L.J.; Gordon, S. Lipopolysaccharide (LPS) down-regulates CD4 expression in primary human macrophages through induction of endogenous tumour necrosis factor (TNF) and IL-1 β . *Clin. Exp. Immunol.* **1995**, *102*, 430–437. [[CrossRef](#)] [[PubMed](#)]
25. Di Marzio, P.; Tse, J.; Landau, N.R. Chemokine receptor regulation and HIV type 1 tropism in monocyte-macrophages. *AIDS Res. Hum. Retrovir.* **1998**, *14*, 129–138. [[CrossRef](#)] [[PubMed](#)]
26. Herbein, G.; Gordon, S. 55- and 75-kilodalton tumor necrosis factor receptors mediate distinct actions in regard to human immunodeficiency virus type 1 replication in primary human macrophages. *J. Virol.* **1997**, *71*, 4150–4156. [[PubMed](#)]
27. Coffey, M.J.; Woffendin, C.; Phare, S.M.; Strieter, R.M.; Markovitz, D.M. RANTES inhibits HIV-1 replication in human peripheral blood monocytes and alveolar macrophages. *Am. J. Physiol.* **1997**, *272*, L1025–L1029. [[PubMed](#)]
28. Simmons, G.; Clapham, P.R.; Picard, L.; Offord, R.E.; Rosenkilde, M.M.; Schwartz, T.W.; Buser, R.; Wells, T.N.; Proudfoot, A.E. Potent inhibition of HIV-1 infectivity in macrophages and lymphocytes by a novel CCR5 antagonist. *Science* **1997**, *276*, 276–279. [[CrossRef](#)] [[PubMed](#)]
29. Lane, B.R.; Markovitz, D.M.; Woodford, N.L.; Rochford, R.; Strieter, R.M.; Coffey, M.J. TNF- α inhibits HIV-1 replication in peripheral blood monocytes and alveolar macrophages by inducing the production of RANTES and decreasing C-C chemokine receptor 5 (CCR5) expression. *J. Immunol.* **1999**, *163*, 3653–3661. [[PubMed](#)]
30. McManus, C.M.; Brosnan, C.F.; Berman, J.W. Cytokine induction of MIP-1 α and MIP-1 β in human fetal microglia. *J. Immunol.* **1998**, *160*, 1449–1455. [[PubMed](#)]
31. Kitai, R.; Zhao, M.L.; Zhang, N.; Hua, L.L.; Lee, S.C. Role of MIP-1beta and RANTES in HIV-1 infection of microglia: Inhibition of infection and induction by IFN β . *J. Neuroimmunol.* **2000**, *110*, 230–239. [[CrossRef](#)]
32. Gaur, U.; Aggarwal, B.B. Regulation of proliferation, survival and apoptosis by members of the TNF superfamily. *Biochem. Pharmacol.* **2003**, *66*, 1403–1408. [[CrossRef](#)]
33. Ono, K.; Han, J. The p38 signal transduction pathway: Activation and function. *Cell. Signal.* **2000**, *12*, 1–13. [[CrossRef](#)]
34. Lee, J.C.; Laydon, J.T.; McDonnell, P.C.; Gallagher, T.F.; Kumar, S.; Green, D.; McNulty, D.; Blumenthal, M.J.; Heys, J.R.; Landvatter, S.W. A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. *Nature* **1994**, *372*, 739–746. [[CrossRef](#)] [[PubMed](#)]
35. Baud, V.; Karin, M. Signal transduction by tumor necrosis factor and its relatives. *Trends Cell Biol.* **2001**, *11*, 372–377. [[CrossRef](#)]
36. Grivennikov, S.I.; Kuprash, D.V.; Liu, Z.-G.; Nedospasov, S.A. Intracellular signals and events activated by cytokines of the tumor necrosis factor superfamily: From simple paradigms to complex mechanisms. *Int. Rev. Cytol.* **2006**, *252*, 129–161. [[PubMed](#)]
37. Nakano, H. Signaling crosstalk between NF- κ B and JNK. *Trends Immunol.* **2004**, *25*, 402–405. [[CrossRef](#)] [[PubMed](#)]
38. Duh, E.J.; Maury, W.J.; Folks, T.M.; Fauci, A.S.; Rabson, A.B. Tumor necrosis factor alpha activates human immunodeficiency virus type 1 through induction of nuclear factor binding to the NF- κ B sites in the long terminal repeat. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 5974–5978. [[CrossRef](#)] [[PubMed](#)]
39. Griffin, G.E.; Leung, K.; Folks, T.M.; Kunkel, S.; Nabel, G.J. Activation of HIV gene expression during monocyte differentiation by induction of NF- κ B. *Nature* **1989**, *339*, 70–73. [[CrossRef](#)] [[PubMed](#)]
40. Okamoto, T.; Matsuyama, T.; Mori, S.; Hamamoto, Y.; Kobayashi, N.; Yamamoto, N.; Josephs, S.F.; Wong-Staal, F.; Shimotohno, K. Augmentation of human immunodeficiency virus type 1 gene expression by tumor necrosis factor α . *AIDS Res. Hum. Retrovir.* **1989**, *5*, 131–138. [[CrossRef](#)] [[PubMed](#)]

41. Osborn, L.; Kunkel, S.; Nabel, G.J. Tumor necrosis factor alpha and interleukin 1 stimulate the human immunodeficiency virus enhancer by activation of the nuclear factor kappa B. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 2336–2340. [[CrossRef](#)] [[PubMed](#)]
42. Takahashi, Y.; Tanaka, Y.; Yamashita, A.; Koyanagi, Y.; Nakamura, M.; Yamamoto, N. OX40 stimulation by gp34/OX40 ligand enhances productive human immunodeficiency virus type 1 infection. *J. Virol.* **2001**, *75*, 6748–6757. [[CrossRef](#)] [[PubMed](#)]
43. Clerici, M.; Galli, M.; Bosis, S.; Gervasoni, C.; Moroni, M.; Norbiato, G. Immunoendocrinologic abnormalities in human immunodeficiency virus infection. *Ann. N. Y. Acad. Sci.* **2000**, *917*, 956–961. [[CrossRef](#)] [[PubMed](#)]
44. Herbein, G.; Khan, K.A. Is HIV infection a TNF receptor signalling-driven disease? *Trends Immunol.* **2008**, *29*, 61–67. [[CrossRef](#)] [[PubMed](#)]
45. Arokium, H.; Kamata, M.; Chen, I. Virion-associated Vpr of human immunodeficiency virus type 1 triggers activation of apoptotic events and enhances fas-induced apoptosis in human T cells. *J. Virol.* **2009**, *83*, 11283–11297. [[CrossRef](#)] [[PubMed](#)]
46. Andersen, J.L.; DeHart, J.L.; Zimmerman, E.S.; Ardon, O.; Kim, B.; Jacquot, G.; Benichou, S.; Planelles, V. HIV-1 Vpr-induced apoptosis is cell cycle dependent and requires Bax but not ANT. *PLoS Pathog.* **2006**, *2*, e127. [[CrossRef](#)] [[PubMed](#)]
47. Kogan, M.; Deshmane, S.; Sawaya, B.E.; Gracely, E.J.; Khalili, K.; Rappaport, J. Inhibition of NF- κ B activity by HIV-1 Vpr is dependent on Vpr binding protein. *J. Cell. Physiol.* **2013**, *228*, 781–790. [[CrossRef](#)] [[PubMed](#)]
48. Conti, L.; Rainaldi, G.; Matarrese, P.; Varano, B.; Rivabene, R.; Columba, S.; Sato, A.; Belardelli, F.; Malomi, W.; Gessani, S. The HIV-1 VPR protein acts as a negative regulator of apoptosis in a human lymphoblastoid T cell line: Possible implications for the pathogenesis of AIDS. *J. Exp. Med.* **1998**, *187*, 403–413. [[CrossRef](#)] [[PubMed](#)]
49. Malim, M.H.; Emerman, M. HIV-1 accessory proteins—Ensuring viral survival in a hostile environment. *Cell Host Microbe* **2008**, *3*, 388–398. [[CrossRef](#)] [[PubMed](#)]
50. Hoshino, S.; Konishi, M.; Mori, M.; Shimura, M.; Nishitani, C.; Kuroki, Y.; Koyanagi, Y.; Kano, S.; Itabe, H.; Ishizaka, Y. HIV-1 Vpr induces TLR4/MyD88-mediated IL-6 production and reactivates viral production from latency. *J. Leukoc. Biol.* **2010**, *87*, 1133–1143. [[CrossRef](#)] [[PubMed](#)]
51. Varin, A.; Decrion, A.-Z.; Sabbah, E.; Quivy, V.; Sire, J.; Van Lint, C.; Roques, B.P.; Aggarwal, B.B.; Herbein, G. Synthetic Vpr protein activates activator protein-1, c-Jun N-terminal kinase, and NF- κ B and stimulates HIV-1 transcription in promonocytic cells and primary macrophages. *J. Biol. Chem.* **2005**, *280*, 42557–42567. [[CrossRef](#)] [[PubMed](#)]
52. Roux, P.; Alfieri, C.; Hrimech, M.; Cohen, E.A.; Tanner, J.E. Activation of transcription factors NF- κ B and NF-IL-6 by human immunodeficiency virus type 1 protein R (VPR) induces interleukin-8 expression. *J. Virol.* **2000**, *74*, 4658–4665. [[CrossRef](#)] [[PubMed](#)]
53. Roesch, F.; Richard, L.; Rua, R.; Porrot, F.; Casartelli, N.; Schwartz, O. VPR Enhances Tumor Necrosis Factor Production by HIV-1-Infected T Cells. *J. Virol.* **2015**, *89*, 12118–12130. [[CrossRef](#)] [[PubMed](#)]
54. Sawaya, B.E.; Khalili, K.; Gordon, J.; Taube, R.; Amini, S. Cooperative interaction between HIV-1 regulatory proteins Tat and VPR modulates transcription of the viral genome. *J. Biol. Chem.* **2000**, *275*, 35209–35214. [[CrossRef](#)] [[PubMed](#)]
55. Muthumani, K.; Choo, A.Y.; Zong, W.-X.; Madesh, M.; Hwang, D.S.; Premkumar, A.; Thieu, K.P.; Emmanuel, J.; Kumar, S.; Thompson, C.B.; et al. The HIV-1 Vpr and glucocorticoid receptor complex is a gain-of-function interaction that prevents the nuclear localization of PARP-1. *Nat. Cell Biol.* **2006**, *8*, 170–179. [[CrossRef](#)] [[PubMed](#)]
56. Herbein, G. TNF and HIV-1 Nef: An Intimate Interplay. *EBioMedicine* **2016**, *13*, 25–26. [[CrossRef](#)] [[PubMed](#)]
57. Lenassi, M.; Cagney, G.; Liao, M.; Vaupotic, T.; Bartholomeeusen, K.; Cheng, Y.; Krogan, N.J.; Plemenitas, A.; Peterlin, B.M. HIV NEF is secreted in exosomes and triggers apoptosis in bystander CD4+ T cells. *Traffic* **2010**, *11*, 110–122. [[CrossRef](#)] [[PubMed](#)]
58. Varin, A.; Manna, S.K.; Quivy, V.; Decrion, A.-Z.; Van Lint, C.; Herbein, G.; Aggarwal, B.B. Exogenous Nef protein activates NF- κ B, AP-1, and c-Jun N-terminal kinase and stimulates HIV transcription in promonocytic cells. Role in AIDS pathogenesis. *J. Biol. Chem.* **2003**, *278*, 2219–2227. [[CrossRef](#)] [[PubMed](#)]

59. Olivetta, E.; Percario, Z.; Fiorucci, G.; Mattia, G.; Schiavoni, I.; Dennis, C.; Jäger, J.; Harris, M.; Romeo, G.; Affabris, E.; et al. HIV-1 Nef induces the release of inflammatory factors from human monocyte/macrophages: Involvement of Nef endocytotic signals and NF- κ B activation. *J. Immunol.* **2003**, *170*, 1716–1727. [[CrossRef](#)] [[PubMed](#)]
60. Mangino, G.; Percario, Z.A.; Fiorucci, G.; Vaccari, G.; Manrique, S.; Romeo, G.; Federico, M.; Geyer, M.; Affabris, E. In vitro treatment of human monocytes/macrophages with myristoylated recombinant Nef of human immunodeficiency virus type 1 leads to the activation of mitogen-activated protein kinases, I κ B kinases, and interferon regulatory factor 3 and to the release of beta interferon. *J. Virol.* **2007**, *81*, 2777–2791. [[PubMed](#)]
61. Olivetta, E.; Tirelli, V.; Chiozzini, C.; Scazzocchio, B.; Romano, I.; Arenaccio, C.; Sanchez, M. HIV-1 Nef impairs key functional activities in human macrophages through CD36 downregulation. *PLoS ONE* **2014**, *9*, e93699. [[CrossRef](#)] [[PubMed](#)]
62. Herbein, G.; Varin, A.; Larbi, A.; Fortin, C.; Mählknecht, U.; Fulop, T.; Aggarwal, B. Nef and TNF α are Coplayers that Favor HIV-1 Replication in Monocytic Cells and Primary Macrophages. *Curr. HIV Res.* **2008**, *6*, 117–129. [[CrossRef](#)] [[PubMed](#)]
63. Khan, K.A.; Abbas, W.; Varin, A.; Kumar, A.; Di Martino, V.; Dichamp, I.; Herbein, G. HIV-1 Nef interacts with HCV Core, recruits TRAF2, TRAF5 and TRAF6, and stimulates HIV-1 replication in macrophages. *J. Innate Immun.* **2013**, *5*, 639–656. [[CrossRef](#)] [[PubMed](#)]
64. Ostalecki, C.; Wittki, S.; Lee, J.-H.; Geist, M.M.; Tibroni, N.; Harrer, T.; Schuler, G.; Fackler, O.T.; Baur, A.S. HIV Nef- and Notch1-dependent Endocytosis of ADAM17 Induces Vesicular TNF Secretion in Chronic HIV Infection. *EBioMedicine* **2016**, *13*, 294–304. [[CrossRef](#)] [[PubMed](#)]
65. Schorey, J.S.; Harding, C.V. Extracellular vesicles and infectious diseases: New complexity to an old story. *J. Clin. Investig.* **2016**, *126*, 1181–1189. [[CrossRef](#)] [[PubMed](#)]
66. Arenaccio, C.; Chiozzini, C.; Columba-Cabezas, S.; Manfredi, F.; Federico, M. Cell activation and HIV-1 replication in unstimulated CD4+ T lymphocytes ingesting exosomes from cells expressing defective HIV-1. *Retrovirology* **2014**, *11*, 46. [[CrossRef](#)] [[PubMed](#)]
67. Arenaccio, C.; Chiozzini, C.; Columba-Cabezas, S.; Manfredi, F.; Affabris, E.; Baur, A.; Federico, M. Exosomes from human immunodeficiency virus type 1 (HIV-1)-infected cells license quiescent CD4+ T lymphocytes to replicate HIV-1 through a Nef- and ADAM17-dependent mechanism. *J. Virol.* **2014**, *88*, 11529–11539. [[CrossRef](#)] [[PubMed](#)]
68. Sampey, G.C.; Saifuddin, M.; Schwab, A.; Barclay, R.; Punya, S.; Chung, M.-C.; Hakami, R.M.; Zadeh, M.A.; Lepene, B.; Klase, Z.A.; et al. Exosomes from HIV-1-infected cells stimulate production of pro-inflammatory cytokines through trans-activating response (TAR) RNA. *J. Biol. Chem.* **2016**, *291*, 1251–1266. [[CrossRef](#)] [[PubMed](#)]
69. Kumar, A.; Manna, S.K.; Dhawan, S.; Aggarwal, B.B. HIV-Tat protein activates c-Jun N-terminal kinase and activator protein-1. *J. Immunol.* **1998**, *161*, 776–781. [[PubMed](#)]
70. Planès, R.; Ben Haij, N.; Leghmari, K.; Serrero, M.; BenMohamed, L.; Bahraoui, E. HIV-1 Tat protein activates both the MyD88 and TRIF pathways to induce tumor necrosis factor α and interleukin-10 in human monocytes. *J. Virol.* **2016**, *90*, 5886–5898. [[CrossRef](#)] [[PubMed](#)]
71. Abbas, W.; Herbein, G. T-Cell Signaling in HIV-1 Infection. *Open Virol. J.* **2013**, *7*, 57–71. [[CrossRef](#)] [[PubMed](#)]
72. Rieckmann, P.; Poli, G.; Fox, C.H.; Kehrl, J.H.; Fauci, A.S. Recombinant gp120 specifically enhances tumor necrosis factor- α production and Ig secretion in B lymphocytes from HIV-infected individuals but not from seronegative donors. *J. Immunol.* **1991**, *147*, 2922–2927. [[PubMed](#)]
73. Merrill, J.E.; Koyanagi, Y.; Chen, I.S. Interleukin-1 and tumor necrosis factor α can be induced from mononuclear phagocytes by human immunodeficiency virus type 1 binding to the CD4 receptor. *J. Virol.* **1989**, *63*, 4404–4408. [[PubMed](#)]
74. Khanna, K.V.; Yu, X.F.; Ford, D.H.; Ratner, L.; Hildreth, J.K.; Markham, R.B. Differences among HIV-1 variants in their ability to elicit secretion of TNF- α . *J. Immunol.* **2000**, *164*, 1408–1415. [[CrossRef](#)] [[PubMed](#)]
75. Lee, C.; Tomkowicz, B.; Freedman, B.D.; Collman, R.G. HIV-1 gp120-induced TNF- α production by primary human macrophages is mediated by phosphatidylinositol-3 (PI-3) kinase and mitogen-activated protein (MAP) kinase pathways. *J. Leukoc. Biol.* **2005**, *78*, 1016–1023. [[CrossRef](#)] [[PubMed](#)]

76. Zheng, W.; Ouyang, H.; Zheng, X.; Liu, S.; Mata, M.; Fink, D.J.; Hao, S. Glial TNF α in the spinal cord regulates neuropathic pain induced by HIV gp120 application in rats. *Mol. Pain* **2011**, *7*, 40. [[CrossRef](#)] [[PubMed](#)]
77. Furler, R.L.; Uittenbogaart, C.H. Signaling through the P38 and ERK pathways: A common link between HIV replication and the immune response. *Immunol. Res.* **2010**, *48*, 99–109. [[CrossRef](#)] [[PubMed](#)]
78. Rychert, J.; Strick, D.; Bazner, S.; Robinson, J.; Rosenberg, E. Detection of HIV gp120 in plasma during early HIV infection is associated with increased proinflammatory and immunoregulatory cytokines. *AIDS Res. Hum. Retrovir.* **2010**, *26*, 1139–1145. [[CrossRef](#)] [[PubMed](#)]
79. Herbein, G.; Mahlknecht, U.; Batliwalla, F.; Gregersen, P.; Pappas, T.; Butler, J.; O'Brien, W.A.; Verdin, E. Apoptosis of CD8+ T cells is mediated by macrophages through interaction of HIV gp120 with chemokine receptor CXCR4. *Nature* **1998**, *395*, 189–194. [[PubMed](#)]
80. Alfano, M.; Alfano, M.; Banki, Z.; Borde, C.; Bristow, C.L.; Caruz, A.; Chang, T.L.; Cortes, J.; Eugen-Olsen, J.; Fibla, J.; et al. *Soluble Factors Mediating Innate Immune Responses to HIV Infection*; Bentham Science Publishers: Chennai, India, 2010.
81. Zangerle, R.; Gallati, H.; Sarcelletti, M.; Wachter, H.; Fuchs, D. Tumor necrosis factor α and soluble tumor necrosis factor receptors in individuals with human immunodeficiency virus infection. *Immunol. Lett.* **1994**, *41*, 229–234. [[CrossRef](#)]
82. Croft, M.; Benedict, C.A.; Ware, C.F. Clinical targeting of the TNF and TNFR superfamilies. *Nat. Rev. Drug Discov.* **2013**, *12*, 147–168. [[CrossRef](#)] [[PubMed](#)]
83. Wendling, D.; Herbein, G. TNF α antagonist therapy in patients with joint disease and chronic viral infection. *Jt. Bone Spine* **2007**, *74*, 407–409. [[CrossRef](#)] [[PubMed](#)]
84. Knight, D.M.; Trinh, H.; Le, J.; Siegel, S.; Shealy, D.; McDonough, M.; Scallon, B.; Moore, M.A.; Vilcek, J.; Daddona, P. Construction and initial characterization of a mouse-human chimeric anti-TNF antibody. *Mol. Immunol.* **1993**, *30*, 1443–1453. [[CrossRef](#)]
85. Goel, N.; Stephens, S. Certolizumab pegol. *MAbs* **2010**, *2*, 137–147. [[CrossRef](#)] [[PubMed](#)]
86. Aukrust, P.; Liabakk, N.B.; Müller, F.; Lien, E.; Espevik, T.; Frøland, S.S. Serum levels of tumor necrosis factor- α (TNF α) and soluble TNF receptors in human immunodeficiency virus type 1 infection—correlations to clinical, immunologic, and virologic parameters. *J. Infect. Dis.* **1994**, *169*, 420–424. [[CrossRef](#)] [[PubMed](#)]
87. Whalen, C.; Horsburgh, C.R.; Hom, D.; Lahart, C.; Simberkoff, M.; Ellner, J. Accelerated course of human immunodeficiency virus infection after tuberculosis. *Am. J. Respir. Crit. Care Med.* **1995**, *151*, 129–135. [[CrossRef](#)] [[PubMed](#)]
88. Sereti, I.; Krebs, S.J.; Phanuphak, N.; Fletcher, J.L.; Slike, B.; Pinyakorn, S.; O'Connell, R.J.; Rupert, A.; Chomont, N.; Valcour, V.; et al. Persistent, Albeit Reduced, Chronic Inflammation in Persons Starting Antiretroviral Therapy in Acute HIV Infection. *Clin. Infect. Dis.* **2017**, *64*, 124–131. [[CrossRef](#)] [[PubMed](#)]
89. Wilson, E.M.P.; Singh, A.; Hullsiek, K.H.; Gibson, D.; Henry, W.K.; Lichtenstein, K.; Önen, N.F.; Kojic, E.; Patel, P.; Brooks, J.T.; et al. Study to understand the natural history of HIV/AIDS in the era of effective therapy (SUN study) investigators monocyte-activation phenotypes are associated with biomarkers of inflammation and coagulation in chronic HIV infection. *J. Infect. Dis.* **2014**, *210*, 1396–1406. [[CrossRef](#)] [[PubMed](#)]
90. Castillo-Mancilla, J.R.; Brown, T.T.; Erlandson, K.M.; Palella, F.J.; Gardner, E.M.; Macatangay, B.J.C.; Breen, E.C.; Jacobson, L.P.; Anderson, P.L.; Wada, N.I. Suboptimal adherence to combination antiretroviral therapy is associated with higher levels of inflammation despite HIV suppression. *Clin. Infect. Dis.* **2016**, *63*, 1661–1667. [[CrossRef](#)] [[PubMed](#)]
91. Wada, N.I.; Jacobson, L.P.; Margolick, J.B.; Breen, E.C.; Macatangay, B.; Penugonda, S.; Martínez-Maza, O.; Bream, J.H. The effect of HAART-induced HIV suppression on circulating markers of inflammation and immune activation. *AIDS* **2015**, *29*, 463–471. [[CrossRef](#)] [[PubMed](#)]
92. Dezube, B.J.; Lederman, M.M.; Chapman, B.; Georges, D.L.; Dogon, A.L.; Mudido, P.; Reis-Lishing, J.; Cheng, S.L.; Silberman, S.L.; Crumpacker, C.S. The effect of tenidap on cytokines, acute-phase proteins, and virus load in human immunodeficiency virus (HIV)-infected patients: Correlation between plasma HIV-1 RNA and proinflammatory cytokine levels. *J. Infect. Dis.* **1997**, *176*, 807–810. [[CrossRef](#)] [[PubMed](#)]
93. Valdez, H.; Lederman, M.M. Cytokines and cytokine therapies in HIV infection. *AIDS Clin. Rev.* **1997**, *187*–228.

94. Beyer, M.; Abdullah, Z.; Chemnitz, J.M.; Maisel, D.; Sander, J.; Lehmann, C.; Thabet, Y.; Shinde, P.V.; Schmidleithner, L.; Köhne, M.; et al. Tumor-necrosis factor impairs CD4(+) T cell-mediated immunological control in chronic viral infection. *Nat. Immunol.* **2016**, *17*, 593–603. [[CrossRef](#)] [[PubMed](#)]
95. Aderka, D. The potential biological and clinical significance of the soluble tumor necrosis factor receptors. *Cytokine Growth Factor Rev.* **1996**, *7*, 231–240. [[CrossRef](#)]
96. Brockhaus, M. Soluble TNF receptor: What is the significance? *Intensive Care Med.* **1997**, *23*, 808–809. [[CrossRef](#)] [[PubMed](#)]
97. Xanthoulea, S.; Pasparakis, M.; Kousteni, S.; Brakebusch, C.; Wallach, D.; Bauer, J.; Lassmann, H.; Kollias, G. Tumor necrosis factor (TNF) receptor shedding controls thresholds of innate immune activation that balance opposing TNF functions in infectious and inflammatory diseases. *J. Exp. Med.* **2004**, *200*, 367–376. [[CrossRef](#)] [[PubMed](#)]
98. Decrion, A.Z.; Dichamp, I.; Varin, A.; Herbein, G. HIV and inflammation. *Curr. HIV Res.* **2005**, *3*, 243–259. [[CrossRef](#)] [[PubMed](#)]
99. Marriott, J.B.; Cookson, S.; Carlin, E.; Youle, M.; Hawkins, D.A.; Nelson, M.; Pearson, M.; Vaughan, A.N.; Gazzard, B.; Dalglish, A.G. A double-blind placebo-controlled phase II trial of thalidomide in asymptomatic HIV-positive patients: Clinical tolerance and effect on activation markers and cytokines. *AIDS Res. Hum. Retrovir.* **1997**, *13*, 1625–1631. [[CrossRef](#)] [[PubMed](#)]
100. Wallis, R.S.; Nsubuga, P.; Whalen, C.; Mugerwa, R.D.; Okwera, A.; Oette, D.; Jackson, J.B.; Johnson, J.L.; Ellner, J.J. Pentoxifylline therapy in human immunodeficiency virus-seropositive persons with tuberculosis: A randomized, controlled trial. *J. Infect. Dis.* **1996**, *174*, 727–733. [[CrossRef](#)] [[PubMed](#)]
101. Haraguchi, S.; Day, N.K.; Kamchaisatian, W.; Beigier-Pompadre, M.; Stenger, S.; Tangsinmankong, N.; Sleasman, J.W.; Pizzo, S.V.; Cianciolo, G.J. LMP-420, a small-molecule inhibitor of TNF- α , reduces replication of HIV-1 and *Mycobacterium tuberculosis* in human cells. *AIDS Res. Ther.* **2006**, *3*, 8. [[CrossRef](#)] [[PubMed](#)]
102. Ting, P.T.; Koo, J.Y. Use of etanercept in human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) patients. *Int. J. Dermatol.* **2006**, *45*, 689–692. [[CrossRef](#)] [[PubMed](#)]
103. Calabrese, L.H.; Zein, N.; Vassilopoulos, D. Safety of antitumor necrosis factor (anti-TNF) therapy in patients with chronic viral infections: Hepatitis C, hepatitis B, and HIV infection. *Ann. Rheum. Dis.* **2004**, *63* (Suppl. 2), ii18–ii24. [[CrossRef](#)] [[PubMed](#)]
104. Sha, B.E.; Valdez, H.; Gelman, R.S.; Landay, A.L.; Agosti, J.; Mitsuyasu, R.; Pollard, R.B.; Mildvan, D.; Namkung, A.; Ogata-Arakaki, D.M.; et al. Effect of etanercept (Enbrel) on interleukin 6, tumor necrosis factor α , and markers of immune activation in HIV-infected subjects receiving interleukin 2. *AIDS Res. Hum. Retrovir.* **2002**, *18*, 661–665. [[CrossRef](#)] [[PubMed](#)]
105. Wallis, R.S.; Kyambadde, P.; Johnson, J.L.; Horter, L.; Kittle, R.; Pohle, M.; Ducar, C.; Millard, M.; Mayanja-Kizza, H.; Whalen, C.; et al. A study of the safety, immunology, virology, and microbiology of adjunctive etanercept in HIV-1-associated tuberculosis. *AIDS* **2004**, *18*, 257–264. [[CrossRef](#)] [[PubMed](#)]
106. Walker, R.E.; Spooner, K.M.; Kelly, G.; McCloskey, R.V.; Woody, J.N.; Falloon, J.; Baseler, M.; Piscitelli, S.C.; Davey, R.T.; Polis, M.A.; et al. Inhibition of immunoreactive tumor necrosis factor- α by a chimeric antibody in patients infected with human immunodeficiency virus type 1. *J. Infect. Dis.* **1996**, *174*, 63–68. [[CrossRef](#)] [[PubMed](#)]
107. Gaylis, N. Infliximab in the treatment of an HIV positive patient with Reiter's syndrome. *J. Rheumatol.* **2003**, *30*, 407–411. [[PubMed](#)]
108. Gallitano, S.M.; McDermott, L.; Brar, K.; Lowenstein, E. Use of tumor necrosis factor (TNF) inhibitors in patients with HIV/AIDS. *J. Am. Acad. Dermatol.* **2016**, *74*, 974–980. [[CrossRef](#)] [[PubMed](#)]
109. Cepeda, E.J.; Williams, F.M.; Ishimori, M.L.; Weisman, M.H.; Reveille, J.D. The use of anti-tumor necrosis factor therapy in HIV-positive individuals with rheumatic disease. *Ann. Rheum. Dis.* **2008**, *67*, 710–712. [[CrossRef](#)] [[PubMed](#)]
110. Wangsiricharoen, S.; Ligon, C.; Gedmintas, L.; Dehrab, A.; Tungsiripat, M.; Bingham, C.; Lozada, C.; Calabrese, L. The rates of serious infections in HIV-infected patients who received tumor necrosis factor (TNF)- α inhibitor therapy for concomitant autoimmune diseases. *Arthritis Care Res.* **2016**, *69*, 449–452. [[CrossRef](#)] [[PubMed](#)]
111. Chun, T.W.; Fauci, A.S. Latent reservoirs of HIV: Obstacles to the eradication of virus. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 10958–10961. [[CrossRef](#)] [[PubMed](#)]

112. Chomont, N.; El-Far, M.; Ancuta, P.; Trautmann, L.; Procopio, F.A.; Yassine-Diab, B.; Boucher, G.; Boulassel, M.-R.; Ghattas, G.; Brechley, J.M.; et al. HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. *Nat. Med.* **2009**, *15*, 893–900. [[CrossRef](#)] [[PubMed](#)]
113. Abbas, W.; Herbein, G. Molecular understanding of HIV-1 latency. *Adv. Virol.* **2012**, *2012*, 574967. [[CrossRef](#)] [[PubMed](#)]
114. Siliciano, J.D.; Siliciano, R.F. Recent developments in the effort to cure HIV infection: Going beyond N = 1. *J. Clin. Investig.* **2016**, *126*, 409–414. [[CrossRef](#)] [[PubMed](#)]
115. Saleh, S.; Lu, H.K.; Evans, V.; Harisson, D.; Zhou, J.; Jaworowski, A.; Sallmann, G.; Cheong, K.Y.; Mota, T.M.; Tennakoon, S.; et al. HIV integration and the establishment of latency in CCL19-treated resting CD4(+) T cells require activation of NF- κ B. *Retrovirology* **2016**, *13*, 49. [[CrossRef](#)] [[PubMed](#)]
116. Abbas, W.; Khan, K.A.; Kumar, A.; Tripathy, M.K.; Dichamp, I.; Keita, M.; Mahlknecht, U.; Rohr, O.; Herbein, G. Blockade of BFA-mediated apoptosis in macrophages by the HIV-1 Nef protein. *Cell Death Dis.* **2014**, *5*, e1080. [[CrossRef](#)] [[PubMed](#)]
117. Abbas, W.; Tariq, M.; Iqbal, M.; Kumar, A.; Herbein, G. Eradication of HIV-1 from the macrophage reservoir: An uncertain goal? *Viruses* **2015**, *7*, 1578–1598. [[CrossRef](#)] [[PubMed](#)]
118. Chun, T.W.; Engel, D.; Mizell, S.B.; Ehler, L.A.; Fauci, A.S. Induction of HIV-1 replication in latently infected CD4+ T cells using a combination of cytokines. *J. Exp. Med.* **1998**, *188*, 83–91. [[CrossRef](#)] [[PubMed](#)]
119. Reuse, S.; Calao, M.; Kabeya, K.; Guiguen, A.; Gatot, J.-S.; Quivy, V.; Vanhulle, C.; Lamine, A.; Vaira, D.; Demonte, D.; et al. Synergistic activation of HIV-1 expression by deacetylase inhibitors and prostratin: Implications for treatment of latent infection. *PLoS ONE* **2009**, *4*, e6093. [[CrossRef](#)] [[PubMed](#)]
120. Chan, J.K.L.; Greene, W.C. NF- κ B/Rel: Agonist and antagonist roles in HIV-1 latency. *Curr. Opin. HIV AIDS* **2011**, *6*, 12–18. [[CrossRef](#)] [[PubMed](#)]
121. Burnett, J.C.; Lim, K.-I.; Calafi, A.; Rossi, J.J.; Schaffer, D.V.; Arkin, A.P. Combinatorial latency reactivation for HIV-1 subtypes and variants. *J. Virol.* **2010**, *84*, 5958–5974. [[CrossRef](#)] [[PubMed](#)]
122. Cummins, N.W.; Badley, A.D. Mechanisms of HIV-associated lymphocyte apoptosis: 2010. *Cell Death Dis.* **2010**, *1*, e99. [[CrossRef](#)] [[PubMed](#)]
123. Quivy, V.; Adam, E.; Collette, Y.; Demonte, D.; Chariot, A.; Vanhulle, C.; Berkhout, B.; Castellano, R.; de Launoit, Y.; Burny, A.; et al. Synergistic activation of human immunodeficiency virus type 1 promoter activity by NF- κ B and inhibitors of deacetylases: Potential perspectives for the development of therapeutic strategies. *J. Virol.* **2002**, *76*, 11091–11103. [[CrossRef](#)] [[PubMed](#)]
124. Vandergeeten, C.; Quivy, V.; Moutschen, M.; Van Lint, C.; Piette, J.; Legrand-Poels, S. HIV-1 protease inhibitors do not interfere with provirus transcription and host cell apoptosis induced by combined treatment TNF- α TSA. *Biochem. Pharmacol.* **2007**, *73*, 1738–1748. [[CrossRef](#)] [[PubMed](#)]
125. Demonté, D.; Quivy, V.; Colette, Y.; Van Lint, C. Administration of HDAC inhibitors to reactivate HIV-1 expression in latent cellular reservoirs: Implications for the development of therapeutic strategies. *Biochem. Pharmacol.* **2004**, *68*, 1231–1238. [[CrossRef](#)] [[PubMed](#)]
126. El Kharroubi, A.; Piras, G.; Zensen, R.; Martin, M.A. Transcriptional activation of the integrated chromatin-associated human immunodeficiency virus type 1 promoter. *Mol. Cell. Biol.* **1998**, *18*, 2535–2544. [[CrossRef](#)] [[PubMed](#)]
127. Berger, S.L. Histone modifications in transcriptional regulation. *Curr. Opin. Genet. Dev.* **2002**, *12*, 142–148. [[CrossRef](#)]
128. Adam, E.; Quivy, V.; Bex, F.; Chariot, A.; Collette, Y.; Vanhulle, C.; Schoonbroodt, S.; Goffin, V.; Nguyễn, T.L.-A.; Gloire, G.; et al. Potentiation of tumor necrosis factor-induced NF- κ B activation by deacetylase inhibitors is associated with a delayed cytoplasmic reappearance of I κ B α . *Mol. Cell. Biol.* **2003**, *23*, 6200–6209. [[CrossRef](#)] [[PubMed](#)]
129. Bouchat, S.; Gatot, J.-S.; Kabeya, K.; Cardona, C.; Colin, L.; Herbein, G.; De Wit, S.; Clumeck, N.; Lambotte, O.; Rouzioux, C.; et al. Histone methyltransferase inhibitors induce HIV-1 recovery in resting CD4(+) T cells from HIV-1-infected HAART-treated patients. *AIDS* **2012**, *26*, 1473–1482. [[CrossRef](#)] [[PubMed](#)]
130. Kulkosky, J.; Culnan, D.M.; Roman, J.; Dornadula, G.; Schnell, M.; Boyd, M.R.; Pomerantz, R.J. Prostratin: Activation of latent HIV-1 expression suggests a potential inductive adjuvant therapy for HAART. *Blood* **2001**, *98*, 3006–3015. [[CrossRef](#)] [[PubMed](#)]

131. Xing, S.; Bullen, C.K.; Shroff, N.S.; Shan, L.; Yang, H.-C.; Manucci, J.L.; Bhat, S.; Zhang, H.; Margolick, J.B.; Quinn, T.C.; et al. Disulfiram reactivates latent HIV-1 in a Bcl-2-transduced primary CD4+ T cell model without inducing global T cell activation. *J. Virol.* **2011**, *85*, 6060–6064. [[CrossRef](#)] [[PubMed](#)]
132. Doyon, G.; Zerbato, J.; Mellors, J.W.; Sluis-Cremer, N. Disulfiram reactivates latent HIV-1 expression through depletion of the phosphatase and tensin homolog. *AIDS* **2013**, *27*, F7–F11. [[CrossRef](#)] [[PubMed](#)]
133. Darcis, G.; Kula, A.; Bouchat, S.; Fujinaga, K.; Corazza, F.; Ait-Ammar, A.; Delacourt, N.; Melard, A.; Kabeya, K.; Vanhulle, C.; et al. An in-depth comparison of latency-reversing agent combinations in various in vitro and ex vivo HIV-1 latency models identified Bryostatin-1+JQ1 and Ingenol-B+JQ1 to potently reactivate viral gene expression. *PLoS Pathog.* **2015**, *11*, e1005063. [[CrossRef](#)] [[PubMed](#)]
134. International AIDS Society Scientific Working Group on HIV Cure; Deeks, S.G.; Autran, B.; Berkhout, B.; Benkirane, M.; Cairns, S.; Chomont, N.; Chun, T.-W.; Churchill, M.; Di Mascio, M.; et al. Towards an HIV cure: A global scientific strategy. *Nat. Rev. Immunol.* **2012**, *12*, 607–614. [[CrossRef](#)] [[PubMed](#)]
135. Bullen, C.K.; Laird, G.M.; Durand, C.M.; Siliciano, J.D.; Siliciano, R.F. New ex vivo approaches distinguish effective and ineffective single agents for reversing HIV-1 latency in vivo. *Nat. Med.* **2014**, *20*, 425–429. [[CrossRef](#)] [[PubMed](#)]
136. Chun, T.-W.; Moir, S.; Fauci, A.S. HIV reservoirs as obstacles and opportunities for an HIV cure. *Nat. Immunol.* **2015**, *16*, 584–589. [[CrossRef](#)] [[PubMed](#)]
137. Darcis, G.; Van Driessche, B.; Van Lint, C. HIV Latency: Should We Shock or Lock? *Trends Immunol.* **2017**, *38*, 217–228. [[CrossRef](#)] [[PubMed](#)]
138. Besnard, E.; Hakre, S.; Kampmann, M.; Lim, H.W.; Hosmane, N.N.; Martin, A.; Bassik, M.C.; Verschueren, E.; Battivelli, E.; Chan, J.; et al. The mTOR Complex Controls HIV Latency. *Cell Host Microbe* **2016**, *20*, 785–797. [[CrossRef](#)] [[PubMed](#)]
139. Kumar, A.; Abbas, W.; Colin, L.; Khan, K.A.; Bouchat, S.; Varin, A.; Larbi, A.; Gatot, J.-S.; Kabeya, K.; Vanhulle, C.; et al. Tuning of AKT-pathway by Nef and its blockade by protease inhibitors results in limited recovery in latently HIV infected T-cell line. *Sci. Rep.* **2016**, *6*, 24090. [[CrossRef](#)] [[PubMed](#)]
140. Kumar, A.; Abbas, W.; Bouchat, S.; Gatot, J.-S.; Pasquereau, S.; Kabeya, K.; Clumeck, N.; De Wit, S.; Van Lint, C.; Herbein, G. Limited HIV-1 reactivation in resting CD4(+) T cells from aviremic patients under protease inhibitors. *Sci. Rep.* **2016**, *6*, 38313. [[CrossRef](#)] [[PubMed](#)]
141. Vranckx, L.S.; Demeulemeester, J.; Saleh, S.; Boll, A.; Vansant, G.; Schrijvers, R.; Weydert, C.; Battivelli, E.; Verdin, E.; Cereseto, A.; et al. LEDGIN-mediated inhibition of integrase-LEDGF/p75 interaction reduces reactivation of residual latent HIV. *EBioMedicine* **2016**, *8*, 248–264. [[CrossRef](#)] [[PubMed](#)]
142. Messiaen, P.; Wensing, A.M.J.; Fun, A.; Nijhuis, M.; Brusselaers, N.; Vandekerckhove, L. Clinical use of HIV integrase inhibitors: A systematic review and meta-analysis. *PLoS ONE* **2013**, *8*, e52562. [[CrossRef](#)] [[PubMed](#)]
143. Withers, D.R.; Jaansson, E.; Gaspal, F.; McConnell, F.M.; Eksteen, B.; Anderson, G.; Agace, W.W.; Lane, P.J.L. The survival of memory CD4+ T cells within the gut lamina propria requires OX40 and CD30 signals. *J. Immunol.* **2009**, *183*, 5079–5084. [[CrossRef](#)] [[PubMed](#)]
144. Mahlknecht, U.; Deng, C.; Lu, M.C.; Greenough, T.C.; Sullivan, J.L.; O'Brien, W.A.; Herbein, G. Resistance to apoptosis in HIV-infected CD4+ T lymphocytes is mediated by macrophages: Role for Nef and immune activation in viral persistence. *J. Immunol.* **2000**, *165*, 6437–6446. [[CrossRef](#)] [[PubMed](#)]



5. Traitement des infections virales par des thérapies ciblant la régulation épigénétique.

5.1. VIH et épigénétique

L'infection par le VIH est devenue, grâce aux thérapies cART, une maladie chronique. Malgré l'amélioration de la survie et de la qualité de vie des patients, ces traitements ne permettent pas l'éradication des réservoirs de latence virale. Ces réservoirs sont constitués d'un provirus intégré au génome cellulaire et soumis à un silence transcriptionnel par les enzymes de modification de la chromatine. Le ciblage de ces enzymes a entraîné l'émergence de deux stratégies.

La stratégie « shock and kill » est basée sur la réactivation du provirus, pour permettre son éradication par la réponse immunitaire. Pour cela, des agents d'inversion de la latence ont été proposés, incluant des inhibiteurs des histones déacétylases, des histones méthyltransférases et des ADN méthyltransférases.

La stratégie « block and lock » repose sur un verrouillage du silence transcriptionnel en verrouillant le promoteur du VIH dans un état de super latence. Pour cela, des inhibiteurs de Tat, qui est nécessaire pour la transcription virale, sont utilisés pour bloquer celle-ci. L'addition d'inhibiteurs d'Akt, dont l'activation favorise la réactivation et la survie cellulaire, augmentera l'efficacité du blocage, tout en réduisant la survie des cellules infectées.

5.2. Cytomégalovirus humain et épigénétique

Le cytomégalovirus humain (CMV), membre de la famille des *herpesviridae*, est responsable, chez les individus immunocompromis, de complications importantes. Le CMV entraîne également des complications chez les nouveaux-nés infectés de manière congénitale. L'infection par le CMV comprend deux phases : un cycle lytique et un cycle latent. La cascade d'expression génique du CMV est hautement contrôlée, notamment en raison de l'association de l'ADN viral avec les histones cellulaires. Le ciblage des histones par des inhibiteurs d'histone deméthylase pourrait permettre de bloquer le virus en phase latente. A l'inverse, l'utilisation d'inhibiteurs des histones deacétylases, associés aux inhibiteurs de PRC2 et aux traitements antiviraux classiques, pourrait permettre de forcer la réactivation virale pour éradiquer le virus.

5.3. Thérapie épigénétique des infections virales

Bien que le VIH soit le modèle principal pour l'utilisation de thérapies épigénétiques, d'autres infections virales pourraient également bénéficier de ce type d'approche.

Le virus de l'hépatite B persiste malgré les traitements sous la forme d'un minichromosome extrêmement stable. La transcription et la réplication virale sont régulées par les DNA méthyltransférases et par les histones désacétylases. L'inhibition des DNMT entraîne la restauration de l'expression des protéines virales. L'utilisation d'inhibiteurs d'histone acétyltransférase semble réduire la transcription et la réplication.

Le virus de l'hépatite C est responsable d'infections chroniques pouvant évoluer vers des cirrhoses et des hépatocarcinomes. Bien que les traitements antiviraux récents aient considérablement amélioré l'évolution de l'infection, certains patients restent difficiles à traiter. Un lien entre les modifications épigénétiques induites par l'infection et la survenue des hépatocarcinomes a été démontré. L'utilisation d'inhibiteurs des HDAC a montré une suppression de la réplication virale. De plus, une hyperméthylation de l'ADN a été associée à l'infection par le VHC, ce qui suggère que l'utilisation d'inhibiteurs des DNMT pourrait réduire l'infection.

Le virus herpes simplex 1, à l'instar d'autres membres des *herpesviridae*, possèdent une phase de latence, sous la forme d'un épisome associé au nucléosome. Les promoteurs viraux et les gènes transcrits sont associés aux histones, y compris durant le cycle lytique. La transcription et la réplication virale sont réduites par l'augmentation de la méthylation de l'ADN. L'utilisation d'inhibiteurs des HDAC semble permettre de réduire la réplication virale, en maintenant la méthylation de l'ADN.

Le virus d'Epstein-Barr (EBV) est un autre membre des *herpesviridae* qui possède une phase latente sous la forme d'un épisome circulaire. La latence est principalement sous le contrôle des HDAC, qui maintiennent un faible niveau d'acétylation de l'ADN, et une méthylation du promoteur Zp. L'utilisation d'inhibiteurs des HDAC a montré la réactivation virale. L'infection par EBV est également associée à plusieurs cancers, incluant les lymphomes de Burkitt et d'Hodgkin et le carcinome nasopharyngé. Dans le contexte de l'oncogenèse associée à EBV, les modifications épigénétiques liées à la latence virale affectent également des promoteurs et gènes cellulaires. L'utilisation d'inhibiteurs des HDAC et des DNMT ont montré une réversion des effets de la latence virale sur les protéines et promoteurs cellulaires.

5.4. Article paru dans la revue *Clinical Epigenetics* en 2019 « Control of viral infections by epigenetic-targeted therapy »

REVIEW

Open Access

Control of viral infections by epigenetic-targeted therapy

Zeina Nehme^{1,2}, Sébastien Pasquereau¹ and Georges Herbein^{1,3*}**Abstract**

Epigenetics is defined as the science that studies the modifications of gene expression that are not owed to mutations or changes in the genetic sequence. Recently, strong evidences are pinpointing toward a solid interplay between such epigenetic alterations and the outcome of human cytomegalovirus (HCMV) infection. Guided by the previous possibly promising experimental trials of human immunodeficiency virus (HIV) epigenetic reprogramming, the latter is paving the road toward two major approaches to control viral gene expression or latency. Reactivating HCMV from the latent phase ("shock and kill" paradigm) or alternatively repressing the virus lytic and reactivation phases ("block and lock" paradigm) by epigenetic-targeted therapy represent encouraging options to overcome latency and viral shedding or otherwise replication and infectivity, which could lead eventually to control the infection and its complications. Not limited to HIV and HCMV, this concept is similarly studied in the context of hepatitis B and C virus, herpes simplex virus, and Epstein-Barr virus. Therefore, epigenetic manipulations stand as a pioneering research area in modern biology and could constitute a curative methodology by potentially consenting the development of broad-spectrum antivirals to control viral infections *in vivo*.

Keywords: Virus, HIV, HCMV, Epigenetics, Treatment, Cancer

Background

Since its emergence for the first time in 1940, the epigenetic field is witnessing a continuous surge over the last decades [1]. Although the epigenetic term is well thought out to be a large umbrella under which falls concepts related to development, heredity, and evolution [2], recent technical advancements have narrowed the term's definition in the standpoint of molecular biology [3]. Hence, epigenetics could be defined as "the study of heritable changes in gene expression that are not due to changes in DNA sequence" [4]. Recent numerous literature is showing a correlation between epigenetic modifications and a wide array of human diseases including—but not limited to—cancer, neurological and psychiatric disorders (Alzheimer's disease, schizophrenia), autoimmune disorders (rheumatoid arthritis, systemic lupus erythematosus), and others [5–7]. However, this association was converted and extended to the clinical level first in the cancerology field

with the FDA-approved DNA methyltransferase (DNMT) inhibitors (azacitidine, decitabine) and histone deacetylase (HDAC) inhibitors (vorinostat, romidepsin, belinostat, panobinostat) [8]. In fact, in addition to DNMT and HDAC, the epigenetic machinery entails composite complexes that each of which could constitute a valuable target for the development of potential new epigenetic antiviral drugs [9]. This review examines and discusses the involvement and the role of various epigenetic players throughout the different viral life cycle stages and highlights their potential implications in the clinical management of several viral infections, especially human immunodeficiency virus (HIV) and human cytomegalovirus (HCMV), in addition to hepatitis viruses, herpes simplex virus-1 (HSV-1), and Epstein-Barr virus (EBV).

HIV and epigenetics, a leading example as a proof of concept

Since the introduction of combination antiretroviral therapy (cART), survival and quality of life among HIV-infected patients significantly improved [10], with a more favorable outcome with therapy initiation in the setting of early asymptomatic infection [11]. This shifted

* Correspondence: georges.herbein@univ-fcomte.fr

¹Department Pathogens & Inflammation-EPILAB, UPRES EA4266, University of Franche-Comté, University of Bourgogne Franche-Comté, 16 route de Gray, F-25030 Besançon cedex, France

²Department of Virology, CHRU Besançon, F-25030 Besançon, France

Full list of author information is available at the end of the article



© The Author(s). 2019 **Open Access** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated.

HIV conception from a non-curable devastating fatal illness to a possibly manageable chronic one. However, cART is yet not the ideal road map for HIV management, as physical and psychological burden are still imposed by this therapy [12, 13], leading sometimes to a reduced compliance or even discontinuation [14]. Markedly, a major limitation associated with cART cessation is viral rebound [15]. This is due to the presence of HIV reservoirs, mainly in the latently infected resting CD4+ memory T cells and myeloid cells such as macrophages and microglia, that are difficult to be targeted by cART or immune effector mechanisms [16–18]. Interestingly, the integrated provirus in those cells is subjected to transcriptional silencing by host chromatin-modifying enzymes, comprising deacetylases, methyltransferases, and others [19]. This paved the road to the emergence of two new epigenetic therapeutic approaches, namely the “shock and kill” and the “block and lock” strategies [20–23]. Here, we present general points about these two anti-HIV therapeutic strategies (Fig. 1, Table 1).

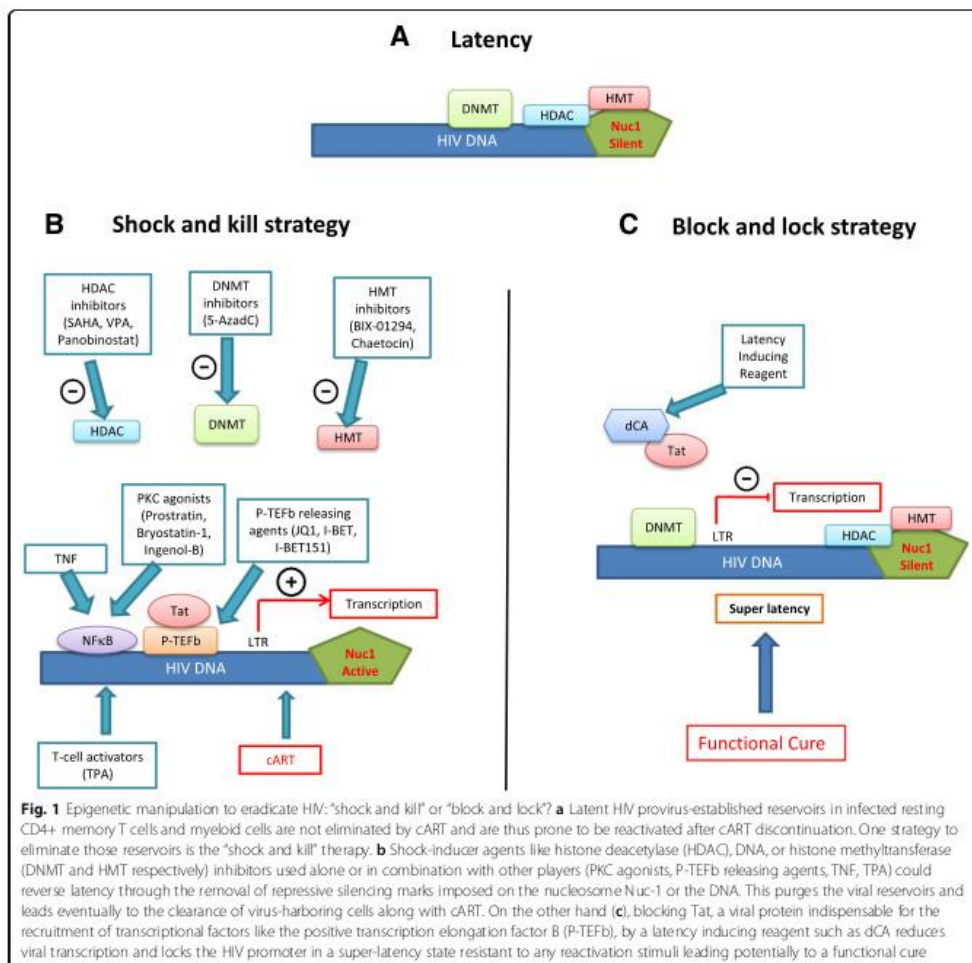
Shock and kill strategy

This strategy is grounded on the concept that the latent HIV provirus could be switch on from latency (shock) into an active form prone to eradication (kill) through the humoral immune response, CD8+ T cells-mediated lysis, virus-induced apoptosis, or activation-induced cell death [24]. Several latency-reversing agents (LRA) or “shock” inducers have been proposed [25, 26] including histone deacetylase (HDAC), histone methyltransferase (HMT), and DNA methyltransferase (DNMT) inhibitors. Histone deacetylases family is composed of 18 enzymes that are gathered into four major groups: HDAC I–IV [27]. HDAC enzymes are responsible of removing acetyl groups from histones, which favors the formation of a compacted, transcriptionally repressed chromatin structure [28]. HDACs have gained an ascending attention after the FDA approval of HDACs inhibitors for cancer treatment [29], such as vorinostat or suberanilohydroxamic acid (SAHA) for the management of cutaneous T cell lymphoma [30] and panobinostat in relapsed multiple myeloma [31]. HDAC inhibitors like SAHA or DNMT inhibitors could be used alone to reactivate HIV gene expression along with efficient cART [32]. For instance, co-treatment with the HDAC inhibitor SAHA and the global T cell activator 12-O-tetradecanoylphorbol-13-acetate (TPA) revealed a significant synergistic effect on purging HIV-1 proviruses in HIV-1 latently infected cells [26]. Other activators of NF- κ B such as prostratin have been also used in combination with HDAC inhibitors to reactivate HIV, these former players reactivating HIV in the absence of immune activation [33]. The concomitant use of protein kinase C (PKC) agonists (prostratin, bryostatins-1, and ingenol-B), which are known to activate NF- κ B signaling pathway as

well as the positive transcription elongation factor B (P-TEFb), used alone or in combination with P-TEFb-releasing agents (HMBA and Bromodomain and Extraterminal (BET) inhibitors JQ1, I-BET and I-BET151) leads to synergistic HIV reactivation from latency [34]. Moreover, sequential treatment with the DNMT inhibitor 5-aza-2'-deoxycytidine (5-Aza-dC) and HDAC inhibitors reactivates HIV-1 from latency [35]. In addition, it has been shown that the use of chaetocin and BIX-01294, specific inhibitors of HMT Suv39H1 and G9a respectively, resulted in HIV-1 recovery in resting CD4+ T cells in highly active antiretroviral therapy (HAART)-treated patients with undetectable viral load [36]. Another therapeutic approach could be considering tumor necrosis factor alpha (TNF)-based therapies, where combining HDAC inhibitors or HMT inhibitors with TNF, disrupts HIV-1 latency by triggering the activation of transcriptional activators like NF- κ B and preventing the formation of heterochromatin, enhancing thus HIV-1 long terminal repeat (LTR) transcription and viral purge [37]. In fact, targeting several cellular proteins involved in the epigenetic control of viral gene expression usually amplifies HIV-1 reactivation. Although this approach is facing several hurdles, including—but not limited to—reactivating and possibly eliminating only a small subset of the latent HIV genome, it constitutes however one tactic that could be used in parallel to other approaches to achieve a fully effective cure [38].

Block and lock strategy

In contrast to LRA, chemical agents could “block” the ongoing viremia during cART, by “locking” the HIV promoter in a super latency state resistant to reactivation stimuli. In fact, the “block and lock” strategy is emerging as a new approach to functionally cure HIV. Didehydrocortistatin A (dCA), a specific and potent Tat inhibitor [39] has been studied in this context. Briefly, binding of the transactivator of transcription Tat to the HIV-1 mRNA results in the recruitment of indispensable transcriptional factors like the P-TEFb to induce sustained transcriptional elongation from the viral promoter LTR [40]. dCA binds specifically to the TAR-binding domain [41], reduces residual levels of viral transcription in several models of HIV latency, establishes a nearly permanent state of latency [42], and delays viral rebound after cART interruption in HIV+ humanized BLT mice [43]. This could be especially beneficial in cases of therapy non-compliance or short period discontinuation as dCA addition to cART regimens can limit the continual replenishment of the CD4+ T cell reservoir [44]. This could potentially stop the increased longevity and persistence of the latent viral reservoir observed in cART-treated patients by inhibiting new rounds of infection in CD4+ T cells [45]. Interestingly, as Tat is a



HIV-specific viral protein with no cellular homolog, using dCA to “block and lock” HIV should not silence other regulatory pathways essential to fight other infections. In addition, Akt activation favors HIV-1 reactivation from resting CD4+ T cells and monocytes/macrophages, the two major HIV-1 cellular reservoirs [46–48]. Thus, Akt inhibitors, but also HIV protease inhibitors which display an anti-Akt activity [47], inhibit Akt activation in HIV-1 infected cells thereby favoring a “lock” stage, decreasing cell viability, and opening thus the door to the clearance of infected cells under Akt blockade. These results strongly encourage and open new insights to the possible addition of the “block and

lock” approach as an additional potential therapeutic management strategy.

Human cytomegalovirus and epigenetics

Human cytomegalovirus (HCMV) is a ubiquitous pathogen also denoted as human herpesvirus 5 (HHV5). It is a member of betaherpesvirinae, a subfamily of the *Herpesviridae* family [49]. HCMV infection is very common, as 40 to 95% of the population is seropositive [50]. However, the pathological outcomes depend on the host's immune status, where infection in immunocompetent individuals rarely causes evident manifestations at the clinical level [51]. Conversely, HCMV infection significantly affects

Table 1 Functional outcomes of epigenetic regulation in viral infections

Target class	Target	Inhibitor	Virus studied	Functional outcome	
HDM	JMJD2	ML324	HCMV	Repression of viral IE gene expression and viral yields [95, 97]	
		DMOG	HCMV	Decrease in the expression of HCMV IE genes UL37, UL72, and US3 [97]	
		DMOG and ML342	HSV-1	Significant decreased in the viral titers in trigeminal ganglia of HSV-1 latently infected mice [97]	
	LSD1	OG-L002	HCMV	Repression of HCMV IE expression [96]	
			HCMV	Decrease in the expression of HCMV IE genes UL37, UL72, and US3 [97]	
		TCP	HSV-1	Repression of HSV IE gene expression and genome replication in vivo Decrease in the severity of a virus-induced encephalitis and corneal blindness in mouse models Blockage of viral reactivation in trigeminal ganglia	
HDAC	Class II HDAC4	MC1568	Adenovirus	Reduction in E1A gene expression [96]	
		HCMV	Induction of transient expression of the viral lytic IE antigens without full virus reactivation [104]		
	Histone deacetylase	Sodium butyrate	HSV-1	Production of infectious progeny in quiescently infected cells [154]	
			EBV, KSHV	Latency reversal [179]	
			HSV-1	Reduction in the number of HSV-1 genomes that initiate replication [164]	
	Histone deacetylase 3	SAHA, VPA, and suberoylanilide hydroxamic acid	HBV	Increase in HBV transcripts Cytoplasmic accumulation of HBV replicative intermediates Increase in secreted HBV viral particles [128]	
				HCV	Suppression of HCV replication without affecting cell viability [135]
				HCV	Reduction of viral replication in Huh7 cells and an in vivo model of humanized transgenic mice [141]
	Histone deacetylase 6	Tubastatin A	HCV	Suppression of HCV replication in HepG2 cells [137]	
				Pan-histone deacetylase	SAHA + TPA
HMT	EZH2	(DZnep)	HCMV		
		GSK126 and GSK343	HSV-1	Blockage of lytic viral replication in latently infected ganglion explant model [169]	
	Suv39H	Chaetocin	HIV	HIV-1 recovery in resting CD4 ⁺ T cells [36]	
G9a	BIX-01294	HIV	HIV-1 recovery in resting CD4 ⁺ T cells [36]		
HAT	p300/CBP	C646	HBV	Reduction in HBV transcription in a dose-dependent manner [111]	
DNMT	DNMT	Azacitidine	HBV	Tumor growth inhibition and decreased aggressiveness in vitro and in vivo [123]	
			HCV	Inhibition of HCV infection [150]	
Viral protein	Tat (transactivator of transcription)	Didehydro-cortistatin A (dCA)	HIV	Reduction of residual levels of viral transcription in several models of HIV latency Establishment of a nearly permanent state of latency [42] Suppression of viral rebound after ART interruption in HIV+ humanized BLT mice [43]	

morbidity and mortality in solid organ or stem cell transplantation recipients and immunocompromised individuals such HIV patients [52, 53], where infection could result in interstitial pneumonia, retinitis, gastrointestinal tract complications like gastroenteritis, hepatitis, and graft failure [54]. Added to the previously mentioned hosts, HCMV infection poses a real burden in congenitally

infected newborns with immature immune system, resulting possibly in deafness and neurodevelopmental delay [55]. HCMV exhibits two modes of viral infection: a lytic and a latent one [56]. The lytic phase is a highly regulated stage that ensures the production and the release of the new viral progeny outside the infected cells. This is followed by latency, a state characterized by a lifelong

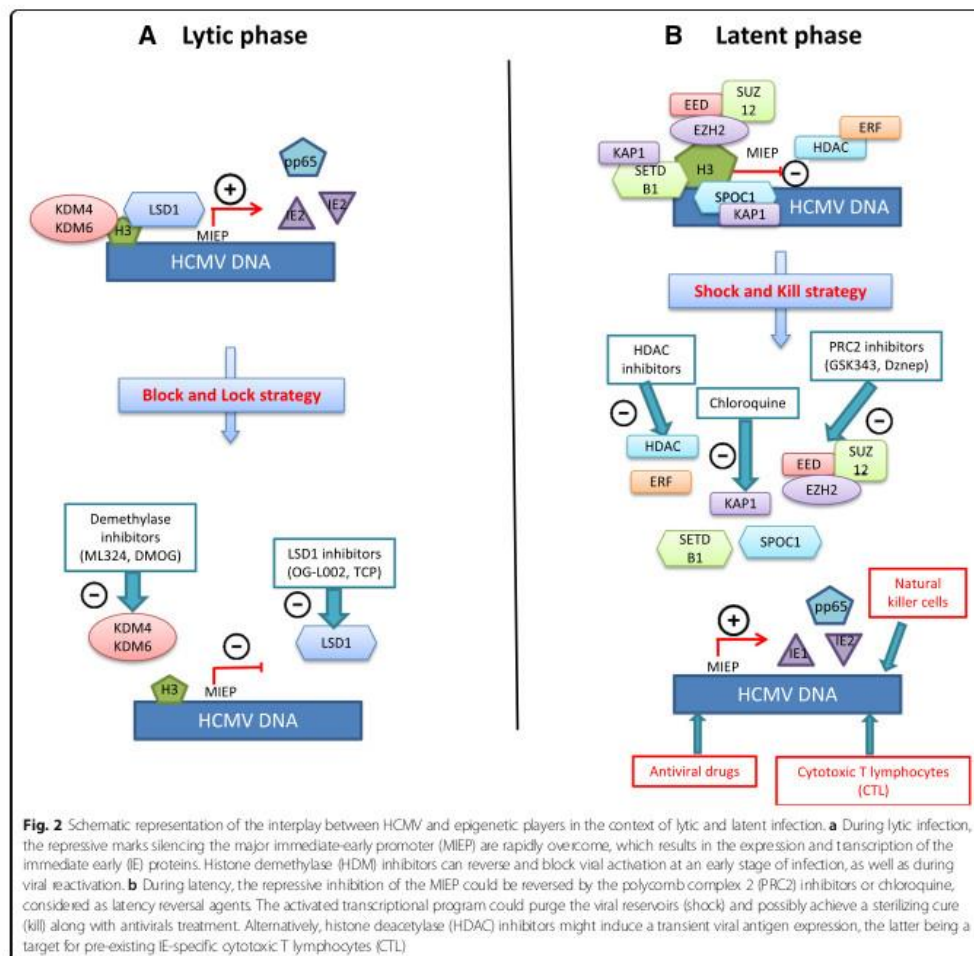
persistence in the host with the ability to reactivate under certain circumstances [53, 54]. During lytic infection, HCMV endures a well-regulated cascade of gene expression that starts with the expression of the immediate early viral genes [57] via the interaction of various cellular factors with the major immediate-early promoter (MIEP) [58]. This is followed by the expression of the early viral genes that play a role in the cellular modulation to favor viral replication [59] and later on by the late viral gene expression that ensures viral progeny assembly and release [60]. Recent studies have shown that epigenetic modifications play a role in the early productive infection events [61]. In fact, after viral entry, viral DNA rapidly becomes associated with histones, which makes it a vulnerable candidate to epigenetic modifications [62]. Such modifications usually result in a silent repressive state to viral gene expression as an intrinsic cellular defense mechanism. However, this repression is ultimately overcome, allowing the sequential expression cascade of lytic viral genes mentioned previously [63].

Although the few available antiviral drugs have granted chief advances in HCMV disease treatment and prophylaxis, their clinical applicability and utility confront several barriers [64]. First, resistance to antivirals is documented after prolonged use [65]. Added to this is the poor oral bioavailability and the dose-limited hematologic and renal toxicities reported with their use [66]. Moreover, they do not target the latent viral form in the host, which leaves the door open for viral shedding and transmission in saliva, urine, milk, vaginal secretions, and other bodily fluids [67]. It is worth to mention that the current antivirals used in HCMV management are ganciclovir, and its oral prodrug valganciclovir, cidofovir, and foscavir that target the viral DNA polymerase, in addition to fomivirsen, an antisense antiviral drug used in the treatment of CMV retinitis [68], and the recently FDA-approved letermovir used to prevent viral infection following allogenic hematopoietic stem cell transplant [69]. Since those antivirals target the viral DNA replication step, the function and expression of the immediate early (IE) and early (E) HCMV genes during the early stages of infection are not blocked, paving the road to immunopathology and raise the risk of graft rejection [70]. Thus, this mandates and sheds the light on the urgent necessity of developing new antiviral drugs with novel mechanisms of action based on new potential viral or cellular targets. This is particularly conceivable with the enhanced understanding of HCMV molecular biology and the epigenetic mechanisms involved with its regulation (Fig. 2, Table 1).

Reactivating HCMV from the latent phase the "shock and kill" paradigm

In contrast to the virus' wide tropism during productive infection [71], several laboratories have pinpointed the

myeloid lineage CD14⁺ monocytes and their CD34⁺ hematopoietic progenitor cells as important sites of HCMV latency in vivo [72]. While much is known about lytic infection, mechanisms of latency establishment and maintenance are still not fully elucidated [73]. This is due in part to difficulties of studying this process in vivo [74]. Indeed, extensive research showed that what was thought to be a quiescent state is in fact a highly active process characterized by the expression of a number of latency-associated viral genes [75]. Interestingly, a strong interplay exists between HCMV latency and epigenetic regulation through alterations of histones and other factors interacting directly or indirectly with the genome [76]. It has been shown that the removal of the repressive modifications associated with the latent phase can set in motion the lytic cycle and favor lytic gene expression [77]. The polycomb repressive complex 2 (PRC2) is a multi-subunit chromatin-modifying complex that plays a central role in regulating cellular differentiation, development, stem cell maintenance, and lineage specification [78, 79]. PRC2 complex is composed of the following core subunits: the embryonic ectoderm development (EED), the zinc finger protein suppressor of zeste 12 (SUZ12), and the enhancer of zeste homolog 2 (EZH2) that is responsible of the catalytic activity [80]. EZH2 protein is a histone methyltransferase (HMT) responsible for the tri-methylation of the lysine 27 residues of histone H3 (H3K27me3), which is generally associated with transcriptional silencing [81]. Several studies have linked HCMV latency to PRC2 activity [82–84]. In one study, chemical inhibitors of PRC2 were studied in THP1 monocytes and NT2D1 embryonal carcinoma cells, as models of HCMV quiescence. The methyltransferase inhibitor 3-deazaneplanocin A (DZnep) resulted in significant activation of the lytic transcriptional program, detected through the temporal regulated increase in viral transcript and antigen levels [85]. KAP1 (KRAB-associated protein 1) is a transcriptional co-repressor protein whose C-terminal effector end interacts with the H3K9me3-specific HMT SETDB1 (SET domain bifurcated 1) [86] and recruits it to the genome, which triggers H3K9 methylation and heterochromatin formation. It has been shown that during HCMV lytic infection, KAP1 is unable to repress transcription due to its suppression by mTOR-mediated phosphorylation. Pharmacological induction of KAP1 phosphorylation on serine 824 by the ATM activator chloroquine released HCMV from its latent state by blocking its ability to bind to SETDB1 and recruit it [87]. This approach could be added to the previously mentioned strategies to purge the HCMV latent reservoirs. Interestingly, survival time-associated PHD finger protein in ovarian cancer 1 (SPOC1), a recently identified restriction factor



against HCMV, associates with the proximal enhancer region of the MIEP and promotes heterochromatin condensation possibly through the recruitment of co-repressors that SPOC1 is known to interact with notably the previously mentioned KAP-1 and H3K9 methyltransferase [88]. On the other hand, Ets-2 repressor factor (ERF) is a cellular protein that physically interacts with the HCMV MIEP and functions as a transcriptional repressor of the latter by suppressing IE gene expression [89]. GST fusion assays showed a strong interaction between ERF and the N-terminus of HDAC1, a result that was further confirmed *in vivo*, suggesting that the physical interaction

between ERF and HDAC1 could mediate repression of the MIEP [90]. Moreover, ying-yang 1 (YY1), a zinc finger DNA-binding protein and a multifunctional transcription factor [91], has been shown to repress the HCMV MIEP [92] partly by indirectly recruiting HDACs to the promoter via the nuclear protein SAP30, a component of the human HDAC complex [93]. In fact, forcing HCMV out of latency along with the conventional antiviral drug use in an attempt to eradicate it and establish a sterilizing cure is in correspondence with the "shock and kill" concept currently studied in HIV. Nevertheless, a better understanding of the molecular mechanisms involved

in HCMV latency and reactivation could open future avenues for HCMV infection control.

Repressing both HCMV lytic and reactivation phases by epigenetic-targeted therapy, "blocking and locking" the virus

HCMV lytic cycle and reactivation from latency are under the control of several epigenetic mechanisms. For example, histone demethylases (HDMs), due to their ability to remove the repressive marks, will promote productive infection. HDMs constitute a large family of more than 20 demethylases that are divided into two functional enzymatic families: the Lys-specific demethylase (LSD), also known as KDM1A and the Jumonji C (JMJC) protein families [94]. Two HDMs were studied in the context of HCMV lytic infection: KDM4 (JMJD2) and KDM6 (UTX/JMJD3), which demethylase histone H3-lysine 9 and lysine 27, respectively [95]. By using the JMJD2 demethylase inhibitor ML324, viral IE gene expression and viral yields were potently repressed, which could suggest that targeting these histone demethylases may potentially block viral gene expression and viral replication at a very early stage of infection and possibly abrogate it. In the same perspective, the LSD1 inhibitor OG-L002 repressed the expression of HCMV IE expression in HCMV-infected MRC5 cells [96]. In addition, the use of another LSD1 inhibitor, tranilcypromine (TCP), or alternatively the JMJD2 inhibitors, dimethylallylglycine (DMOG) or the previously mentioned ML324, resulted in a decrease in the expression of HCMV IE genes UL37, UL72, and US3 with a noted potent inhibition of IE gene expression with ML324 [97]. Thus, HDMs inhibitors could provide a therapeutic tool to target the initiation of infection or the spontaneous reactivation by blocking the viral cycle at an early stage, as a mimic for the proposed HIV "block and lock" strategy. This could be highly beneficial in the context of HCMV infection, as the expression or functions of viral IE and E gene products has shown their potential ability to elicit immuno-inflammatory responses that can lead to tissue rejection [98]. Not limited to inflammatory damage, some IE gene products can significantly interfere with important oncogenic signaling pathways and exhibit oncomodulatory properties, such as in glioblastoma cells [99]. In the context of oncomodulation, the PRC2 complex is associated with HCMV latency by the induction of transcriptional silencing [81–84]. HCMV-infected cells have been showed to exhibit enhanced expression of cellular oncogenic pathways, including c-Myc, c-Fos, c-Jun, Akt, and NF- κ B [100–103]. This transcriptional activation will in turn lead to an increased expression of EZH2, resulting in an auto-amplifying loop. This oncomodulatory effect of HCMV infection could be targeted by PRC2 inhibitors and

HDAC inhibitors that could both block cellular transformation and induce the activation of the viral lytic transcriptional program. This latter effect could allow through the expression of IE antigens the infected cells to be cleared by CMV-specific cytotoxic T cells (CTLs) [104].

Epigenetic therapy, a general approach to cure viral infections?

Besides infection with HIV and HCMV, any viral infection might be potentially treated by new therapeutics targeting the epigenetic mechanisms (Table 1). We present below several examples of viral infections which could benefit from such new therapies.

Hepatitis B virus

Hepatitis B virus (HBV) is a highly transmissible double-stranded DNA virus responsible of acute and chronic hepatitis B (CHB) in humans. Worldwide, about 240 million people are chronically infected, which could ultimately lead to liver fibrosis, cirrhosis, and the development of hepatocellular carcinoma (HCC) [105]. Despite the availability of several FDA-approved drugs like nucleos(t)ide analogs and pegylated interferon, clinical management remains problematic as cure is rarely achieved and the risk of resistance with long-term use and relapse after therapy discontinuation are common [106]. This is partly attributable to HBV persistence, despite treatment, as an episomal non-integrated covalently closed circular (ccc) DNA in the hepatocyte nucleus where it forms a highly stable minichromosome susceptible for epigenetic modifications [107]. Recently, as with the aforementioned viruses, the role of the epigenetic machinery in HBV persistence is gaining much attention [108]. Genome-wide maps of de novo infected HepG2-NTCP1 cells, primary human hepatocytes (PHH), and HBV-infected liver tissue have shown that posttranslational modifications (PTM) that set in motion active transcriptional states are enriched at specific sites within the HBV covalently closed circular DNA (cccDNA) chromatin. Precisely, those PTM encompass high levels of H3K4me3, found at the transcription start site (TSS) of actively transcribed genes, as well as H3K27ac and H3K122ac, indicative of active gene enhancers [109]. This suggests that targeting epigenetic regulation could offer a new insight into new therapeutic approach to treat CHB. In fact, several options targeting epigenetic regulation are being studied with different desired end points: either a complete silencing of cccDNA or its complete elimination through reactivation and subsequent eradication. For instance, SIRT3, a class III HDAC, restricted HBV cccDNA transcription in PHH cells, possibly by increasing the recruitment of the HMT SUV39H1 and decreasing SETD1A recruitment,

resulting in a marked increase of H3K9me3 and a decrease of H3K4me3 on cccDNA [110]. Moreover, treatment with the small molecule C646 that specifically inhibits p300/CBP, the histone acetyltransferases (HAT) for H3K27ac and H3K122ac reduced HBV transcription in a dose-dependent manner in the absence of measurable toxicity [111]. Likewise, PRMT5, a protein arginine methyltransferase 5, restricted HBV transcription and replication partly through regulation of symmetric dimethylation of arginine 3 on H4 on cccDNA exclusively [109]. In the same context, PRMT1, another arginine methyltransferase, is directly recruited to cccDNA, where its overexpression results in a 60% inhibition of HBV transcription in HepG2 cells. It is worthy to mention that this transcription inhibitory effect is limited to PRMT1 as PRMT3 overexpression did not affect transcription. Interestingly, PRMT1 was shown to interact with the regulatory hepatitis B virus X protein (HBx), which in turn inhibits PRMT1 methyltransferase activity [112]. HBx protein is a multifunctional regulatory protein that enhances HBV replication in vitro and in vivo [113] and affects numerous cellular processes including apoptosis [114], DNA repair mechanism [115], mitochondrial function [116], and cell signaling [117, 118]. Importantly, HBx could epigenetically influence cccDNA transcription through its recruitment onto the cccDNA minichromosome where it modulates the recruitment of chromatin-modifying enzymes such as the acetyltransferase p300 and HDACs including Sirt1 and HDAC1 [119]. HBx could also induce epigenetic aberrations that may lead to HBV-related HCC [120]. Those abnormalities include hypermethylation of several tumor suppressor genes, including—but not limited to—IGFBP-3 by DNMT3A1 and DNMT3A2 [121] and the E-cadherin promoter by DNMT1 [122]. Treatment with the DNMT inhibitor AZA restored the expression of the HBx-mediated epigenetically repressed secreted frizzled-related protein 1 (SFRP1), resulting in tumor growth inhibition and decreased aggressiveness in vitro and in vivo through negatively regulating the Wnt/ β -catenin signaling pathway. This effect was further synergized by the use of the HDAC inhibitor trichostatin A (TSA) [123]. HBx-induced upregulation of SIRT2 expression promotes HBV replication in HepAD38 cells and enhances cell migration and invasion in the human hepatoma Huh7 cells, facilitating thus hepatocarcinogenesis [124]. The HBx protein upregulates the insulin-like growth factor 2 (IGF2) oncogene through hypomethylation of its promoter resulting in a poorer clinical outcome for HBV-related HCC patients [125]. Further understanding of the exact mechanisms of HBx-induced epigenetic alterations is highly needed, as those modifications could be used as biomarkers for the detection of early malignant transformation or as potential targets to

treat persistent infection or HBV-related HCC. Nevertheless, for other epigenetic players such as DNMT, a critical balance in the context of HBV-related HCC should be maintained. Although DNMTs induce a decrease in the viral gene expression and replication [126], it could also result in silencing of tumor suppressor genes through DNA methylation, contributing thus to hepatocarcinogenesis [127]. On the other hand, treatment of HBV-transfected HuH7 cells with class I/II HDAC inhibitors, valproic acid (VPA) and TSA resulted in an increase in HBV transcripts, cytoplasmic accumulation of HBV replicative intermediates, and an increase in secreted HBV viral particles [128]. This was on controversy to a study that showed that some HDAC inhibitors like TSA and apicidin, a class I HDAC-specific inhibitor, suppressed cccDNA transcription in a duck hepatitis B virus (DHBV)-transfected chicken hepatoma cell line [129], which could be possibly due to some specificities or differences at the cellular level between the avian and the human cell model or at the viral level between the human and the duck virus.

Hepatitis C virus

Infection with hepatitis C virus (HCV), a small enveloped RNA virus [130], is considered a health concern worldwide [131]. Although acute HCV infection can develop into a chronic condition with life-threatening complications including cirrhosis and HCC [132], recent antiviral treatments have highly improved the disease outcome. Even though previous treatment options were limited to interferon and ribavirin regimens, direct-acting antiviral (DAA) therapies based on HCV protease inhibitors have granted a definitive cure in more of 95% of patients [133]. Nevertheless, HCV-infected patients with genotype 3 represent up to 30% of all HCV infections worldwide and belong to the difficult-to-treat subgroup patients [134]. Therefore, epigenetic-targeted therapy could be a useful therapeutic tool not only for these HCV-infected difficult-to-treat patients, but also in HCV-HBV and HCV-HIV co-infected patients. Interestingly, recent studies are revealing a possible link between the molecular mechanisms of HCC carcinogenesis and host epigenetic alterations induced by HCV infection. The HDAC inhibitor SAHA suppressed HCV replication without affecting cell viability in *Cellosaurus* OR6 cell line. This suppression was linked to changes in gene expression through a SAHA-mediated increase in H3 acetylation levels of the promoter regions of several genes, resulting in an increased expression of osteopontin OPN [135]. Interestingly, OPN is a key cytokine that initiates Th1 immune response through regulating IL-12 and IL-10 cytokine expression [136]. Thus, SAHA-induced increased expression of OPN could possibly eliminate

HCV by activating the Th1-type immune system. Correspondingly, tubastatin A, a selective inhibitor of HDAC6 suppressed HCV replication in HepG2 cells, along with α -tubulin hyperacetylation [137]. Although α -tubulin is known to be deacetylated by the histone deacetylase HDAC6 [138], the latter also controls the acetylation levels of other targets such as peroxiredoxins Prx1/2 [139] and the chaperone Hsp90 [140]. Thus, studying the effects of tubastatin A on those targets would be necessary to unveil the mechanism(s) by which this inhibitor is mediating its antiviral activity. Similarly, the HDAC3 inhibitor RGFP966 reduced viral replication in Huh7 cells and in vivo model of humanized transgenic mice [141] with a downregulation in Apo-A1 expression, an indispensable protein for HCV infectivity maintenance [142], leading possibly to HCV secretion suppression. On the other hand, it has been shown that HCV infection could result in the DNA hypermethylation of some epigenetic markers [143]. For example, the methylation of the suppressor of cytokine signaling 1 (SOCS1), a negative regulator of the JAK/STAT pathway regarded as a tumor suppressor gene [144], was found to be positively associated with HCV infection status [145]. In the same context, the promoter of the tumor suppressor gene GADD45 (growth arrest and DNA damage-inducible gene 45) was detected to be hypermethylated in the context of HCV infection in mice transgenic for the entire HCV open reading frame, notwithstanding that the exact HCV-altered methylation mechanisms during infection remain to be explored [146]. The hypermethylation of GADD45 promoter by HCV downregulates GADD45 gene expression and interferes with its ability to block proliferation and tumorigenesis [147]. In addition, hepatitis C virus core protein has been linked to E-cadherin and p16 downregulation through upregulation of DNMT1 and DNMT3b [148, 149]. Thus DNMT inhibitors could constitute a novel approach for the treatment HCV-associated HCC. In this context, 5-Aza-C and 5-Aza-dC, two well-known DNMT inhibitors, significantly inhibited HCV infection. Interestingly, this effect is due not only to a decreased DNMT expression, but also through DNMT1 degradation [150]. It is worthy to mention that DNMTs expression could be varied between the different HCV genotypes 1b, 2a, 3a, 4h, and 5a. For example, DNMT3b mRNA is upregulated in genotype 1b HCV but not changed in genotypes 2a, 3a, 4h, and 5a [151]. Hence, the identification of HCV-induced epigenetic regulation that may actively participate in tumorigenesis and linking their prevalence to different HCV genotypes could possibly decipher new therapeutic targets for HCV infection and HCC management.

Herpes simplex virus

Herpes simplex virus 1 (HSV-1), a double-stranded DNA virus [152], is a ubiquitous pathogen which infects more than 50% of the population in the USA and Europe [153]. As with other members of the *Herpesviridae* family, HSV-1 conserves its ability to remain latent in the sensory neurons of the trigeminal ganglion as a nucleosome-associated episome in the nucleus of the host cell [154], which makes it vulnerable to epigenetic posttranslational modifications. Not limited to latent phase, viral promoters and transcribed genes were shown to be associated with histones during lytic infection [155]. A transcriptional factor, the CCCTC binding factor CTCF extensively binds to HSV-1 DNA during lytic infection where it promotes HSV-1 lytic transcription. CTCF knockdown increased the repressive histone marks H3K27me3 and H3K9me3 and reduced viral transcription and virus yield [156]. In the same context, treatment with the protein methylation inhibitor, 5'-deoxy-5'-methylthioadenosine (MTA), reduced the level of H3K4me3 mediated by the methyltransferase Set1, along with a decrease in the transcription and replication of HSV-1 [157]. Interestingly, the transcriptional coactivator host cell factor-1 (HCF-1) was found to be involved with transcriptional activation through the recruitment of the histone methyltransferases Set1 and MLL1, leading consequently to H3K4 trimethylation [158]. Also, HCF-1 interacts with three sets of acetyltransferases: MOF/NSL [159], ATAC [160], and CLOCK [161]. In addition to multiple acetyltransferase complexes, HCF-1 induces transcriptional activation during the G1-to-S phase transition [162]. Not only essential to the expression of immediate-early genes and initiation of lytic infection, HCF-1 could be also involved in the reactivation process. This is demonstrated by HCF-1 translocation and recruitment to IE promoters upon ex vivo reactivation in trigeminal ganglia neurons [163]. Given the multiple complexes with which HCF-1 is associated, discerning the dynamic mechanistic process and the possible interactions between HCF-1 and various transcription factors, coactivators, and chromatin modulation components could define a new aspect of HSV-1 lytic infection and thus identify new possible targets to control it. In fact, epigenetic marks could be manipulated to reinforce latency and prevent reactivation cycles, or to purge the viral reservoirs. Treatment of the quiescently infected PC12 (QIF-PC12) cells, an in vitro accepted latency model for HSV, by HDAC inhibitors sodium butyrate and TSA resulted in the production of infectious progeny [164]. However, the end point of HDACs interaction with the viral genome is more complex than initially presumed. In fact, four HDACi, TSA, VPA, SAHA, and suberohydroxamic acid (SBHA), reduced the number of HSV-1 genome that initiate replication in human foreskin fibroblasts (HFF) cells and human female osteosarcoma (U2OS) cells [165]. This

is possibly due to the HDACi-induced increase in the levels of some intrinsic immunity proteins known to exhibit antiviral immunity like promyelocytic leukemia (PML) bodies [166]. Same was shown with the nucleosome remodeler chromodomain helicase DNA-binding 3 protein (CHD3) that mediates repression of HSV genome upon infection. The CHD3 protein conserves its ability to identify and bind the repressive histone marks H3K27-trimethyl and H3K9-trimethyl, promoting the formation of heterochromatin [167, 168]. Surprisingly, the EZH2/1 inhibitors GSK126 and GSK343 suppressed productive viral lytic phase and decreased viral yields instead of inducing activation *in vitro* and *in vivo* [169]. This was attributable to the fact that treatment with those inhibitors enhanced cellular antiviral state by triggering antipathogen pathways. In the same context, other epigenetic players could negatively impact the reactivation of HSV-1 latent or quiescent infection. The JMJD2 inhibitors DMOG or ML342 significantly decreased the viral titers in trigeminal ganglia of HSV-1 latently infected mice through suppressing IE gene transcription and expression [97]. Attractively, the monoamine oxidase inhibitor (MAOI) tranylcypromine repressed HSV IE gene expression and genome replication *in vivo*, decreased the severity of a virus-induced encephalitis and corneal blindness in mouse models, and blocked viral reactivation in trigeminal ganglia [170, 171]. This is explained by the ability of MAOis to inhibit the LSD-1 mediated demethylation of lysine residues via a flavin-adenine-dinucleotide-dependent reaction [172, 173], resulting in the accumulation of repressive H3K9 chromatin marks at the IE promoters. Further studies are definitely needed to identify additional possible components and mechanisms involved in the epigenetic regulation of HSV-1 infection.

Epstein-Barr virus

Epstein-Barr virus (EBV), or human herpesvirus 4 (HH4), is a DNA virus [174] that belongs to the *Herpesviridae* family, subfamily *Gammaherpesvirinae* [175]. Like other herpes viruses, EBV persists predominantly in the latently infected B lymphocytes as a covalently closed circular episome [176]. It has been shown that reactivation from latency is coupled to and initiated by expression of the viral BZLF1 gene [177]. During latency, BZLF1 promoter is silenced partly by the recruitment of repressive factors, such as YY1 and the zinc finger E-box-binding factor (ZEB), that block the access of some transcriptional activators and ease the binding or function of repressive transcriptional co-factors like HDAC, maintaining thus a low level of histone acetylation [178, 179]. Therefore, it is not surprising that HDAC inhibitors, like sodium butyrate, can reverse latency [180]. Not limited to low acetylation level, BZLF1 gene proximal promoter Z_p is also silenced due to

methylation. Treatment with DZNep, an H3K27me3 and H4K20me3 inhibitor, along with TSA stimulated BZLF1 expression level [181]. In fact, recognizing the mechanisms behind lytic reactivation is gaining an increased attention, due to the potential use of epigenetic inducing agents as sensitizers to conventional antivirals for the treatment of EBV-associated lymphomas [182]. Other than establishing a persistent latent infection, EBV infection is associated with several malignancies including Burkitt's lymphoma (BL) [183], nasopharyngeal carcinoma (NPC) [184], Hodgkin's lymphoma (HL) [185], and others [186, 187]. In the context of BL pathogenesis, regulation of Bim protein appears to be of high importance [188]. BIM is a member of the proapoptotic BH3-only family that acts as a cellular inducer of programmed cell death (PCD) by inactivating the function of the antiapoptotic BCL-2 and its homologs through binding or by directly activating the function of BAX and BAK [189]. During EBV latent infection, BIM expression is repressed, which increases the likelihood of B lymphomagenesis. Interestingly, latent EBV reduces acetylation of histones associated with Bim promoter and increases DNA methylation of the Bim promoter since the use of HDAC inhibitor TSA and the DNMT inhibitor AZA resulted in an increase in Bim mRNA and protein levels in EBV infected cells. In addition, the methylation of the CpG dinucleotides in the large CpG island located at the 5' end of Bim in EBV-positive BL biopsies has been reported [190]. Besides Bim, p53 up-regulated modulator of apoptosis (PUMA) is another proapoptotic BH3-only protein prone to regulation by EBV in the setting of Burkitt's lymphoma. It was shown that EBV latency 1 genes, EBNA1, EBEBs, or miR-BARTs, act cooperatively together to inhibit apoptosis by repressing PUMA [191]. For example, miR-BART5 binds PUMA in its 3' untranslated region (UTR) which induces a decrease in PUMA transcription [192]. In addition, an *in vitro* model of continuously proliferating lymphoblastoid cell lines (LCLs) showed that EBV infection triggered CpG islands methylation of 40 tumor suppressor genes (TSG) including genes responsible of DNA-damage repair, cell cycle, and apoptosis regulation, resulting in a global transcriptional repression [193]. A comprehensive understanding of B cell reprogramming and epigenetic modifications could have important implications in the perception of EBV persistence and EBV-induced tumorigenesis, as well as potential therapeutic approaches in EBV-associated diseases. This could be exemplified by the fact that the epigenetic silencing through hypermethylation and deacetylation of the previously mentioned BIM gene is correlated with chemotherapeutic resistance (especially to doxorubicin) and lower complete remission rates in Burkitt lymphoma/leukemia. Interestingly, BIM could be re-expressed

by the use of the HDAC inhibitor vorinostat, in a xenograft mouse model, resulting in cells sensitization to doxorubicin and cyclophosphamide, as evidenced by the increased survival rate [194].

Epigenetic players in viral infections: filling the gap between basic research and clinical application

Powered by the supportive results in cell cultures and mouse models, epigenetic drug candidates are recently being clinically evaluated as potential antiviral drugs. In this section, some completed and ongoing clinical trials are cited, as an attempt to present preliminary data about the use of epigenetic drugs to manipulate viral

infections or viral infection-related malignancies (Table 2). As most trials are being published recently, analyzing toxicity, schedules, doses, and measuring clinical response stand up as the main aim. In the setting of HIV infection, several HDAC inhibitors were tested as a combination with antiretroviral therapy: panobinostat (NCT01680094), vorinostat (NCT01319383), and romidepsin (NCT02092116, NCT01933594), in addition to VPA (NCT00289952). Although with varying degrees, all HDAC inhibitors showed an increase in viral transcription with no significant effect on the size of the HIV-1 functional reservoir, as no inhibitor has demonstrated complete clearance of latent infection [195–198]. This is possibly attributable to incomplete latency reversal or

Table 2 Clinical trials of histone deacetylase inhibitors in viral infections and viral-associated malignancies

Drug	Combination	Indication	Clinical result	Study phase and status	Trial*	Reference
Panobinostat	Antiretroviral therapy	HIV infection	-Increase in unspliced HIV RNA -No reduction in integrated HIV DNA -Safe, well tolerated	Phases I–II	NCT01680094	[195]
	-Antiretroviral therapy -Interferon-alpha2a	HIV infection	Ongoing	Phases I–II	NCT02471430	
Vorinostat	Antiretroviral therapy	HIV infection	-Increase in cell-associated HIV RNA with no effective depletion of persistent HIV reservoir -Safe, well tolerated	Phases I–II	NCT01319383	[196]
	-Antiretroviral therapy -Autologous dendritic cell vaccine (AGS 004)	HIV infection	No published results yet	Phase I	NCT02707900	
	-Antiretroviral therapy -Disulfiram	HIV infection	Suspended	Phases I–II	NCT03198559	
Romidepsin	Antiretroviral therapy	HIV infection	-Increase in cell-associated un-spliced HIV-1 RNA -No effect on the number of HIV-specific T cells -No severe adverse events	Phases I–II	NCT02092116	[197]
	Antiretroviral therapy	HIV infection	No published results yet	Phases I–II	NCT01933594	
	MVA.HM/consv vaccine	HIV infection	No published results yet	Phase I	NCT02616874	
	Broadly neutralizing antibody (3BNC117)	HIV infection	Ongoing	Phase II	NCT03041012	
Valproic acid	Antiretroviral therapy	HIV infection	No significant reductions in the frequency of CD4+ T cells harboring replication-competent HIV	Phase II	NCT00289952	[198]
Belinostat	None	Unresectable hepatocellular carcinoma	-Tumor stabilization -Well tolerated	Phases I–II	NCT00321594	[200]
Mocetinostat	None	Relapsed and refractory classical Hodgkin lymphoma	-Decrease in tumor measurements -Grade 3 and 4 adverse events (neutropenia and pneumonia)	Phase II	NCT00358982	[201]
Tractinostat	Valganciclovir	EBV-associated lymphoid malignancies	Ongoing	Phase Ib/II	NCT03397706	

*Retrieved from Clinicaltrials.gov

insufficient clearance of latency-reactivated cells. It is suggested that the clearance of HIV latent reservoir could be enhanced by adding immune enhancement treatments, such as the immunomodulatory cytokine interferon- α 2a with panobinostat (NCT02471430); the therapeutic vaccine MVA.HIVconsV (NCT02616874); the bNAb-based therapeutic HIV vaccine 3BNC117 (NCT03041012) with romidepsin; alternatively, the autologous dendritic cell vaccine AGS 004 (NCT02616874); or disulfiram (NCT03198559) with vorinostat, as disulfiram was shown to reactivate latent HIV-1 in a primary CD4+ T cell model [199]. Importantly, caution should be engaged as the increased efficacy implicated by those combinations could be complemented with adverse effects not noted with the use of HDAC inhibitors alone. Not limited to HIV infection, some HDAC inhibitors have been tested in the context of viral-induced malignancies. Treatment with belinostat demonstrated tumor stabilization in unresectable hepatocellular carcinoma (NCT00321594) [200]. In addition, mocetinostat (MGCD0103) showed promising disease control in patients with relapsed classical Hodgkin lymphoma (NCT00358982) [201]. Tractinostat (VRx-3996) in combination with valganciclovir is currently under investigation in EBV-associated lymphoid malignancies (NCT03397706). Interestingly, HDAC inhibitors could induce EBV lytic-phase gene expression and act as sensitizers to antivirals for the treatment of EBV-associated lymphomas [202] as in the case of arginine butyrate and ganciclovir [203]. Another epigenetic player, the DNMT inhibitor azacitidine, was shown to reverse the dense CpG methylation and potentially triggering gene re-expression in patients with EBV-positive tumors [204]. Although those inhibitors might be future promising candidates for inclusion in the current therapeutic management, caution should be taken as they could reactivate some latent DNA viruses like HBV or EBV in the setting of other conditions treatment [205]. For instance, the use of SAHA or TSA aggravated the severity of myocarditis induced by coxsackievirus B3 (CVB3) through CVB3-induced myocardial apoptosis [206]. Thus, a careful assessment before the use of HDAC inhibitors is highly needed.

Conclusion and future perspectives

Over the past years, epigenetic studies have revealed novel principles and profoundly broadened our knowledge about the interplay between viruses, cellular transcription factors, histones, and nonhistones modifying enzymes. As most of those modifications are reversible, manipulating this complex machinery could have a critical role in determining an active lytic or latent viral infection and subsequent viral reactivation from latency. This diverts the end goal to permanently silence the virus in latent reservoirs so that the

possibility of reactivation is diminished, or to eradicate it through purging the viral reservoirs after reactivating it. Advantageously, and in contrast to the conventional antivirals, it is hypothesized that the emergence of resistant strains is minimized, as those chromatin modulation components target the host, rather than viral-encoded factors. Furthermore, epigenetic therapy could exemplify the "two birds, one stone" concept in the scenario of viral co-infection, e.g., with HCMV-HSV, HIV-HCV, and HIV-HBV. However, this tremendous array for new targets is a double-edged sword. In fact, the available epigenetics therapies lack specificity, which raises questions about their cytotoxic side effects due to unintended global epigenetic modifications and complicates the achievement of a therapeutic index within the acceptable toxicity levels. Intriguingly, designing and testing target-specific inhibitors (specific HDAC inhibitors rather than pan-inhibitors for example) could improve therapeutic outcomes through dropping the off-target undesired effects. This could be partly achieved through studying structure-activity relationship (SAR) to select a potent and selective compound for further mechanistic studies. In addition, advancement in epigenetic analysis tools such as epigenome microarray and combining chromatin immune-precipitation (ChIP) to next-generation sequencing (NGS) could provide a useful tool to decipher the multiprotein complexes involved in the epigenetic control of viral infections. In addition, addressing the role of the less studied post-translational modifications such as phosphorylation or sumoylation can shed light on new aspects of the dynamic host-viral interplay. Altogether, new therapeutic approaches are actively needed to fight viral infections and drugs targeting epigenetic players could lead to major therapeutic breakthroughs in the future.

Abbreviations

5-Aza: 5-Azacytidine; BL: Burkitt's lymphoma; cART: Combination antiretroviral therapy; cccDNA: Covalently closed circular DNA; CHB: Chronic hepatitis B; CTL: Cytotoxic T cells; CVB3: Coxsackievirus B3; DAA: Direct-acting antiviral; dCA: Didehydro-cortistatin A; DHBV: Duck hepatitis B virus; DMOG: Dimethylxalylglycine; DNMT: DNA methyltransferase; EBV: Epstein-Barr virus; EED: Embryonic ectoderm development; ERF: Ets-2 repressor factor; EZH2: Enhancer of zeste homolog 2; GADD45: Growth arrest and DNA damage-inducible gene 45; HAART: Highly active antiretroviral therapy; HAT: Histone acetyltransferases; HBV: Hepatitis B virus; HBx: Hepatitis B virus X protein; HCC: Hepatocellular carcinoma; HCF-1: Host cell factor-1; HCMV: Human cytomegalovirus; HCV: Hepatitis C virus; HDAC: Histone deacetylase; HDM: Histone demethylase; HHV4: Human herpesvirus 4; HHV5: Human herpesvirus 5; HIV: Human immunodeficiency virus; HL: Hodgkin's lymphoma; HMT: Histone methyltransferase; HSV-1: Herpes simplex virus 1; IE: Immediate early; IGF2: Insulin-like growth factor 2; JMJ3: Jumonji C; KAP1: KRAB-associated protein 1; LCLs: Lymphoblastoid cell lines; LRA: Latency reversing agent; LSD: Lys-specific demethylase; LTR: Long terminal repeat; MAOI: Monoamine oxidase inhibitor; MIEP: Major immediate-early promoter; MTA: Methylthioadenosine; NPC: Nasopharyngeal carcinoma; PCD: Programmed cell death; PHH: Primary human hepatocytes; PKC: Protein kinase C; PRC2: Polycomb repressive complex 2; P-TEFb: Positive transcription elongation factor 2; PTM: Posttranslational modifications; PUMA: p53 upregulated modulator of apoptosis; SAHA: Suberanilohydroxamic acid; SBHA: Suberohydroxamic acid; SETDB1: SET domain bifurcated 1; SFRP1: Secreted frizzled-related protein 1; SOCS1: Suppressor of cytokine signaling 1; SPOCI: Survival time-associated PHD finger protein in ovarian

cancer 1; SUZ12: Suppressor of zeste 12; Tat: Transactivator of transcription; TCP: Tranlycypromine; TNF: Tumor necrosis factor; TPA: 12-O-Tetradecanoylphorbol-13-acetate; TSG: Tumor suppressor gene; TSS: Transcription start site; UTR: Untranslated region; VPA: Valproic acid; YY1: Ying-yang 1

Acknowledgements

Not applicable.

Funding

This work was supported by grants from the University of Franche-Comté (UFC). Zeina Nehme is a recipient of a doctoral scholarship from the municipality of Habbouch.

Availability of data and materials

Not applicable.

Authors' contributions

ZN, SP, and GH wrote the paper. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details

¹Department Pathogens & Inflammation-EPILAB, UPRES EA4266, University of Franche-Comté, University of Bourgogne Franche-Comté, 16 route de Gray, F-25030 Besançon cedex, France. ²Université Libanaise, Beirut, Lebanon. ³Department of Virology, CHRU Besançon, F-25030 Besançon, France.

Received: 20 November 2018 Accepted: 13 March 2019

Published online: 27 March 2019

References

- Deans C, Maggert KA. What do you mean, "epigenetic"? *Genetics*. 2015;199:887–96.
- Osborne A. The role of epigenetics in human evolution. *Biosci Horiz*. 2017;1:01–8.
- Stotz K, Griffiths P. Epigenetics: ambiguities and implications. *Hist Philos Life Sci*. 2016;38:22.
- Eccleston A, DeWitt N, Gunter C, Marte B, Nath D. Epigenetics. *Nature*. 2007;447:395.
- Shamsi MB, Firoz AS, Imam SN, Alzaman N, Samman MA. Epigenetics of human diseases and scope in future therapeutics. *J Taibah Univ Med Sci*. 2017;12:205–11.
- Moosavi A, Ardekani AM. Role of epigenetics in biology and human diseases. *Iran Biomed J*. 2016;20:246.
- Egger G, Liang G, Aparicio A, Jones PA. Epigenetics in human disease and prospects for epigenetic therapy. *Nature*. 2004;429:457–63.
- Li J, Hao D, Wang L, Wang H, Wang Y, Zhao Z, et al. Epigenetic targeting drugs potentiate chemotherapeutic effects in solid tumor therapy. *Sci Rep*. 2017;7:4035.
- Allis CD, Jenuwein T. The molecular hallmarks of epigenetic control. *Nat Rev Genet*. 2016;17:487–500.
- Sainz B, LaMarca HL, Garry RF, Morris CA. Synergistic inhibition of human cytomegalovirus replication by interferon-alpha/beta and interferon-gamma. *Viral J*. 2005;2:14.
- Panos G, Samonis G, Alexiou VG, Kavaimou GA, Charatsis G, Falagas ME. Mortality and morbidity of HIV infected patients receiving HAART: a cohort study. *Curr HIV Res*. 2008;6:257–60.
- Lifson AR, Grund B, Gardner EM, Kaplan R, Denning E, Engen N, et al. Improved quality of life with immediate versus deferred initiation of antiretroviral therapy in early asymptomatic HIV infection. *AIDS*. 2017;31:953–63.
- Chen W-T, Shiu C-S, Yang J-P, Simon J-M, Friedriksen-Goldsen KJ, Lee T-S-H, et al. Antiretroviral therapy (ART) side effect impacted on quality of life, and depressive symptomatology: a mixed-method study. *J AIDS Clin Res*. 2013;4:218.
- Montessori V, Press N, Harris M, Akagi L, Montaner JSG. Adverse effects of antiretroviral therapy for HIV infection. *CMAJ*. 2004;170:229–38.
- d'Arminio Monforte A, Lepri AC, Rezza G, Pezzotti P, Antinori A, Phillips AN, et al. Insights into the reasons for discontinuation of the first highly active antiretroviral therapy (HAART) regimen in a cohort of antiretroviral naïve patients. I.CO.N.A. Study Group. Italian Cohort of Antiretroviral-Naïve Patients. *AIDS*. 2000;14:499–507.
- Hamlyn E, Ewings FM, Porter K, Cooper DA, Tambussi G, Schechter M, et al. Plasma HIV viral rebound following protocol-indicated cessation of ART commenced in primary and chronic HIV infection. *PLoS One*. 2012;7:e43754.
- Abbas W, Tariq M, Iqbal M, Kumar A, Herbein G. Eradication of HIV-1 from the macrophage reservoir: an uncertain goal? *Viruses*. 2015;7:1578–98.
- Le Douce V, Herbein G, Rohr O, Schwartz C. Molecular mechanisms of HIV-1 persistence in the monocyte-macrophage lineage. *Retrovirology*. 2010;7:32.
- Churchill MJ, Deeks SG, Margolis DM, Siliciano RF, Swanstrom R. HIV reservoirs: what, where and how to target them. *Nat Rev Microbiol*. 2016;14:55–60.
- Mbonye U, Karn J. Transcriptional control of HIV latency: cellular signaling pathways, epigenetics, happenstance and the hope for a cure. *Virology*. 2014;454–455:328–39.
- Darcis G, Van Driessche B, Van Lint C. HIV latency: should we shock or lock? *Trends Immunol*. 2017;38:217–28.
- Kumar A, Darcis G, Van Lint C, Herbein G. Epigenetic control of HIV-1 post integration latency: implications for therapy. *Clin Epigenetics*. 2015;7:103.
- Khan S, Iqbal M, Tariq M, Baig SM, Abbas W. Epigenetic regulation of HIV-1 latency: focus on polycomb group (PcG) proteins. *Clin Epigenetics*. 2018;10:14.
- Archin NM, Margolis DM. Emerging strategies to deplete the HIV reservoir. *Curr Opin Infect Dis*. 2014;27:29–35.
- Deeks SG. HIV: shock and kill. *Nature*. 2012;487:439–40.
- Archin NM, Liberty AL, Kashuba AD, Choudhary SK, Kuruc JD, Crooks AM, et al. Administration of vorinostat disrupts HIV-1 latency in patients on antiretroviral therapy. *Nature*. 2012;487:482–5.
- Seto E, Yoshida M. Erasers of histone acetylation: the histone deacetylase enzymes. *Cold Spring Harb Perspect Biol*. 2014;6:a018713.
- Delcuve GP, Khan DH, Davie JR. Roles of histone deacetylases in epigenetic regulation: emerging paradigms from studies with inhibitors. *Clin Epigenetics*. 2012;4:5.
- West AC, Johnstone RW. New and emerging HDAC inhibitors for cancer treatment. *J Clin Invest*. 2014;124:30–9.
- Bubna AK. Vorinostat—an overview. *Indian J Dermatol*. 2015;60:419.
- Raedler LA. Farydak (Panobinostat): first HDAC inhibitor approved for patients with relapsed multiple myeloma. *Am Health Drug Benefits*. 2016;9:84–7.
- du Chéné I, Basyuk E, Lin Y-L, Triboulet R, Knezevich A, Chable-Bessia C, et al. Suv39H1 and HPIgamma are responsible for chromatin-mediated HIV-1 transcriptional silencing and post-integration latency. *EMBO J*. 2007;26:424–35.
- Reuse S, Calao M, Kabeya K, Guiguen A, Gatot J-S, Quivy V, et al. Synergistic activation of HIV-1 expression by deacetylase inhibitors and prostratin: implications for treatment of latent infection. *PLoS One*. 2009;4:e6093.
- Darcis G, Kula A, Bouchat S, Fujinaga K, Corazza F, Ait-Ammar A, et al. An in-depth comparison of latency-reversing agent combinations in various in vitro and ex vivo HIV-1 latency models identified bryostatins-1+JQ1 and ingenol-B+JQ1 to potentially reactivate viral gene expression. *PLoS Pathog*. 2015;11:e1005063.
- Bouchat S, Delacourt N, Kula A, Darcis G, Van Driessche B, Corazza F, et al. Sequential treatment with 5-aza-2'-deoxycytidine and deacetylase inhibitors reactivates HIV-1. *EMBO Mol Med*. 2016;8:117–38.
- Boehm D, Ott M. Host methyltransferases and demethylases: potential new epigenetic targets for HIV cure strategies and beyond. *AIDS Res Hum Retrovir*. 2017;33:58–22.
- Thorlund K, Horwitz MS, Fife BT, Lester R, Cameron DW. Landscape review of current HIV "kick and kill" cure research - some kicking, not enough killing. *BMC Infect Dis*. 2017;17:595.

38. Park SY, Kim K-C, Hong K-J, Kim SS, Choi B-S. Histone deacetylase inhibitor SAHA induces a synergistic HIV-1 reactivation by 12-O-tetradecanoylphorbol-13-acetate in latently infected cells. *Intervirology*. 2013;56:242–8.
39. Pasquereau S, Kumar A, Herbein G. Targeting TNF and TNF receptor pathway in HIV-1 infection: from immune activation to viral reservoirs. *Viruses*. 2017;9:64.
40. Das AT, Harwig A, Berkhout B. The HIV-1 Tat protein has a versatile role in activating viral transcription. *J Virol*. 2011;85:9506–16.
41. Laspia MF, Rice AP, Mathews MB. HIV-1 Tat protein increases transcriptional initiation and stabilizes elongation. *Cell*. 1989;59:283–92.
42. Mousseau G, Clement MA, Bakeman WN, Nagarsheth N, Cameron M, Shi J, et al. An analog of the natural steroidal alkaloid cortistatin a potently suppresses Tat-dependent HIV transcription. *Cell Host Microbe*. 2012;12:97–108.
43. Mousseau G, Kessing CF, Fromentin R, Trautmann L, Chomont N, Valente ST. The Tat inhibitor didehydro-cortistatin A prevents HIV-1 reactivation from latency. *MBio*. 2015;6:e00465.
44. Chun T-W, Nickle DC, Justement JS, Large D, Semerjian A, Curlin ME, et al. HIV-infected individuals receiving effective antiviral therapy for extended periods of time continually replenish their viral reservoir. *J Clin Invest*. 2005;115:3250–5.
45. Gandhi RT, Bosch RJ, Aga E, Albrecht M, Demeter LM, Dykes C, et al. No evidence for decay of the latent reservoir in HIV-1-infected patients receiving intensive enfuvirtide-containing antiretroviral therapy. *J Infect Dis*. 2010;201:293–6.
46. Kumar A, Abbas W, Bouchat S, Gatot J-S, Pasquereau S, Kabeya K, et al. Limited HIV-1 reactivation in resting CD4(+) T cells from aviremic patients under protease inhibitors. *Sci Rep*. 2016;6:38313.
47. Kumar A, Abbas W, Colin L, Khan KA, Bouchat S, Varin A, et al. Tuning of Akt-pathway by Nef and its blockade by protease inhibitors results in limited recovery in latently HIV infected T-cell line. *Sci Rep*. 2016;6:24090.
48. Pasquereau S, Kumar A, Abbas W, Herbein G. Counteracting Akt activation by HIV protease inhibitors in monocytes/macrophages. *Viruses*. 2018;10:190.
49. Schottstedt V, Blümel J, Burger R, Diosten C, Gröner A, Güntler L, et al. Human cytomegalovirus (HCMV) – revised*. *Transfus Med Hemother*. 2010;37:365–75.
50. Mocarski ES, Shenk T, Griffiths PD, Pass RF. Cytomegaloviruses. In: Knipe DM, Howley PM, editors. *Fields virology*. 6th ed. Philadelphia: Wolters Kluwer Lippincott Williams & Wilkins; 2013. p. 1960–2014.
51. Emery VC. Investigation of CMV disease in immunocompromised patients. *J Clin Pathol*. 2001;54:84–8.
52. Rubin RH. Impact of cytomegalovirus infection on organ transplant recipients. *Rev Infect Dis*. 1990;12(Suppl 7):S754–66.
53. Klatt EC, Shibata D. Cytomegalovirus infection in the acquired immunodeficiency syndrome. Clinical and autopsy findings. *Arch Pathol Lab Med*. 1988;112:540–4.
54. Manicklal S, Emery VC, Lazzarotto T, Boppana SB, Gupta RK. The “silent” global burden of congenital cytomegalovirus. *Clin Microbiol Rev*. 2013;26:86–102.
55. Ljungman P, Hakki M, Boeckh M. Cytomegalovirus in hematopoietic stem cell transplant recipients. *Hematol Oncol Clin North Am*. 2011;25:151–69.
56. Sinclair J, Sissons P. Latency and reactivation of human cytomegalovirus. *J Gen Virol*. 2006;87:1763–79.
57. Wathen MW, Stinski MF. Temporal patterns of human cytomegalovirus transcription: mapping the viral RNAs synthesized at immediate early, early, and late times after infection. *J Virol*. 1982;41:462–77.
58. Torres L, Tang Q. Immediate-early (IE) gene regulation of cytomegalovirus: IE1- and pp71-mediated viral strategies against cellular defenses. *Virol Sin*. 2014;29:343–52.
59. Compton T, Feire A. Early events in human cytomegalovirus infection. In: Arvin A, Campadelli-Fiume G, Mocarski E, Moore PS, Roizman B, Whitley R, et al., editors. *Human herpesviruses: biology, therapy, and immunoprophylaxis*. Cambridge: Cambridge University Press; 2007.
60. Anders DG, Kerry JA, Pait GS. DNA synthesis and late viral gene expression. In: Arvin A, Campadelli-Fiume G, Mocarski E, Moore PS, Roizman B, Whitley R, et al., editors. *Human herpesviruses: biology, therapy, and immunoprophylaxis*. Cambridge: Cambridge University Press; 2007.
61. Sinclair J. Chromatin structure regulates human cytomegalovirus gene expression during latency, reactivation and lytic infection. *Biochim Biophys Acta*. 2010;1799:286–95.
62. Reeves MB. Chromatin-mediated regulation of cytomegalovirus gene expression. *Viruses*. 2011;157:134–43.
63. Woodhall DL, Groves LJ, Reeves MB, Wilkinson G, Sinclair JH. Human Daxx-mediated repression of human cytomegalovirus gene expression correlates with a repressive chromatin structure around the major immediate early promoter. *J Biol Chem*. 2006;281:37652–60.
64. Biron KK. Antiviral drugs for cytomegalovirus diseases. *Antivir Res*. 2006;71:154–63.
65. Gilbert C, Boivin G. Human cytomegalovirus resistance to antiviral drugs. *Antimicrob Agents Chemother*. 2005;49:873–83.
66. Jacobsen T, Sifontis N. Drug interactions and toxicities associated with the antiviral management of cytomegalovirus infection. *Am J Health Syst Pharm*. 2010;67:1417–25.
67. Cannon MJ, Hyde TB, Schmid DS. Review of cytomegalovirus shedding in bodily fluids and relevance to congenital cytomegalovirus infection. *Rev Med Virol*. 2011;21:240–55.
68. Andrei G, De Clercq E, Snoeck R. Drug targets in cytomegalovirus infection. *Infect Disord Drug Targets*. 2009;9:201–22.
69. Marty FM, Ljungman P, Chermaly RF, Maertens J, Dadwal SS, Duarte RF, et al. Letermovir prophylaxis for cytomegalovirus in hematopoietic-cell transplantation. *N Engl J Med*. 2017;377:2433–44.
70. Carbone J. The immunology of posttransplant CMV infection: potential effect of CMV immunoglobulins on distinct components of the immune response to CMV. *Transplantation*. 2016;100:511.
71. Sinzger C, Digel M, Jahn G. Cytomegalovirus cell tropism. In: Shenk TE, Stinski MF, editors. *Human cytomegalovirus*. Berlin-Heidelberg: Springer-Verlag; 2008. p. 63–83.
72. Poole E, Willis M, Sinclair J. Human cytomegalovirus latency: targeting differences in the latently infected cell with a view to clearing latent infection. *New J Sci*. 2014;2014:313761.
73. Dupont L, Reeves MB. Cytomegalovirus latency and reactivation: recent insights into an age old problem. *Rev Med Virol*. 2016;26:75–89.
74. Liu X, Wang X, Yan S, Zhang Z, Abecassis M, Hummel M. Epigenetic control of cytomegalovirus latency and reactivation. *Viruses*. 2013;5:1325–45.
75. Slobedman B, Cao JZ, Avdic S, Webster B, McAlley S, Cheung AK, et al. Human cytomegalovirus latent infection and associated viral gene expression. *Future Microbiol*. 2010;5:883–900.
76. Kumar A, Herbein G. Epigenetic regulation of human cytomegalovirus latency: an update. *Epigenomics*. 2014;6:533–46.
77. Sourvinos G, Morou A, Sanidas I, Codruta I, Ezeil SA, Dowaki C, et al. The downregulation of GF11 by the EZH2-NFY1/KDM2B-JARID2 axis and by human cytomegalovirus (HCMV) associated factors allows the activation of the HCMV major IE promoter and the transition to productive infection. *PLoS Pathog*. 2014;10:e1004136.
78. Moritz LE, Trivelp RC. Structure, mechanism, and regulation of polycomb-repressive complex 2. *J Biol Chem*. 2018;293:13805–14.
79. Rajasekhar VK, Begemann M. Concise review: roles of polycomb group proteins in development and disease: a stem cell perspective. *Stem Cells*. 2007;25:2498–510.
80. Chittock EC, Latwiel S, Miller TCR, Müller CW. Molecular architecture of polycomb repressive complexes. *Biochem Soc Trans*. 2017;45:193–205.
81. Margueron R, Reinberg D. The Polycomb complex PRC2 and its mark in life. *Nature*. 2011;469:343–9.
82. Abraham CG, Kulesza CA. Polycomb repressive complex 2 targets murine cytomegalovirus chromatin for modification and associates with viral replication centers. *PLoS One*. 2012;7:e29410.
83. Kirmizis A, Bartley SM, Kuzmichev A, Margueron R, Reinberg D, Green R, et al. Silencing of human polycomb target genes is associated with methylation of histone H3 Lys 27. *Genes Dev*. 2004;18:1592–605.
84. Kuzmichev A, Nishioka K, Erdjument-Bromage H, Tempst P, Reinberg D. Histone methyltransferase activity associated with a human multiprotein complex containing the enhancer of zeste protein. *Genes Dev*. 2002;16:2893–905.
85. Abraham CG, Kulesza CA. Polycomb repressive complex 2 silences human cytomegalovirus transcription in quiescent infection models. *J Virol*. 2013;87:13193–205.
86. Rosenfeld MG, Lunyak W, Glass CK. Sensors and signals: a coactivator/corepressor/epigenetic code for integrating signal-dependent programs of transcriptional response. *Genes Dev*. 2006;20:1405–28.
87. Schultz DC, Ayyanathan K, Negorev D, Maul GG, Rauscher FJ. SETDB1: a novel KAP-1-associated histone H3, lysine 9-specific methyltransferase that contributes to HP1-mediated silencing of euchromatic genes by KRAB zinc-finger proteins. *Genes Dev*. 2002;16:919–32.

88. Reichel A, Stilp A-C, Scherer M, Reuter N, Lukassen S, Kasmour B, et al. Chromatin-remodeling factor SPOC1 acts as a cellular restriction factor against human cytomegalovirus by repressing the major immediate early promoter. *J Virol*. 2018;92(14):e00342–18.
89. Bain M, Mendelson M, Sinclair J. Ets-2 repressor factor (ERF) mediates repression of the human cytomegalovirus major immediate-early promoter in undifferentiated non-permissive cells. *J Gen Virol*. 2003;84:41–9.
90. Wright E, Bain M, Teague L, Murphy J, Sinclair J. Ets-2 repressor factor recruits histone deacetylase to silence human cytomegalovirus immediate-early gene expression in non-permissive cells. *J Gen Virol*. 2005;86:535–44.
91. Gordon S, Akopyan G, Garban H, Bonavida B. Transcription factor YY1: structure, function, and therapeutic implications in cancer biology. *Oncogene*. 2006;25:1125–42.
92. Liu R, Bailly J, Sissons JG, Sinclair JH. The transcription factor YY1 binds to negative regulatory elements in the human cytomegalovirus major immediate early enhancer/promoter and mediates repression in non-permissive cells. *Nucleic Acids Res*. 1994;22:2453–9.
93. Huang NE, Lin CH, Lin YS, Yu WCY. Modulation of YY1 activity by SAP30. *Biochem Biophys Res Commun*. 2003;306:267–75.
94. Koista SM, Helin K. Molecular mechanisms and potential functions of histone demethylases. *Nat Rev Mol Cell Biol*. 2012;13:297–311.
95. Gan X, Wang H, Yu Y, Yi W, Zhu S, Li E, et al. Epigenetically repressing human cytomegalovirus lytic infection and reactivation from latency in THP-1 model by targeting H3K9 and H3K27 histone demethylases. *PLoS One*. 2017;12:e0175390.
96. Liang Y, Quenelle D, Vogel JL, Mascaro C, Ortega A, Kristie TM. A novel selective LSD1/KDM1A inhibitor epigenetically blocks herpes simplex virus lytic replication and reactivation from latency. *MBio*. 2013;4:e00558–12.
97. Liang Y, Vogel JL, Arbutckle J, Rai G, Jadhav A, Simeonov A, et al. Targeting the JMJD2 histone demethylase to epigenetically control herpesvirus infection and reactivation from latency. *Sci Transl Med*. 2013;5:167a5.
98. Craigen JL, Grundy JE. Cytomegalovirus induced up-regulation of LFA-3 (CD58) and ICAM-1 (CD54) is a direct viral effect that is not prevented by ganciclovir or foscarnet treatment. *Transplantation*. 1996;62:1102–8.
99. Cobbs CS, Sorocceanu L, Denham S, Zhang W, Kraus MH. Modulation of oncogenic phenotype in human glioma cells by cytomegalovirus IE1-mediated mitogenicity. *Cancer Res*. 2008;68:724–30.
100. Boldogh I, AbuBakar S, Deng CZ, Albrecht T. Transcriptional activation of cellular oncogenes fos, jun, and myc by human cytomegalovirus. *J Virol*. 1991;65:1568–71.
101. Khan KA, Coaquette A, Davrinche C, Herbein G. Bcl-3-regulated transcription from major immediate-early promoter of human cytomegalovirus in monocyte-derived macrophages. *J Immunol*. 2009;182:7784–94.
102. Kumar A, Tripathy MK, Pasquereau S, Al Moussawi F, Abbas W, Coquard L, et al. The human cytomegalovirus strain DB activates oncogenic pathways in mammary epithelial cells. *EBioMedicine*. 2018;30:167–83.
103. Herbein G. The human cytomegalovirus, from oncomodulation to oncogenesis. *Viruses*. 2018;10(8):408.
104. Krishna BA, Lau B, Jackson SE, Willis MR, Sinclair JH, Poole E. Transient activation of human cytomegalovirus lytic gene expression during latency allows cytotoxic T cell killing of latently infected cells. *Sci Rep*. 2016;6:24674.
105. MacLachlan JH, Cowie BC. Hepatitis B virus epidemiology. *Cold Spring Harb Perspect Med*. 2015;5:a021410.
106. Yuen M-F, Chen D-S, Dusheiko GM, Janssen HLA, Lau DTY, Locamini SA, et al. Hepatitis B virus infection. *Nat Rev Dis Primers*. 2018;4:18035.
107. Bahmania M, Feld J, Arif A, Janssen HLA. New therapeutic agents for chronic hepatitis B. *Lancet Infect Dis*. 2016;16:e10–21.
108. Nassal M. HBV cccDNA: viral persistence reservoir and key obstacle for a cure of chronic hepatitis B. *Gut*. 2015;64:1972–84.
109. Tropberger P, Mercier A, Robinson M, Zhong W, Ganem DE, Holdorf M. Mapping of histone modifications in episomal HBV cccDNA uncovers an unusual chromatin organization amenable to epigenetic manipulation. *Proc Natl Acad Sci U S A*. 2015;112:E5715–24.
110. Hensel KO, Rendon JC, Navas M-C, Rots MG, Postberg J. Virus-host interplay in hepatitis B virus infection and epigenetic treatment strategies. *FEBS J*. 2017;284:3550–72.
111. Ren J-H, Hu J-L, Cheng S-T, Yu H-B, Wong VKW, Law BYK, et al. SIRT3 restricts hepatitis B virus transcription and replication through epigenetic regulation of covalently closed circular DNA involving suppressor of variegation 3-9 homolog 1 and SET domain containing 1A histone methyltransferases. *Hepatology*. 2018;68:1260–76.
112. Benhenda S, Ducroux A, Rivière L, Sobhian B, Ward MD, Dion S, et al. Methyltransferase PRMT1 is a binding partner of HBx and a negative regulator of hepatitis B virus transcription. *J Virol*. 2013;87:4360–71.
113. Keasler VV, Hodgson AJ, Madden CR, Slagle BL. Enhancement of hepatitis B virus replication by the regulatory X protein in vitro and in vivo. *J Virol*. 2007;81:2656–62.
114. Tang R-X, Kong F-Y, Fan B-F, Liu X-M, You H-J, Zhang P, et al. HBx activates FasL and mediates HepG2 cell apoptosis through MLK3-MKK7-JNKs signal module. *World J Gastroenterol*. 2012;18:1485–95.
115. Lee ATC, Ren J, Wong E-T, Ban KHK, Lee LA, Lee CGL. The hepatitis B virus X protein sensitizes HepG2 cells to UV light-induced DNA damage. *J Biol Chem*. 2005;280:33525–35.
116. Lee YI, Hwang JM, Im JH, Lee YI, Kim NS, Kim DG, et al. Human hepatitis B virus-X protein alters mitochondrial function and physiology in human liver cells. *J Biol Chem*. 2004;279:15460–71.
117. Li C, Lin C, Gong X, Jiang Y. PDK1-WNK1 signaling is affected by HBx and involved in the viability and metastasis of hepatic cells. *Oncol Lett*. 2018;15:5940–6.
118. Kongkaviton P, Tangkijvarich P, Hirankarn N, Palaga T. Hepatitis B virus HBx activates notch signaling via delta-like 4/Notch1 in hepatocellular carcinoma. *PLoS One*. 2016;11:e0146696.
119. Belloni L, Pollicino T, De Nicola F, Guerrieri F, Raffa G, Fanciulli M, et al. Nuclear HBx binds the HBV minichromosome and modifies the epigenetic regulation of cccDNA function. *Proc Natl Acad Sci U S A*. 2009;106:19975–9.
120. Kgatle M. The role of HBx-mediated transcriptional activities and epigenetic alterations in hepatitis B virus induced hepatocellular carcinoma. *J Emerg Dis Virol*. 2017;3.
121. Park Y, Sohn BH, Yu E, Suh DJ, Chung Y-H, Lee J-H, et al. Aberrant epigenetic modifications in hepatocarcinogenesis induced by hepatitis B virus X protein. *Gastroenterology*. 2007;132:1476–94.
122. Lee J-O, Kwun HJ, Jung JK, Choi KH, Min DS, Jiang KL. Hepatitis B virus X protein represses E-cadherin expression via activation of DNA methyltransferase 1. *Oncogene*. 2005;24:6617–25.
123. Xie Q, Chen L, Shan X, Shan X, Tang J, Zhou F, et al. Epigenetic silencing of SFRP1 and SFRP5 by hepatitis B virus X protein enhances hepatoma cell tumorigenicity through Wnt signaling pathway. *Int J Cancer*. 2014;135:635–46.
124. Cheng S-T, Ren J-H, Cai X-F, Jiang H, Chen J. HBx-elevated SIRT2 promotes HBV replication and hepatocarcinogenesis. *Biochem Biophys Res Commun*. 2018;496:904–10.
125. Tang S, Hu W, Hu J, Wu S, Li J, Luo Y, et al. Hepatitis B virus X protein promotes P3 transcript expression of the insulin-like growth factor 2 gene via inducing hypomethylation of P3 promoter in hepatocellular carcinoma. *Liver Int*. 2015;35:608–19.
126. Zhang W, Chen J, Wu M, Zhang X, Zhang M, Yue L, et al. PRMT5 restricts hepatitis B virus replication through epigenetic repression of covalently closed circular DNA transcription and interference with pregenomic RNA encapsidation. *Hepatology*. 2017;66:398–415.
127. Vivekanandan P, Daniel HD-J, Kannangai R, Martinez-Munillo F, Torbenson M. Hepatitis B virus replication induces methylation of both host and viral DNA. *J Virol*. 2010;84:4321–9.
128. Dong Y, Wang A. Aberrant DNA methylation in hepatocellular carcinoma tumor suppression (review). *Oncol Lett*. 2014;8:963–8.
129. Pollicino T, Belloni L, Raffa G, Pediconi N, Squadrito G, Raimondo G, et al. Hepatitis B virus replication is regulated by the acetylation status of hepatitis B virus cccDNA-bound H3 and H4 histones. *Gastroenterology*. 2005;130:823–37.
130. Shetty S, Kim S, Shimakami T, Lemon SM, Mihalescu M-R. Hepatitis C virus genomic RNA dimerization is mediated via a kissing complex intermediate RNA. *2010;16:913–25*.
131. Lauer GM, Walker BD. Hepatitis C virus infection. *N Engl J Med*. 2001;345:41–52.
132. Chen SL, Morgan TR. The natural history of hepatitis C virus (HCV) infection. *Int J Med Sci*. 2006;3:47–52.
133. Feeney ER, Chung RT. Antiviral treatment of hepatitis C. *BMJ*. 2014;348:g3308.
134. Chan A, Patel K, Naggie S. Genotype 3 infection: the last stand of hepatitis C virus. *Drugs*. 2017;77:131–44.
135. Sato A, Saito Y, Sugiyama K, Sakasegawa N, Muramatsu T, Fukuda S, et al. Suppressive effect of the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA) on hepatitis C virus replication. *J Cell Biochem*. 2013;114:1987–96.

136. Ashkar S, Weber GF, Panoutsakopoulou V, Sanchirico ME, Jansson M, Zawaideh S, et al. Eta-1 (osteopontin): an early component of type-1 (cell-mediated) immunity. *Science*. 2000;287:860–4.
137. Kozlov MV, Keymenova AA, Konduktorov KA, Malikova AZ, Kocherikov SN. Selective inhibitor of histone deacetylase 6 (tubastatin A) suppresses proliferation of hepatitis C virus replicon in culture of human hepatocytes. *Biochemistry (Mosc)*. 2014;79:637–42.
138. Hubbert C, Guardiola A, Shao R, Kawaguchi Y, Ito A, Nixon A, et al. HDAC6 is a microtubule-associated deacetylase. *Nature*. 2002;417:455–8.
139. Parmigiani RB, Xu WS, Venta-Perez G, Erdjument-Bromage H, Yaneva M, Tempst P, et al. HDAC6 is a specific deacetylase of peroxiredoxins and is involved in redox regulation. *Proc Natl Acad Sci U S A*. 2008;105:9633.
140. Kovacs JJ, Murphy PJM, Gaillard S, Zhao X, Wu J-T, Nichchitta CV, et al. HDAC6 regulates Hsp90 acetylation and chaperone-dependent activation of glucocorticoid receptor. *Mol Cell*. 2005;18:601–7.
141. Zhou Y, Wang Q, Yang Q, Tang J, Xu C, Gai D, et al. Histone deacetylase 3 inhibitor suppresses hepatitis C virus replication by regulating Apo-A1 and LEAP-1 expression. *Virology*. 2018;333(5):418–28.
142. Mancone C, Steindler C, Santangelo L, Simonte G, Vlassi C, Longo MA, et al. Hepatitis C virus production requires apolipoprotein A-I and affects its association with nascent low-density lipoproteins. *Gut*. 2011;60:378–86.
143. Nishida N, Nagasaka T, Nishimura T, Ikai I, Boland CR, Goel A. Aberrant methylation of multiple tumor suppressor genes in aging liver, chronic hepatitis, and hepatocellular carcinoma. *Hepatology*. 2008;47:908–18.
144. Zhang J, Li H, Yu J-P, Wang SE, Ren X-B. Role of SOCS1 in tumor progression and therapeutic application. *Int J Cancer*. 2012;130:1971–80.
145. Ko E, Kim S-J, Joh J-W, Park C-K, Park J, Kim D-H. CpG island hypermethylation of SOCS-1 gene is inversely associated with HBV infection in hepatocellular carcinoma. *Cancer Lett*. 2008;271:240–50.
146. Higgs MR, Lerat H, Pawlowsky J-M. Downregulation of Gadd45beta expression by hepatitis C virus leads to defective cell cycle arrest. *Cancer Res*. 2010;70:4901–11.
147. Michaëlis KA, Knox AJ, Xu M, Kiseljak-Vassiliades K, Edwards MG, Gesici M, et al. Identification of growth arrest and DNA-damage-inducible gene beta (GADD45beta) as a novel tumor suppressor in pituitary gonadotrope tumors. *Endocrinology*. 2011;152:3603–13.
148. Aroia P, Kim E-Q, Jung JK, Jang KL. Hepatitis C virus core protein downregulates E-cadherin expression via activation of DNA methyltransferase 1 and 3b. *Cancer Lett*. 2008;261:244–52.
149. Park S-H, Lim JS, Lim S-Y, Tiwari J, Jang KL. Hepatitis C virus core protein stimulates cell growth by down-regulating p16 expression via DNA methylation. *Cancer Lett*. 2011;310:61–8.
150. Chen C, Pan D, Deng A-M, Huang F, Sun B-L, Yang R-G. DNA methyltransferases 1 and 3B are required for hepatitis C virus infection in cell culture. *Virology*. 2013;441(1):57–65.
151. Benegiamo G, Vinciguerra M, Mazzoccoli G, Piepoli A, Andriulli A, Paziienza V. DNA methyltransferases 1 and 3b expression in Huh-7 cells expressing HCV Core protein of different genotypes. *Dig Dis Sci*. 2012;57:1598–603.
152. Lacasse JJ, Schang LM. During lytic infections, herpes simplex virus type 1 DNA is in complexes with the properties of unstable nucleosomes. *J Virol*. 2010;84:1920–33.
153. Wald A, Corey L. Persistence in the population: epidemiology, transmission. In: Arvin A, Campadelli-Fiume G, Mocarski E, Moore PS, Roizman B, Whitley R, et al, editors. *Human herpesviruses: biology, therapy, and immunoprophylaxis*. Cambridge: Cambridge University Press; 2007.
154. Brown JC. Herpes simplex virus latency: the DNA repair-centered pathway. *Adv Virol*. 2017;2017:7028194.
155. Kent JR, Zeng P-Y, Atanasiu D, Gardner J, Fraser MW, Berger SL. During lytic infection herpes simplex virus type 1 is associated with histones bearing modifications that correlate with active transcription. *J Virol*. 2004;78(10):78–86.
156. Lang F, Li X, Vladimirova O, Hu B, Chen G, Xiao Y, et al. CTCF interacts with the lytic HSV-1 genome to promote viral transcription. *Sci Rep*. 2017;7:39861.
157. Huang J, Kent JR, Placek B, Whelan KA, Hollow GM, Zeng P-Y, et al. Trimethylation of histone H3 lysine 4 by Set1 in the lytic infection of human herpes simplex virus 1. *J Virol*. 2006;80:5740–6.
158. Narayanan A, Ruyechan WT, Kristie TM. The coactivator host cell factor-1 mediates Set1 and MLL1 H3K4 trimethylation at herpesvirus immediate early promoters for initiation of infection. *Proc Natl Acad Sci U S A*. 2007;104:10835–40.
159. Cai Y, Jin J, Swanson SK, Cole MD, Choi SH, Florens L, et al. Subunit composition and substrate specificity of a MCF-containing histone acetyltransferase distinct from the male-specific lethal (MSL) complex. *J Biol Chem*. 2010;285:4268–72.
160. Guelman S, Suganuma T, Florens L, Swanson SK, Kiesecker CL, Kusch T, et al. Host cell factor and an uncharacterized SANT domain protein are stable components of ATAC, a novel dAda2A/dGcn5-containing histone acetyltransferase complex in *Drosophila*. *Mol Cell Biol*. 2006;26:871–82.
161. Kalamvoki M, Roizman B. Circadian CLOCK histone acetyl transferase localizes at ND10 nuclear bodies and enables herpes simplex virus gene expression. *Proc Natl Acad Sci U S A*. 2010;107:17721–6.
162. Tyagi S, Chabes AL, Wysocka J, Herr W. E2F activation of S phase promoters via association with HCF-1 and the MLL family of histone H3K4 methyltransferases. *Mol Cell*. 2007;27:107–19.
163. Whitlow Z, Kristie TM. Recruitment of the transcriptional coactivator HCF-1 to viral immediate-early promoters during initiation of reactivation from latency of herpes simplex virus type 1. *J Virol*. 2009;83:9591–5.
164. Danaher RJ, Jacob RJ, Steiner MR, Allen WR, Hill JM, Miller CS. Histone deacetylase inhibitors induce reactivation of herpes simplex virus type 1 in a latency-associated transcript-independent manner in neuronal cells. *J Neuro-Oncol*. 2005;11:306–17.
165. Shapira L, Ralph M, Tomer E, Cohen S, Kobiler O. Histone deacetylase inhibitors reduce the number of herpes simplex virus-1 genomes initiating expression in individual cells. *Front Microbiol*. 2016;7:1970.
166. Everett RD, Rechter S, Papior P, Tavalai N, Stamminger T, Orr A. PML contributes to a cellular mechanism of repression of herpes simplex virus type 1 infection that is inactivated by KPO. *J Virol*. 2006;80:7995–8005.
167. Tencer AH, Cox KL, Di L, Bridgers JB, Lyu J, Wang X, et al. Covalent modifications of histone H3K9 promote binding of CHD3. *Cell Rep*. 2017;21:455–66.
168. Arbuckle JH, Kristie TM. Epigenetic repression of herpes simplex virus infection by the nucleosome remodeler CHD3. *MBio*. 2014;5:e01027–13.
169. Arbuckle JH, Gardina PJ, Gordon DN, Hickman HD, Yewdell JW, Pierson TC, et al. Inhibitors of the histone methyltransferases EZH2/1 induce a potent antiviral state and suppress infection by diverse viral pathogens. *MBio*. 2017;8(4):e01141–17.
170. Yao H-W, Lin P-H, Shen F-H, Peng G-C, Tung Y-Y, Hsu S-M, et al. Tranylcypromine reduces herpes simplex virus 1 infection in mice. *Antimicrob Agents Chemother*. 2014;58:2807–15.
171. Liang Y, Vogel JL, Narayanan A, Peng H, Kristie TM. Inhibition of the histone demethylase LSD1 blocks alpha-herpesvirus lytic replication and reactivation from latency. *Nat Med*. 2009;15:1312–7.
172. Yang M, Culhane JC, Sawczuk LM, Jallil P, Ball HL, Machius M, et al. Structural basis for the inhibition of the LSD1 histone demethylase by the antidepressant trans-2-phenylcyclopropylamine. *Biochemistry*. 2007;46:8058–65.
173. Fornieri F, Binda C, Vanoni MA, Mattevi A, Battaglioli E. Histone demethylation catalysed by LSD1 is a flavin-dependent oxidative process. *FEBS Lett*. 2005;579:2203–7.
174. Ali AS, Al-Shraim M, Al-Hakami AM, Jones IM. Epstein-Barr virus: clinical and epidemiological revisits and genetic basis of oncogenesis. *Open Virol J*. 2015;9:7–28.
175. Kliszczewska E, Jarzyński A, Boguszewska A, Pasternak J, Polz-Dacewicz M. Epstein-Barr virus—pathogenesis, latency and cancers. *J Pe Clin Clin Res*. 2017;11:142–6.
176. Hurley EA, Thorley-Lawson DA. B cell activation and the establishment of Epstein-Barr virus latency. *J Exp Med*. 1988;168:2059–75.
177. Speck SH, Chatila T, Flemington E. Reactivation of Epstein-Barr virus regulation and function of the BZLF1 gene. *Trends Microbiol*. 1997;5:399–405.
178. Montalvo EA, Cottam M, Hill S, Wang YJ. YY1 binds to and regulates cis-acting negative elements in the Epstein-Barr virus BZLF1 promoter. *J Virol*. 1995;69:4158–65.
179. Yu X, Wang Z, Mertz JE. ZEB1 regulates the latent-lytic switch in infection by Epstein-Barr virus. *PLoS Pathog*. 2007;3:e194.
180. Miller G, El-Guindy A, Countryman J, Ye J, Gradoville L. Lytic cycle switches of oncogenic human gammaherpesviruses. *Adv Cancer Res*. 2007;9:781–109.
181. Murata T, Kondo Y, Sugimoto A, Kawashima D, Saito S, Isomura H, et al. Epigenetic histone modification of Epstein-Barr virus BZLF1 promoter during latency and reactivation in Raji cells. *J Virol*. 2012;86:4752–61.
182. Buslinger M, Tarakhovskiy A. Epigenetic control of immunity. *Cold Spring Harb Perspect Biol*. 2014;6:a019307.

183. Rowe M, Fitzsimmons L, Bell AI. Epstein-Barr virus and Burkitt lymphoma. *Chin J Cancer*. 2014;33:609–19.
184. Chu EA, Wu JM, Tunkel DE, Ishman SL. Nasopharyngeal carcinoma: the role of the Epstein-Barr virus. *Medscape J Med*. 2008;10:165.
185. Flavell KJ, Murray PG. Hodgkin's disease and the Epstein-Barr virus. *Mol Pathol*. 2000;53:262–9.
186. Peabhu SR, Wilson DF. Evidence of EPSTEIN-BARR virus association with head and neck cancers: a review. *J Can Dent Assoc*. 2016;82:g2.
187. Izasa H, Nanbo A, Nishikawa J, Jinushi M, Yoshijama H. Epstein-Barr virus (EBV)-associated gastric carcinoma. *Viruses*. 2012;4:3420–39.
188. Tan T-T, Degenhardt K, Nelson DA, Beaudoin B, Nieves-Neira W, Bouliket P, et al. Key roles of BIM-driven apoptosis in epithelial tumors and rational chemotherapy. *Cancer Cell*. 2005;7:27–38.
189. Shamas-Din A, Kale J, Leber B, Andrews DW. Mechanisms of action of Bcl-2 family proteins. *Cold Spring Harb Perspect Biol*. 2013;5:a00871.
190. Paschos K, Smith P, Anderson E, Middeldorp JM, White RE, Allday MJ. Epstein-Barr virus latency in B cells leads to epigenetic repression and CpG methylation of the tumour suppressor gene *Bim*. *PLoS Pathog*. 2009;5:e1000492.
191. Fitzsimmons L, Boyce AJ, Wei W, Chang C, Croom-Carter D, Tierney RJ, et al. Coordinated repression of *BIM* and *PUMA* by Epstein-Barr virus latent genes maintains the survival of Burkitt lymphoma cells. *Cell Death Differ*. 2018;25:241–54.
192. Choy EY-W, Siu K-L, Kok K-H, Lung RW-M, Tsang CM, To K-F, et al. An Epstein-Barr virus-encoded microRNA targets *PUMA* to promote host cell survival. *J Exp Med*. 2008;205:2551–60.
193. Saha A, Jha HC, Upadhyay SK, Robertson ES. Epigenetic silencing of tumor suppressor genes during in vitro Epstein-Barr virus infection. *Proc Natl Acad Sci U S A*. 2015;112:E5199–207.
194. Richter-Lamea JA, Robles EF, Fresquet V, Beltran E, Rullan AJ, Agirre X, et al. Reversion of epigenetically mediated *BIM* silencing overcomes chemoresistance in Burkitt lymphoma. *Blood*. 2010;116:2531–42.
195. Rasmussen TA, Tolstrup M, Brinkmann CR, Olesen R, Erikstrup C, Solomon A, et al. Panobinostat, a histone deacetylase inhibitor, for latent-virus reactivation in HIV-infected patients on suppressive antiretroviral therapy: a phase 1/2, single group, clinical trial. *Lancet HIV*. 2014;1:e13–21.
196. Archin NM, Kichherr JL, Sung JA, Clutton G, Sholtis K, Xu Y, et al. Interval dosing with the HDAC inhibitor vorinostat effectively reverses HIV latency. *J Clin Invest*. 2017;127:3126–35.
197. Sogaard OS, Graversen ME, Leth S, Olesen R, Brinkmann CR, Nissen SK, et al. The deipeptide romidepsin reverses HIV-1 latency in vivo. *PLoS Pathog*. 2015;11:e1005142.
198. Routy JP, Tremblay CL, Angel JB, Trottier B, Rouleau D, Baril JG, et al. Valproic acid in association with highly active antiretroviral therapy for reducing systemic HIV-1 reservoirs: results from a multicentre randomized clinical study. *HIV Med*. 2012;13:291–6.
199. Xing S, Bullen CK, Shroff NS, Shan L, Yang H-C, Manucci JL, et al. Disulfiram reactivates latent HIV-1 in a Bcl-2-transduced primary CD4+ T cell model without inducing global T cell activation. *J Virol*. 2011;85:6060–4.
200. Yeo W, Chung HC, Chan SL, Wang LZ, Lim R, Picus J, et al. Epigenetic therapy using belinostat for patients with unresectable hepatocellular carcinoma: a multicenter phase III study with biomarker and pharmacokinetic analysis of tumors from patients in the Mayo Phase II Consortium and the Cancer Therapeutics Research Group. *J Clin Oncol*. 2012;30:3361–7.
201. Younes A, Ok J, Bociek RG, Kuruvilla J, Fanale M, Neelapu S, et al. Phase II study of mocetinostat (MGCD0103) in patients with relapsed and refractory classical Hodgkin lymphoma. *Lancet Oncol*. 2011;12:1222–8.
202. Ghosh SK, Perrine SP, Williams RM, Faller DV. Histone deacetylase inhibitors are potent inducers of gene expression in latent EBV and sensitize lymphoma cells to nucleoside antiviral agents. *Blood*. 2012;119:1008–17.
203. Perrine SP, Hermine O, Small T, Suarez F, O'Reilly R, Boulad F, et al. A phase 1/2 trial of arginine butyrate and ganciclovir in patients with Epstein-Barr virus-associated lymphoid malignancies. *Blood*. 2007;109:2571–8.
204. Chan ATC, Tao Q, Robertson KD, Flinn IW, Mann RB, Klencke B, et al. Azacitidine induces demethylation of the Epstein-Barr virus genome in tumors. *JCO*. 2004;22:1373–81.
205. Ritchie D, Piekarz RL, Bomberg P, Karai LI, Pittaluga S, Jaffe ES, et al. Reactivation of DNA viruses in association with histone deacetylase inhibitor therapy: a case series report. *Haematologica*. 2009;94:1618–22.
206. Zhou L, He X, Gao B, Xiong S. Inhibition of histone deacetylase activity aggravates coxsackievirus B3-induced myocarditis by promoting viral replication and myocardial apoptosis. *J Virol*. 2015;89:10512–23.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions



6. Etude de la réactivation virale dans les lymphocytes T CD4+ quiescents de patients avirémiques.

6.1. Comparaison des inhibiteurs de protéase et des inhibiteurs non nucléosidiques de la reverse transcriptase sur la réactivation virale dans les lymphocytes T CD4+ de patients infectées.

Nous avons inclus dans cette étude 47 patients infectés de manière chronique par le VIH et traités par cART, avec une virémie plasmatique sous le seuil de détection (<40 copies/mL). Les patients ont été répartis dans deux groupes : 24 patients ont été inclus dans le groupe « traités par inhibiteurs de protéase » (PI) et 23 patients ont été inclus dans le groupe « traités par inhibiteurs de la transcriptase inverse » (NNRTI).

Nous n'avons pas observé de différences significatives entre les deux groupes de patients pour le compte de lymphocytes T CD4+ au nadir, à l'initiation du traitement et au dernier point mesuré. Nous n'avons pas non plus observé de différences significatives pour la charge virale maximale, pour le nombre d'échecs de traitement, pour la durée de thérapie et pour la durée sans virémie détectable.

6.2. Impact du type de traitement sur la réactivation virale

Nous avons mesuré les niveaux de réactivation virale dans les lymphocytes T CD4+ quiescents de patients traités par thérapie cART. Les lymphocytes ont été purifiés par sélection négative en utilisant des billes magnétiques pour ne conserver que les lymphocytes T CD4+ CD25- CD69- HLA-DR-. La mesure des niveaux de réactivation a été réalisée après stimulation des lymphocytes purifiés par une combinaison d'anticorps anti-CD2 et anti-CD28. Six jours après stimulation, la quantité d'ARN viral a été mesurée.

Nous avons observé une réduction dans la quantité d'ARN viral mesuré après réactivation d'environ 1 log (0.92 log copies/mL) pour les lymphocytes de patients traités par PI (3.01 log copies/mL) par rapport aux lymphocytes de patients traités par NNRTI (3.93 log copies/mL).

6.3. Impact du type de traitement sur la taille du réservoir viral intégré

Nous avons quantifié la quantité d'ADN proviral intégré au génome cellulaire par PCR en nid Alu-Gag. Cette méthode permet l'amplification des provirus intégrés uniquement, par l'utilisation d'un primer reconnaissant le motif Alu présent dans le génome humain.

Nous avons observé une réduction (0.89 log copies/10⁶ cellules) de la quantité d'ADN proviral intégré dans les lymphocytes de patients par PI (2.09 log copies/10⁶ cellules) par rapport aux lymphocytes de patients traités par NNRTI (2.98 log copies/10⁶ cellules).

6.4. Discussion

Les résultats que nous avons obtenus semblent montrer que l'utilisation de PI pour le traitement de l'infection par le VIH limite la réactivation virale chez les patients avirémiques, par rapport à l'utilisation de NNRTI. Ces résultats semblent également montrer une réduction de la taille du réservoir viral intégré chez les patients traités par PI, par rapport aux patients traités par NNRTI

Premièrement, la réduction des niveaux de réactivation virale lors du traitement par les inhibiteurs de protéase peut être expliquée par plusieurs mécanismes. La réduction de la quantité d'ADN viral intégré observée sous inhibiteurs de protéase réduit le nombre génomes viraux intégrés dans des sites transcriptionnellement actifs et donc réactivables. L'inhibition des protéases pourrait impacter l'intégrase virale, qui est le produit d'un clivage protéasique du précurseur Pr160-Gag-Pol. Cela pourrait entraîner une réduction de l'intégration du génome viral et l'expansion du réservoir de latence.

Par ailleurs, il a été montré que les inhibiteurs de protéase réduisent l'activation cellulaire d'Akt [313,314]. Akt étant essentielle à la survie cellulaire, son blocage pourrait entraîner une augmentation de la mort cellulaire des lymphocytes T CD4+ infectées, réduisant ainsi la taille du réservoir viral [182,315]. Akt participe également à l'activation de la voie NFκB, qui est impliqué dans l'intégration et la réactivation du provirus latent [185].

Cette étude a fait l'objet d'une publication dans la revue Scientific Reports en 2016.

6.5. Article paru dans la revue Scientific Reports en 2016 « Limited HIV-1 Reactivation in Resting CD4 + T cells from Aviremic Patients under Protease Inhibitors »

SCIENTIFIC REPORTS

OPEN

Limited HIV-1 Reactivation in Resting CD4⁺ T cells from Aviremic Patients under Protease Inhibitors

Received: 20 September 2016
Accepted: 07 November 2016
Published: 06 December 2016

Amit Kumar^{1,*}, Wasim Abbas^{1,*}, Sophie Bouchat², Jean-Stéphane Gatot^{2,†}, Sébastien Pasquereau¹, Kabamba Kabeya³, Nathan Clumeck³, Stéphane De Wit³, Carine Van Lint^{2,*} & Georges Herbein^{1,*}

A latent viral reservoir that resides in resting CD4⁺ T cells represents a major barrier for eradication of HIV infection. We test here the impact of HIV protease inhibitor (PI) based combination anti-retroviral therapy (cART) over nonnucleoside reverse transcriptase inhibitor (NNRTI)-based cART on HIV-1 reactivation and integration in resting CD4⁺ T cells. This is a prospective cohort study of patients with chronic HIV-1 infection treated with conventional cART with an undetectable viremia. We performed a seven-year study of 47 patients with chronic HIV-infection treated with cART regimens and with undetectable plasma HIV-1 RNA levels for at least 1 year. Of these 47 patients treated with cART, 24 were treated with a PI-based regimen and 23 with a NNRTI-based regimen as their most recent treatment for more than one year. We evaluated the HIV-1 reservoir using reactivation assay and integrated HIV-1 DNA, respectively, in resting CD4⁺ T cells. Resting CD4⁺ T cells isolated from PI-treated patients compared to NNRTI-treated patients showed a limited HIV-1 reactivation upon T-cell stimulation ($p = 0.024$) and a lower level of HIV-1 integration ($p = 0.024$). Our study indicates that PI-based cART could be more efficient than NNRTI-based cART for limiting HIV-1 reactivation in aviremic chronically infected patients.

Among the main features of human immunodeficiency virus-1 (HIV-1) infection are immune suppression and viral persistence¹. Combination anti-retroviral therapy (cART) drives the viral load down to undetectable levels². However, using the ultrasensitive assays, low levels of active viral replication can be detected in the majority of subjects successfully treated with cART³. Indeed, the persistence of latent reservoirs of replication-competent proviruses remains a major obstacle in HIV-1 eradication^{4,5}. Latent reservoirs are established early during acute viral infection and include among others, macrophages and latently infected resting CD4⁺ T cells, these later being the main viral reservoir⁶⁻⁸.

Most of the studies so far have addressed the effect of cART on the decrease of HIV-1 viremia under limit of detection using the classical assays. Usually, a better virologic performance of nonnucleoside reverse transcriptase inhibitor (NNRTI)-based cART compared to protease inhibitor (PI)-including regimens has been reported⁹. Although PI-based regimen have lower rates of HIV suppression compared with the NNRTI-based treatments, greater CD4 cell increases are seen in patients on PI arm and there is also less development of major drug resistance mutations in patients failing PI-based therapy^{10,11}. Recently, cART intensification was assessed and did not reduce residual HIV-1 viremia in patients on cART, indicating that its potential to eradicate the virus appears limited¹².

In contrast to the measurement of viremia in patients on cART, the impact of cART on the size of the cellular reservoirs of HIV-1 has been much less studied. Initiation of cART during primary HIV infection may limit the establishment of viral reservoirs, and very early cART limits the seeding of the HIV reservoir in long-lived central memory CD4⁺ T cells^{6,13,14}. By contrast, the impact of cART on the HIV reservoir especially on viral reactivation

¹Department of Virology, Pathogens & Inflammation Laboratory, University of Franche-Comté, COMUE Bourgogne Franche-Comté University, UPRES EA4266, SFR FED 4234, CHRU Besançon, France. ²Laboratory of Molecular Virology, IBMM, Université Libre de Bruxelles (ULB), Gosselies, Belgium. ³Department of Infectious Diseases, CHU St-Pierre, ULB, Bruxelles, Belgium. *Present address: Service de Génétique, Centre Hospitalier Universitaire (CHU) de Liège, Domaine Universitaire du Sart-Tilman, B23, 4000 Liège, Belgium. †These authors contributed equally to this work. Correspondence and requests for materials should be addressed to G.H. (email: georges.herbein@univ-fcomte.fr) or C.V.L. (email: cvlint@ulb.ac.be)

Biological characteristics	PI	NNRTI	P value
	(n = 24)	(n = 23)	
Age (years), mean (\pm s.d.)	48.6 \pm 12.7	46.2 \pm 10.7	0.495
CD4 ⁺ T cell counts at nadir, absolute (cells per μ l), median (IQR)	298 (201–375)	333 (246–419)	0.579
CD4 ⁺ T cell counts at initiation of treatment, absolute (cells per μ l), median (IQR)	384 (285–461)	349 (265–574)	0.903
CD4 ⁺ T cell counts at the last point, absolute (cells per μ l), median (IQR)	607 (480–832)	624 (492–801)	0.647
pVL at zenith (log copies per ml), median (IQR)	4.92 (4.23–5.07)	4.83 (4.47–5.16)	0.892
Previous treatment failure, median (IQR)	1.00 (0–2.25)	1.00 (0–4)	0.985
Time with therapy (years), mean \pm s.d.	8.50 \pm 3.63	8.52 \pm 3.49	0.936
Time with undetectable pVL (years), mean \pm s.d.	4.83 \pm 2.97	5.39 \pm 2.73	0.581
HIV-1 reactivation assay			
Resting CD4 ⁺ T cells	(n = 10)	(n = 11)	
HIV-1 RNA levels after reactivation assay (log copies per ml), median (IQR)	3.01 (1.84–3.45)	3.93 (3.31–4.87)	0.024*
HIV-1 integration assay			
Resting CD4 ⁺ T cells	(n = 14)	(n = 12)	
Integrated HIV-1 DNA (log copies per 10 ⁶ cells), median (IQR)	2.09 (1.53–2.52)	2.98 (2.55–3.48)	0.024*

Table 1. Comparison of biological and virological characteristics of HIV⁺ patient groups under PI-cART and NNRTI-cART. IQR, interquartile range; pVL, plasma viral load * $P < 0.05$.

from resting CD4⁺ T cells in aviremic chronically infected patients is so far unknown. We report here a study indicating a higher efficiency of PI-based cART over NNRTI-based cART for limiting HIV-1 reactivation in CD4⁺ T cells from aviremic chronically HIV-1 infected patients.

Results

Forty-seven patients with chronic HIV-1 infection treated with cART (treatment range: 2 years to 16 years) and with undetectable plasma HIV-1 RNA levels (<40 copies/ml) for at least 1 year were included in the study between 2008 and 2014. Of these 47 patients (mean age 47.4 years; range 27–93 years) treated with cART, 24 were treated with PI-based cART and 23 with NNRTI-based cART (as their most recent treatment) for more than one year (Table 1, Supplementary Tables S1 and S2). We did not observe significant differences for nadir median CD4 counts (298·10⁶ versus 333·10⁶ cells/l, $P = 0.579$), median CD4 counts at initiation of treatment (384·10⁶ versus 349·10⁶ cells/l, $P = 0.903$), median CD4 counts at the last point (607·10⁶ versus 624·10⁶ cells/l, $P = 0.647$), and median HIV RNA load at zenith (4.92 versus 4.83 log/ml, $P = 0.892$) between PI-treated and NNRTI-treated patients (Table 1). We did not find significant differences between PI-treated and NNRTI-treated patients for the number of failures of treatment (1 versus 1, $P = 0.985$) and the duration of therapy (8.50 versus 8.52 years, $P = 0.936$). The duration with undetectable plasma HIV-1 RNA levels was not significantly different in the PI-treated arm compared to the NNRTI-treated arm (4.83 versus 5.39 years, $P = 0.581$) (Table 1). At the last treatment, in 51.1% of individuals cART included a PI (fosamprenavir; saquinavir; atazanavir; lopinavir/ritonavir; darunavir) and in 48.9% a NNRTI (nevirapine; efavirenz; rilpivirine). Of note, among the 47 patients treated with cART, 17 were treated only with PI and 7 only with NNRTI (Supplementary Tables S1 and S2).

In order to evaluate the qualitative and quantitative patient-specific variations in the *ex vivo* reactivation capacity of the HIV-infected cells depending of cART treatment, we analyzed the effect of a known HIV inducer (anti-CD2+anti-CD28 antibodies)^{15,16} on viral reactivation in *ex vivo* cultures of purified resting CD4⁺ T cells isolated from HIV⁺ patients treated with PI-based cART and with NNRTI-based cART. Since latently infected resting CD4⁺ T cells that harbour integrated replication-competent viral DNA represent the primary long-lived source of persistent HIV-1 in patients under cART^{5,6}, we decided to analyze the impact of cART regimen on resting CD4⁺ T cells isolated from HIV⁺ patients. We observed, following reactivation of HIV-1 from latency in purified resting CD4⁺ T cells, that HIV-1 recovery was 0.92 log of HIV RNA copies/ml (3.01 versus 3.93 log copies/ml, $P = 0.024$) lower in HIV-1-infected patients treated with PI compared to those treated with NNRTI as their lastly administered treatment (Tables 1 and 2, and Fig. 1A). The impact of PI versus NNRTI treatment, taking into account the last treatment administered, on latently infected CD4⁺ T cells was further assessed by quantifying integrated HIV-1 DNA. The levels of integrated HIV-1 DNA was 0.89 log (2.09 versus 2.98 log copies/10⁶ cells, $P = 0.024$) lower in purified resting CD4⁺ T cells isolated from HIV-1 patients treated with PI-based cART compared to HIV-1-infected patients treated with NNRTI-based cART (Tables 1 and 3, and Fig. 1B).

Discussion

To diminish the size of the viral reservoir, several studies have shown that an early treatment during HIV-1 primo-infection could be beneficial^{13,14}, and recently on-demand preexposure prophylaxis has been shown to be highly efficient to prevent HIV transmission¹⁷. We report here a study indicating that PI-based cART could be more efficient than NNRTI-based cART for limiting HIV-1 reactivation in aviremic chronically infected patients.

Although the respective use of NNRTI-based cART and PI-based cART have been extensively studied in regard to residual viremia, only little is known on their impact on cellular reservoirs of HIV-1. By two approaches, reactivation assay and the Alu-PCR assay that allow evaluating in a qualitative and quantitative manner, respectively, the amount of integrated provirus, we observed that PI-based cART was superior to NNRTI-based cART for limiting the size of the HIV-1 reservoir. Recent studies indicate that raltegravir intensification of PI-based

Patients	CD4+ at last point (cells/microl)	Last treatment	Duration of therapy (years)	Duration with undetectable plasma HIV-1 RNA levels (years)	HIV-1 RNA levels after reactivation assay (copies/ml)	HIV-1 RNA levels after reactivation assay (log copies/ml)
PI at the last treatment (n = 10)						
WSH 229	617	ABC 3TC SQV	13	12	25862	4.41
WSH 231	770	ABC 3TC ATV	10	7	40	1.60
WSH 232	899	TDF FTC IAPV	7	1	3106	3.49
WSH 233	465	ATV EFV	8	8	40	1.60
WSH 235	797	DDI AZT 3TC IAPV	15	5	1108	3.04
WSH 236	402	TDF FTC ATV	13	1	2862	3.46
WSH 238	1240	TDF FTC ATV	5	5	40	1.60
WSH 239	999	TDF FTC ATV	9	1	954	2.98
WSH 240	663	TDF FTC ATV	9	4	2548	3.41
WSH 241	524	TDF FTC IAPV	2	2	372	2.57
Mean	738		9.10	4.60	3693	2.82
SD	246		3.73	3.44	7477	0.91
Median	717		9.00	4.50	1031	3.01
1st Quartile	547		7.25	1.25	123	1.84
3rd Quartile	874		12.3	6.50	2784	3.45
NNRTI at the last treatment (n = 11)						
WSH 227	733	EFV FTC TDF	9	8	146	2.16
WSH 228	1324	TDF FTC NVP	10	7	229674	5.36
WSH 230	444	EFV FTC TDF	8	1	8492	3.93
WSH 242	428	DDI TDF ABC 3TC EFV	10	3	1668	3.22
WSH 243	794	EFV FTC TDF	6	1	2514	3.40
WSH 244	479	EFV FTC TDF	8	8	122268	5.09
WSH 245	478	TDF FTC NVP	12	3	43614	4.64
WSH 246	629	EFV FTC TDF	3	1	374578	5.57
WSH 247	498	ABC 3TC NVP	16	7	31976	4.50
WSH 248	1017	ABC 3TC EFV	10	7	4224	3.63
WSH 249	519	AZT 3TC NVP	13	11	668	2.82
Mean	668		9.55	5.18	74529	4.03
SD	270		3.31	3.33	116748	1.05
Median	519		10.0	7.00	8492	3.93
1st Quartile	479		8.00	2.00	2091	3.31
3rd Quartile	764		11.0	7.50	82941	4.87
MW p-value	0.461		0.768	0.684	0.024	0.024

Table 2. Reactivation assay on HLA DR⁻ CD25⁻ CD69⁻ CD4⁺ T cells (resting CD4⁺ T cells) isolated from HIV⁺ patients with undetectable viremia treated with PI (n = 10) and NNRTI (n = 11) as their lastly administered treatment.

cART results in a specific and transient increase in episomal HIV DNA that might represent lower efficiency of HIV integration under PI^{18,19}. Recent publications also indicate that the size of the HIV-1 reservoir measured by total cell-associated HIV-1 DNA correlates positively with the frequency of positive recovery measurements in response to various latency-reversing agents²⁰. Additionally, the low frequency of infected cells in the HIV-1

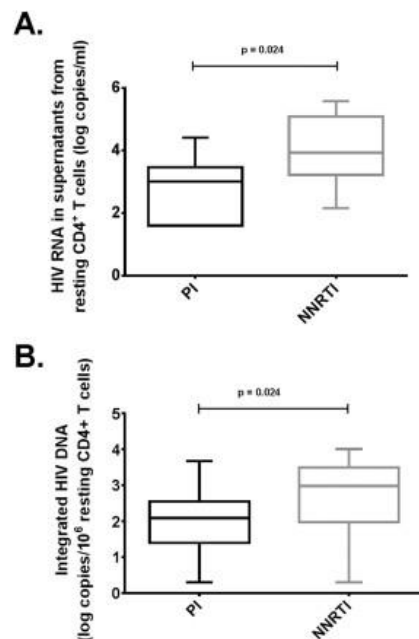


Figure 1. Resting CD4⁺ T cells isolated from PI-treated patients compared to NNRTI-treated patients showed a limited HIV-1 reactivation upon T-cell stimulation and a lower level of HIV-1 integration. (A) Comparison of reactivation assay on resting CD4⁺ T cells isolated from HIV⁺ patients with undetectable viremia treated with PI (n = 10) and NNRTI (n = 11) as their last administered treatment. (B) Comparison of measurement of integrated HIV-1 DNA in resting CD4⁺ T cells isolated from HIV⁺ patients with undetectable viremia treated with PI (n = 14) and NNRTI (n = 12) as their last administered treatment.

controllers is associated with less efficient viral reactivation²¹. Our data are in line with these findings indicating that limited reactivation could be a consequence of lower integration events.

Several molecular mechanisms could explain the lower viral reactivation under PIs. First, low frequency of viral integration in transcriptionally active sites could explain the limited reactivation observed under PIs. Second, since integrase is produced by protease-mediated cleavage of the Pr160^{Gag-Pol} precursor protein, PIs might inhibit HIV-1 integration by preventing the formation of a functional integrase¹⁹. Impaired integrase activity and/or impairment of the integrase-LEDGF/p75 interaction could result in a decreased number of integrated provirus in transcriptionally active sites, but also could favor the viral integration in less transcriptionally active sites which will result in lower viral reactivation²². Third, HIV PIs have been reported to block Akt activation²³, and we recently observed that PIs, by blocking Akt activation especially triggered by Nef, limit HIV-1 recovery from latently-infected T cells²⁴. Additionally HIV integration and the establishment of latency in CCL19-treated resting CD4⁺ T cells has been shown to require activation of NF- κ B and increased HIV integrase stability²⁵. Interestingly, PIs block NF- κ B activation induced by Toll-like receptor 2 (TLR2) and TLR4²⁶. We also observed that PIs, but not NNRTIs, block Akt activation in PBLs treated with recombinant HIV-1 Nef *in vitro* and that the blockade of Nef-mediated Akt activation by Akt inhibitors decreased dramatically NF- κ B activation²⁴. Therefore, we cannot exclude that PIs by blocking HIV-mediated Akt activation in T cells decrease NF- κ B activation and ultimately limit HIV integration in T cells.

Since Akt activation is critical for cell survival, the blockade of Akt activation by PIs in latently infected resting CD4⁺ T cells could help to clear these cells through accelerated cell death. Additionally, PIs have been shown to trigger the death of CD4⁺ T cells through the generation of Casp8p41, a fragment obtained by cleavage of pro-caspase 8 by PIs, that lacks caspase activity but nonetheless contributes to T cell apoptosis²⁷. Thus PIs could participate to decrease the size of the viral reservoir in infected patients through the clearance of infected CD4⁺ T cells.

Low HIV-1 reservoirs found in HIV-1 controllers contributes to temporary control of infection without antiretroviral therapy²¹. Along this line, control of HIV-1 viremia or delayed viral rebound after discontinuation of antiretroviral treatment has been consistently associated with low levels of cell associated HIV-DNA at the time of treatment interruption^{28,29}. Therefore, lower levels of integrated HIV-1 in purified resting CD4⁺ T cells isolated from HIV-1 patients treated with PI-based cART compared to HIV-1-infected patients treated with NNRTI-based cART could be critical to achieve control of infection.

Patients	CD4+ at the last point (cells/microl)	Last treatment	Duration of therapy (years)	Duration with undetectable plasma HIV-1 RNA levels (years)	Integrated HIV-1 DNA (copies/10 ⁶ cells)	Integrated HIV-1 DNA (log copies/10 ⁶ cells)
PI at the last treatment (n = 14)						
B9	485	TDF FTC ATV	11	8	95	1.97
B13	512	ABC 3TC ATV	14	8	85	1.92
B15	995	TDF FTC ATV	12	8	4731	3.67
B27	529	ABC 3TC ATV	3	2	4	0.6
B33	404	TDF FTC DRV	8	2	175	2.24
B34	127	TDF FTC DRV	2	1	17	1.23
B38	423	TDF FTC ATV	8	5	2	0.3
B39	997	ABC 3TC DRV	8	3	409	2.61
B40	394	IAPV AZT 3TC	8	5	56	1.74
B41	596	TDF FTC DRV	3	2	29	1.46
B42	638	DRV TDF ABC 3TC	8	5	307	2.48
B43	505	ABC 3TC ATV	10	8	163	2.21
B45	809	ABC TDF DRV	12	8	344	2.53
B46	906	ABC 3TC ATV	6	5	635	2.8
Mean	594		8.07	5.00	504	1.98
SD	242		3.49	2.56	1186	0.85
Median	521		8.00	5.00	129	2.09
1st Quartile	439		6.50	2.25	35.8	1.53
3rd Quartile	766		10.8	8.00	335	2.52
NNRTI at the last treatment (n = 12)						
B2	548	EFV FTC TDF	5	4	6174	3.79
B3	927	ABC 3TC NVP	7	5	2	0.3
B6	479	NVP TDF FTC	8	7	682	2.83
B8	975	EFV FTC TDF	3	2	985	2.99
B10	580	EFV TDF FTC	6	5	2963	3.47
B14	486	NVP TDF FTC	5	4	944	2.97
B16	928	TDF FTC NVP	15	8	2879	3.46
B18	624	TDF FTC RPV	5	4	10218	4.01
B21	607	TDF FTC RPV	12	8	49	1.69
B22	793	TDF FTC NVP	5	4	38	1.58
B29	807	TDF FTC RPV	10	8	3185	3.5
B37	790	TDF FTC RPV	10	8	771	2.88
Mean	712		7.58	5.58	2408	2.79
SD	171		3.38	2.02	2931	1.04
Median	707		6.50	5.00	965	2.98
1st Quartile	572		5.00	4.00	524	2.55
3rd Quartile	837		10.0	8.00	3019	3.48
MW p-value	0.210		0.583	0.719	0.024	0.024

Table 3. Measurement of integrated HIV-1 DNA in HLA DR⁻ CD25⁻ CD69⁻ CD4⁺ T cells (resting CD4⁺ T cells) isolated from HIV⁺ patients with undetectable viremia treated with PI (n = 14) and NNRTI (n = 12) as their lastly administered treatment.

In conclusion, in aviremic chronically HIV-1-infected patients our study indicates that PI-based cART limits HIV-1 recovery from latently infected resting CD4⁺ T cells and curtails the amount of integrated HIV-1 DNA. Altogether, our results support the need to further assess the therapeutic use of HIV-1 protease inhibitors in view of their impact on persistence of HIV-1 reservoirs and might provide and/or accompany new therapeutic strategies for a remission.

Methods

Ethics approval and consent to participate. All the patients who were enrolled at the St-Pierre University Hospital (Brussels, Belgium) and the Besançon University Hospital (France) gave their written informed consent to participate in the study according to the Helsinki declaration. The study was approved by the local ethics committees of the Saint-Pierre University Hospital (Brussels, Belgium) and the Besançon University Hospital (Besançon, France) (CPP EST-2). To perform our study, we used blood samples that were part of a routine care. It was an observational study and not a clinical trial. According to the French Regulatory Authority for clinical studies, prospective and retrospective studies with observation analysis only are not evaluated by Human Protection Committees. The Human Protection Committee East Area II from France was consulted and issued a

formal waiver of approval. This study did not rely solely on medical records. The authors did not have any contact with the study subjects and performed tests on patient blood samples that were part of a routine care. The blood samples were anonymized before being used by the authors.

Patients. We enrolled 47 chronically HIV-1 infected individuals at the St-Pierre Hospital (Brussels, Belgium) and the Besançon University Hospital (Besançon, France). The patients were treated with cART (treatment range: 2 years to 16 years) and had undetectable plasma HIV-1 RNA levels (<40 copies/ml) for at least 1 year. Characteristics of these patients were well documented and presented in Supplementary Tables S1 and S2. Of these 47 patients treated with cART, 24 were treated with PI and 23 with NNRTI as their most recent treatment for more than one year.

Isolation of resting CD4⁺ T lymphocytes. Purified CD25⁻, CD69⁻, HLA-DR⁻ CD4⁺ T cells (resting CD4⁺ T cells) were obtained from the peripheral blood of HIV⁺ patients using a negative selection assay using magnetic beads (LD columns, Miltenyi Biotec, Bergisch Gladbach, Germany) as described previously³⁰.

Viral reactivation assay. One day after isolation, 5×10^5 purified CD25⁻, CD69⁻, HLA-DR⁻ CD4⁺ T cells (resting CD4⁺ T cells) were mock-treated or treated with anti-CD2+ anti-CD28 antibodies as a positive control for global T-cell activation^{15,36}. For the measurement of reactivation of HIV-1 expression in resting CD4⁺ T cells isolated from the peripheral blood of HIV-infected patients, six days after treatment, culture supernatants were tested for quantitative HIV-1 RNA levels using the COBAS AmpliPrep/COBAS Amplicor HIV-1 Monitor Test, according to the manufacturer's instructions.

Quantification of integrated HIV-1 DNA. Integrated HIV-1 DNA was measured by using a previously described *Alu-gag* PCR with some modifications³¹. This assay detects only integrated provirus because it relies on initial amplification in which one primer hybridizes with conserved sequences in *Alu* elements that are present in the human genome. The level of HIV-1 integration was measured by comparing the detection signal to an integration standard curve that was prepared from U1 cells. ACH2 cells were used for calibration. The sequences of first step PCR amplification primers were as follows: genomic *Alu* forward, 5'-GCCTCCCAAAGTCTGGGATTACAG-3'; and HIV-1 gag reverse, 5'-GCTCTCGCACCCATCTCTCTCC-3'. The reaction conditions were 1x Tfi PCR reaction buffer, 1.5 mM MgCl₂, 1 mM dNTPs, 100 nM *Alu* forward primer, 600 nM gag reverse primer and 5 U of Tfi DNA polymerase (Invitrogen). The thermal cycler (Eppendorf AG, Hamburg, Germany) was programmed to perform a 2 min hot start at 94 °C, followed by 20 cycles of following: denaturation at 94 °C for 30 sec, annealing at 50 °C for 1 min, and extension at 72 °C for 1 min 40 sec. The second round of real time quantitative PCR was performed using 21 µl of Nested *Alu-gag* PCR along with standard curve amplification products. The sequences of the primers were as follows: LTR forward, 5'-GCCCAATAAAGCTTGCCTTGA-3'; and LTR reverse, 5'-TCCACACTGACTAAAAGGGTCTGA-3'. The LTR Taqman probe, labeled at its 5' terminus with the reporter fluorophore 6-carboxyfluorescein (FAM) and at its 3' terminus with the quencher 4-(4-dimethylamino-phenylazo)-benzene (DABCYL), had the following sequence: 5'-FAM-GCGAGTGCCCGTCTGTGTGTGACTCTGGTAACTAGTCGC-DABCYL-3'. The reaction was carried out in a volume of 50 µl containing 1 X Taqman Master mix (Applied Biosystems, Foster City, CA), 250 nM concentration of LTR forward and reverse primers, and 200 nM Taqman probe. The reactions were performed on Stratagene Mx2005 P. The thermal program was 2 min hot start at 95 °C, followed by 40 cycles of denaturation at 95 °C for 15 sec and annealing and extension at 60 °C for 1 min.

Data analysis. The program used for plotting and statistical analysis was Prism version 6.0 (GraphPad Software). Results from *ex vivo* reactivation studies and HIV integration using patient cell cultures of resting CD4⁺ T cells are shown as medians. Data sets were analyzed using an unpaired nonparametric t-test (Mann-Whitney test). Differences were considered significant at a value of $p < 0.05$.

References

- Chun, T. W., Moir, S. & Fauci, A. S. HIV reservoirs as obstacles and opportunities for an HIV cure. *Nat. Immunol.* **16**, 584–589 (2015).
- Perelson, A. S. *et al.* Decay characteristics of HIV-1-infected compartments during combination therapy. *Nature* **387**, 188–191 (1997).
- Palmer, S. *et al.* Low-level viremia persists for at least 7 years in patients on suppressive antiretroviral therapy. *Proc. Natl. Acad. Sci. USA.* **105**, 3879–3884 (2008).
- Ho, Y. C. *et al.* Replication-competent noninduced proviruses in the latent reservoir increase barrier to HIV-1 cure. *Cell* **155**, 540–551 (2013).
- Van Lint, C., Bouchat, S. & Marcello, A. HIV-1 transcription and latency: an update. *Retrovirology* **10**, 67 (2013).
- Siliciano, R. F. Opening fronts in HIV vaccine development: targeting reservoirs to clear and cure. *Nat. Med.* **20**, 480–481 (2014).
- Le Douce, V. *et al.* Molecular mechanisms of HIV-1 persistence in the monocyte-macrophage lineage. *Retrovirology* **7**, 32 (2010).
- Kumar, A., Darcis, G., Van Lint, C. & Herbein, G. Epigenetic control of HIV-1 post integration latency: implications for therapy. *Clin. Epigenetics* **7**, 103 (2015).
- INITIO Trial International Co-ordinating Committee *et al.* Virological and immunological outcomes at 3 years after starting antiretroviral therapy with regimens containing non-nucleoside reverse transcriptase inhibitor, protease inhibitor, or both in INITIO: open-label randomised trial. *Lancet* **368**, 287–298 (2006).
- Cummins, N. W., Saini, A. M., Nate Sampillai, S., Bren, G. D. & Badley, A. D. Choice of antiretroviral therapy differentially impacts survival of HIV-infected CD4 T cells. *Mol. Cell. Ther.* **2**, 1 (2014).
- Cheret, A. *et al.* Combined ART started during acute HIV infection protects central memory CD4⁺ T cells and can induce remission. *J. Antimicrob. Chemother.* **70**, 2108–2120 (2015).
- Dinso, J. B. *et al.* Treatment intensification does not reduce residual HIV-1 viremia in patients on highly active antiretroviral therapy. *Proc. Natl. Acad. Sci. USA.* **106**, 9403–9408 (2009).

13. Sáez-Cirión, A. *et al.* Post-treatment HIV-1 controllers with a long-term virological remission after the interruption of early initiated antiretroviral therapy ANRS VISCONTI study. *Plos Pathog.* **9**, e1003211 (2013).
14. Buzón, M. J. *et al.* HIV-1 replication and immune dynamics are affected by raltegravir intensification of HAART-suppressed subjects. *Nat. Med.* **16**, 460–465 (2010).
15. Costello, R. *et al.* Activation of primary human T-lymphocytes through CD2 plus CD28 adhesion molecules induces long-term nuclear expression of NF- κ B. *Cell Growth Differ.* **4**, 329–339 (1993).
16. Pierrès, A. *et al.* Triggering CD28 molecules synergize with CD2 (T11.1 and T11.2)-mediated T cell activation. *Eur. J. Immunol.* **18**, 685–690 (1988).
17. Molina, J. M. *et al.* On-demand preexposure prophylaxis in men at high risk for HIV-1 infection. *N. Engl. J. Med.* **373**, 2237–2246 (2015).
18. Hatano, H. *et al.* Increase in 2-long terminal repeat circles and decrease in D-dimer after raltegravir intensification in patients with treated HIV infection: a randomized, placebo-controlled trial. *J. Infect. Dis.* **208**, 1436–1442 (2013).
19. Rabi, S. A. *et al.* Multi-step inhibition explains HIV-1 protease inhibitor pharmacodynamics and resistance. *J. Clin. Invest.* **123**, 3848–3860 (2013).
20. Darcis, G. *et al.* Reactivation capacity by latency-reversing agents correlates with the size of the HIV-1 reservoir. *AIDS* PMID: 27755105 (2016).
21. Noel, N. *et al.* Long-term spontaneous control of HIV-1 relates to low frequency of infected cells and inefficient viral reactivation. *J. Virol.* **90**, 6148–6158 (2016).
22. Vranckx, L. S. *et al.* LEDGIN-mediated inhibition of integrase-LEDGF/p75 interaction reduces reactivation of residual latent HIV. *EBioMedicine* **8**, 248–264 (2016).
23. Kourjian, G. *et al.* HIV protease inhibitor-induced cathepsin modulation alters antigen processing and cross-presentation. *J. Immunol.* **196**, 3595–3607 (2016).
24. Kumar, A. *et al.* Fine tuning of AKT-pathway by Nef and its blockade by protease inhibitors results in limited recovery in latently HIV infected T-cell line. *Sci Rep.* **6**, 24090 (2016).
25. Saleh, S. *et al.* HIV integration and the establishment of latency in CCL19-treated resting CD4⁺ T cells require activation of NF- κ B. *Retrovirology* **13**, 49 (2016).
26. Equils, O. *et al.* Human immunodeficiency virus type 1 protease inhibitors block Toll-like receptor 2 (TLR2)- and TLR4-induced NF- κ B activation. *Antimicrob. Agents Chemother.* **48**, 3905–3911 (2004).
27. Sainki, A. M. *et al.* Casp8p1 generated by HIV protease kill CD4 T cells through direct Bak activation. *J Cell Biol* **206**, 867–876 (2014).
28. Assoumou, L. *et al.* A low HIV-DNA level in peripheral blood mononuclear cells at antiretroviral treatment interruption predicts a higher probability of maintaining viral control. *AIDS* **29**, 2003–2007 (2015).
29. Goujard, C. *et al.* HIV-1 control after transient antiretroviral treatment initiated in primary infection: role of patient characteristics and effect of therapy. *Antivir. Ther.* **17**, 1001–1009 (2012).
30. Bouchat, S. *et al.* Histone methyltransferase inhibitors induce HIV-1 recovery in resting CD4(+) T cells from HIV-1-infected HAART-treated patients. *AIDS* **26**, 1473–1482 (2012).
31. Liszewski, M. K., Yu, J. J. & O'Doherty, U. Detecting HIV-1 integration by repetitive-sampling Alu-gag PCR. *Methods* **47**, 254–260 (2009).

Acknowledgements

We thank the HIV-1-infected patients for their willingness to participate in this study and Elodie Goudeseune, Joelle Cailleau and Annick Caestecker who cared for the patients. We thank Christelle Cardona, Kashif Khan, Audrey Varin, Laurie Coquard, Caroline Vanhulle, Nadège Delacourt, and Laurence Colin for excellent technical assistance. This work was supported by grants (to GH) from the University of Franche-Comté, the Région Franche-Comté (RECH-FON12-000013), the France Recherche Nord & Sud Sida-HIV Hépatites (ANRS, n°13543 and 13544) and HIVERA 2013 (EURECA project), (to CVL) from the Université Libre de Bruxelles (ULB), the Belgian Fund for Scientific Research (FRS-FNRS, Belgium), the NEAT program (European AIDS treatment network), the "Fondation Roi Baudouin" (Belgium), the Wallon region (CIBLES Excellence programm) and the ANRS (France). W.A. is a recipient of a doctoral scholarship from the Higher Education Commission, Pakistan. A.K. is a recipient of a postdoctoral grant of Région Franche-Comté (N° 2012C-06102). S.B. is fellowship from the Belgian 'Fonds pour la Recherche dans l'Industrie et l'Agriculture' (FRIA). J.S.G. was a fellow of the Excellence program 'Cibles'. C.V.L. is Research Director of the FRS-FNRS. The University of Franche-Comté, the Région Franche-Comté, ANRS, HIVERA 2013, the Université Libre de Bruxelles, FRS-FNRS, NEAT, the "Fondation Roi Baudouin", and the Wallon region; the funders had no role in the data collection, analysis, patient recruitment, or decision to publish.

Author Contributions

G.H. and C.V.L. conceived and designed the project. A.K., W.A., S.B., S.P. and J.S.G. performed experiments. G.H., K.K., N.C. and S.D.W. participated to the enrolment of HIV-infected patients. Quantification of the integrated viral reservoir: W.A. G.H. and C.V.L. wrote the manuscript.


Additional Information

Supplementary information accompanies this paper at <http://www.nature.com/srep>

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Kumar, A. *et al.* Limited HIV-1 Reactivation in Resting CD4⁺ T Cells from Aviremic Patients under Protease Inhibitors. *Sci. Rep.* **6**, 38313; doi:10.1038/srep38313 (2016).

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

 This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>

© The Author(s) 2016

7. Etude de l'activation de la voie de signalisation Akt par Nef et impact sur la réactivation virale

7.1. Nef exogène entre dans les lymphocytes et augmente l'activation d'Akt.

Nous avons observé une augmentation de la phosphorylation d'Akt dans les lymphocytes traités par la protéine du VIH Nef. Nous avons observé une colocalisation de Nef et d'Akt au niveau membranaire et cytoplasmique. La phosphorylation d'Akt observée lors du traitement des cellules par Nef est bloquée par les inhibiteurs de PI3K, indiquant que l'activité de PI3K est nécessaire à l'activation d'Akt par Nef. Nous avons observé une réduction de l'activation d'Akt dans les lymphocytes infectés avec un clone du VIH délété pour le gène Nef, par rapport au VIH de type sauvage.

7.2. Nef active PI3K et Akt par une interaction directe, entraînant l'activation des voies NFκB et du CD28RE.

Nous avons testé l'interaction de Nef avec Akt et avec PI3K. Nous avons observé la co-précipitation d'Akt et de PI3K avec Nef, indiquant une interaction directe de ces protéines. L'utilisation de protéines Nef recombinantes tronquées nous a permis d'observer la co-précipitation de PI3K avec la partie N-terminal de Nef, tandis que la co-précipitation d'Akt a été observée avec la partie C-terminale de Nef.

Nous avons observé une translocation d'Akt dans les radeaux lipidiques lors du traitement par Nef, qui subit également une translocation dans les radeaux lipidiques. Cette translocation est similaire lors de la stimulation du TCR par une combinaison d'anticorps anti-CD3 anti-CD28. La costimulation TCR/CD28 entraîne l'activation de l'élément de réponse au CD28 (CD28RE) et de NFκB. Nous avons observé l'activation par Nef du CD28RE et de NFκB. Cette activation est bloquée par l'inhibiteur d'Akt VIII, indiquant que cette activation implique Akt.

Nous avons mesuré une augmentation de la production d'IL-2 dans les lymphocytes traités par Nef, ainsi qu'une augmentation de leur prolifération. L'utilisation d'inhibiteurs d'Akt réduit la production d'IL-2 et la prolifération cellulaire. L'activation de la production d'IL-2 et de la prolifération est également bloquée par un anticorps neutralisant anti-Nef. Nef semble donc augmenter la production d'IL-2 dans les lymphocytes et la prolifération cellulaire via l'activation d'Akt.

7.3. Les inhibiteurs de protéase limitent l'activation d'Akt par Nef.

Nous avons testé l'effet des PI sur l'activation d'Akt par Nef dans les lymphocytes. Il a été montré précédemment que les PI inhibent l'activation d'Akt et qu'Akt joue un rôle important dans la réactivation virale [186,314]. Les PI réduisent la phosphorylation d'Akt dans les lymphocytes traités par Nef, ce qui n'est pas le cas des NNRTI.

Nous avons mesuré le taux de phosphorylation d'Akt dans des lymphocytes de patients infectés par le VIH et traités par PI, par NNRTI ou naïfs de traitements. L'activation d'Akt est réduite chez les patients traités par PI par rapport aux patients traités par NNRTI. L'activation d'Akt est également plus élevée chez les patients infectés naïfs de traitement par rapport aux patients sains ou traités par PI.

Nous avons également mesuré la production d'IL-2 et la prolifération des lymphocytes de patients infectés par le VIH et traités par PI, par NNRTI ou naïfs de traitements. Nous avons observé une réduction de la quantité d'IL-2 produite chez les patients traités par PI par rapport aux patients traités par NNRTI ou naïfs de traitement. Nous avons également observé une réduction de la prolifération des lymphocytes de patients traités par PI par rapport aux patients traités par NNRTI ou naïfs de traitement.

7.4. Les inhibiteurs de protéase limitent la réactivation du VIH dans les lymphocytes T CD4+ quiescents.

Nous avons mesuré la réactivation du VIH à partir de cellules infectées de manière latente et traitées par des PI ou des NNRTI. Les cellules ont été stimulées par combinaison de Nef et d'anticorps anti-CD3, ou combinaison d'anticorps anti-CD3 anti-CD28 ou par l'inhibiteur d'histone deacétylase SAHA. Nous avons observé une réduction de la production de nouvelles particules virales pour les cellules traitées avec des PI par rapport aux cellules traitées par des NNRTI ou non-traitées.

7.5. Discussion

Nous avons montré que la protéine Nef induit une activation d'Akt par phosphorylation dans les lymphocytes. Cette activation est réalisée par interaction directe avec Akt et avec PI3K, au niveau des radeaux lipidiques de la membrane cellulaire. Cette activation de la voie PI3K/Akt entraîne une activation du CD28RE et de NFkB. Cette activation entraîne également une augmentation de la production d'IL-2 et de la prolifération des lymphocytes.

Les radeaux lipidiques jouent un rôle important dans la signalisation du TCR, en association avec Akt. Cette activation des lymphocytes par Nef pourrait conduire à une expansion du réservoir viral, en les rendant plus permissifs à l'infection.

Nous avons montré que les PI bloquent l'activation d'Akt par Nef. Cette réduction de l'activation d'Akt par les PI se traduit également par une réduction de la production d'IL-2. Les PI bloquent également la réactivation virale à partir de cellules infectées de manière latente, de manière dépendante d'Akt.

Le blocage de l'activation d'Akt, qui est plus élevée dans les lymphocytes de patients infectés par le VIH par rapport aux patients sains, pourrait être une approche thérapeutique supplémentaire. L'inhibition d'Akt semble pouvoir permettre une réduction de la réactivation virale, ainsi qu'une réduction de la survie cellulaire, ce qui pourrait permettre de réduire davantage la taille du réservoir de latence. La réduction de l'activation d'Akt dans les lymphocytes traités par PI met en avant l'intérêt de ce type de traitement, par rapport à l'utilisation de NNRTI.

L'intérêt thérapeutique des PI est cependant réduit par les effets secondaires adverses importants et l'apparition plus fréquente de résistance au traitement conduisant à des échecs thérapeutiques [316]. De plus, le développement de nouveaux traitements, notamment les inhibiteurs de l'intégrase, réduisent l'intérêt de cette approche.

Cette étude a fait l'objet d'une publication dans la revue Scientific Reports en 2016.

7.6. Article paru dans la revue Scientific Reports en 2016 « Tuning of AKT-pathway by Nef and its blockade by protease inhibitors results in limited recovery in latently HIV infected T-cell line »

SCIENTIFIC REPORTS

OPEN

Tuning of AKT-pathway by Nef and its blockade by protease inhibitors results in limited recovery in latently HIV infected T-cell line

Received: 09 October 2015

Accepted: 18 March 2016

Published: 14 April 2016

Amit Kumar^{1,*}, Wasim Abbas^{1,*}, Laurence Colin^{2,*}, Kashif Aziz Khan¹, Sophie Bouchat², Audrey Varin¹, Anis Larbi³, Jean-Stéphane Gatot², Kabamba Kabeya⁴, Caroline Vanhulle², Nadège Delacourt², Sébastien Pasquereau¹, Laurie Coquard¹, Alexandra Borch⁵, Renate König^{5,6}, Nathan Clumeck⁴, Stéphane De Wit⁴, Olivier Rohr⁷, Christine Rouzioux⁸, Tamas Fulop Jr³, Carine Van Lint^{2,*} & Georges Herbein^{1,*}

Akt signaling plays a central role in many biological processes, which are key players in human immunodeficiency virus 1 (HIV-1) pathogenesis. We found that Akt interacts with HIV-1 Nef protein. In primary T cells treated with exogenous Nef or acutely infected with Nef-expressing HIV-1 *in vitro*, Akt became phosphorylated on serine⁴⁷³ and threonine³⁰⁸. *In vitro*, Akt activation mediated by Nef in T-cells was blocked by HIV protease inhibitors (PI), but not by reverse transcriptase inhibitors (RTI). *Ex vivo*, we found that the Akt pathway is hyperactivated in peripheral blood lymphocytes (PBLs) from cART naive HIV-1-infected patients. PBLs isolated from PI-treated patients, but not from RTI-treated patients, exhibited decreased Akt activation, T-cell proliferation and IL-2 production. We found that PI but not RTI can block HIV-1 reactivation in latently infected J-Lat lymphoid cells stimulated with various stimuli. Using luciferase measurement, we further confirmed that Nef-mediated reactivation of HIV-1 from latency in 1G5 cells was blocked by PI parallel to decreased Akt activation. Our results indicate that PI-mediated blockade of Akt activation could impact the HIV-1 reservoir and support the need to further assess the therapeutic use of HIV-1 PI in order to curtail latently infected cells in HIV-1-infected patients.

The serine/threonine kinase Akt (or protein kinase B) is a key regulator in the phosphoinositide 3-kinase (PI3K) signaling pathway and plays important roles in many cellular processes such as cell survival, metabolism, growth, and proliferation that are involved in HIV-1 pathogenesis^{1,2}. Immune hyperactivation, a hallmark of HIV-1 infection, fuels the progression of the disease and could be critical for the formation of HIV-1 reservoirs in infected individuals^{3,4}.

HIV-1 Nef is a multifunctional viral accessory protein without enzymatic activity that is abundantly expressed early in infection and has been shown to play an important role in numerous aspects of viral pathogenesis^{2,5,6}. Viral pathogenesis and replication is largely attenuated in individuals infected with Nef deficient HIV-1⁷. Endogenous Nef governs the downregulation of CD4+ receptor, major histocompatibility class (MHC) I, MHC II, CD80 and CD86 molecules in infected cells and interferes with several signaling pathways⁸.

¹Department of Virology, Pathogens & Inflammation Laboratory, University of Franche-Comté and COMUE Bourgogne Franche-Comté University, UPRES EA4266, SFR FED 4234, CHRU Besançon, Besançon, France.

²Laboratory of Molecular Virology, Institut de Biologie et de Médecine Moléculaires (IBMM), Université Libre de Bruxelles (ULB), Gosselies, Belgium. ³Department of Medicine, University of Sherbrooke, Sherbrooke, Canada.

⁴Department of Infectious Diseases, CHU St-Pierre, ULB, Bruxelles, Belgium. ⁵Research Group "Host-Pathogen Interactions", Paul-Ehrlich-Institute, Langen, Germany. ⁶Immunity and Pathogenesis Program, Sanford Burnham Preby Medical Discovery Research Institute, La Jolla, CA; German Center for Infection Research (DZIF), Langen, Germany. ⁷Institut de Parasitologie et Pathologie Tropicale, University of Strasbourg, Strasbourg, France.

⁸Department of Virology, Paris University, EA7327 Paris Descartes, APHP Necker Hospital, Paris, France. *These authors contributed equally to this work. Correspondence and requests for materials should be addressed to C.V.L. (email: cvlint@ulb.ac.be) or G.H. (email: georges.herbein@univ-fcomte.fr)

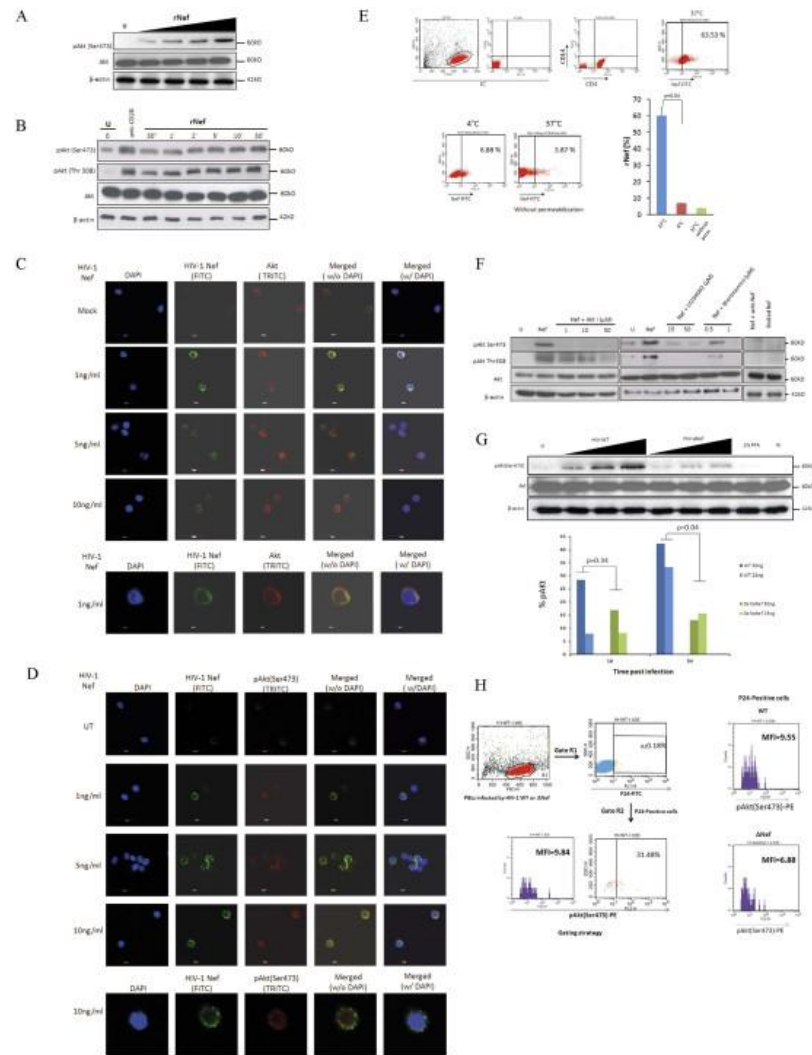


Figure 1. HIV-1 Nef is internalized by CD4⁺ T cells and activates Akt in PBLs which is mediated via PI3K in a dose and time dependent manners. (A), Dose-dependent (n = 3) and (B), Time-dependent activation of Akt (pAkt(Ser473)) in PBLs treated with rNef (n = 3). (B) Five million PBLs were either left untreated or treated with rNef (100 ng/ml) for various period of time (30 seconds to 30 minutes). As a positive control, PBLs were treated with anti-CD28 antibody. Expression of pAkt (Ser473, Thr308), total Akt and β-actin were detected by standard western blotting method as described in materials and methods (n = 3). (C,D) A series of confocal images showing internalization and colocalization of HIV-1 Nef and Akt (C) at serum concentration of Nef (1 to 10 ng/ml) and a dose response of rNef treatment on Akt activation (D) in PBLs isolated from healthy donors. (E), Internalization of rNef by CD4⁺ T cells determined by flow cytometry. Five million CD4⁺ T cells were treated with rNef for 30 min at 37°C and 4°C with and without permeabilization. Expression of rNef was determined by confocal microscopy (n = 3). (F), Activation of Akt in PBLs treated with rNef is mediated via PI3K. Western blot detection of activated pAkt (Ser473, Thr308) in the lysates derived from 5 × 10⁶ PBLs treated with 100 ng/ml of Nef with or without Akt (Akt inhibitor VIII) and PI3K inhibitors (LY294002 and Wortmannin) (n = 3). (G), Akt activation in PBLs by wild-type HIV-1, but not by isogenic HIV-1ΔNef. Five million PBLs were infected with various doses (of p24) of either wild type HIV-1 or ΔNef virus infectious clones. Post infection lysates were prepared and expression of pAkt(Ser473), was determined by western

blotting (upper panel) and flow cytometry ($n=2$) (lower panel). (H), Left panel: Gating strategy for flow cytometry analysis. In this sample gating cells were first gated for PBLs (Gate R1). R1 gate is further analysed for p24 positive PBLs (Gate R2). Gate R2 is analyzed for the expression of pAkt. Right panel is the representative data of pAkt positive cells in p24 positive population obtained by infection of PBLs with WT and Delta Nef virus.

Infection of CD4+ T cells by HIV-1 is largely influenced by their activation state. In activated CD4+ T cells, HIV-1 can readily undergo robust replication whereas resting CD4+ T cells are usually refractory to infection⁹. A number of studies suggest that Nef can reduce the threshold for CD4+ T cell activation, and Nef has been shown to interact with T-cell receptor and various downstream effectors or secondary messengers including Lck, LAT, lipid rafts, PI3K and Ca²⁺^{8,10,11}.

Besides endogenously produced Nef, extracellular Nef protein is found in the serum and cerebrospinal fluid of infected individuals and displays immunomodulatory effects such as the suppression of immunoglobulin class switching in bystander B cells^{12–14}. PI3K/Akt pathway, a key pathway of cell survival is known to be manipulated by a plethora of viral pathogens including human cytomegalovirus, Epstein Barr virus, influenza A virus and HIV-1¹⁵. Few studies also reported the impact of protease inhibitors on Akt signaling in several cell types and in a clinical trial^{16–19}. We hypothesized that the Akt pathway could play a role in HIV-1 reactivation. We found that the Nef protein participates to the hyperactivation of T cells through Akt activation and that blocking Akt activation could limit HIV-1 recovery from latently infected T cells.

Results

Exogenous Nef enters into PBLs and increases Akt phosphorylation. We studied the impact of Nef on Akt activity, which is activated by its phosphorylation on Ser473 and Thr308 residues^{20,21}. We observed that treatment of PBLs with rNef led to Akt phosphorylation (pAkt) in a dose dependent (Fig. 1A) and time dependent manner as determined (Fig. 1B) by western blotting and confocal microscopy (Fig. 1C,D). We did not find any significant toxicity of rNef (1–100 ng/ml) for as long as 30 min as determined by Wst-1 cell viability assay (Supplementary Fig. 1A,B). We observed rapid phosphorylation of Akt by exogenous Nef suggesting the involvement of receptor mediated signaling^{22,23}.

We further investigated the internalization of rNef by PBLs and its cellular colocalization with Akt. We assessed the purity of PBLs using CD14/45, CD8+ and CD4+ T staining by flow cytometry (Supplementary Fig. 2). We incubated PBLs isolated from healthy donors with varying concentration of Nef (1–10 ng/ml) and determined the expression of Nef and Akt by immunofluorescence using confocal microscopy. We detected the expression of Nef both at the cell margins and in the cytoplasm (Fig. 1C). Expression of Akt was found both in cytoplasm as well as in the nucleus (Fig. 1C). In addition, we also observed the colocalization of internalized rNef and Akt mostly at the cell margins. Taken together our results indicate Nef is internalized by PBLs and colocalized with Akt (Fig. 1C).

To further demonstrate the internalization of rNef by PBLs, we incubated CD4+ T cells with rNef for 30 min at 37 °C and 4 °C under permeabilized and non permeabilized conditions. We found that rNef is internalized by CD4+ T at 37 °C and very little internalization was observed at 4 °C (Fig. 1E). To our knowledge this is the first report where internalization of rNef by PBLs has been demonstrated.

Phosphorylation of Akt on Ser473 and Thr308 was blocked by Akt inhibitor VIII (AktI) in PBLs treated with rNef (Fig. 1F). Since Akt activation is partly mediated by PI3K products such as PIP3, we determined whether the effect of HIV-1 Nef on Akt phosphorylation was dependent on cellular PI3K activity. The two PI3K inhibitors LY294002 and Wortmannin hindered the phosphorylation of Akt at Ser473 and Thr308 observed after HIV-1 rNef treatment, demonstrating that endogenous PI3K activity is required for Nef-mediated Akt activation (Fig. 1F). We did not detect any significant cytotoxicity of Akt and PI3K inhibitors used after two hours of treatment (data not shown).

Akt pathway plays a key role in cell survival and homeostasis⁵. Therefore, there are only limited numbers of phosphoproteins unaltered by Akt inhibitors. Phosphorylation of proteins upstream of Akt pathway is generally unaltered upon Akt inhibitors treatment such as focal adhesion kinase (FAK) and proline-rich tyrosine kinase 2 (PYK2). Therefore, to determine the specificity of the AktI inhibitor, we have determined for the phosphorylation level of FAK using western blot. We did not observe any significant change in the levels of pFAK in response to AktI treatment post 2 hours of treatment (Supplementary Fig. 3).

Nef is known to play important role in promoting viral infectivity in a host cell dependent manner in several independent studies^{24–27}. Importantly Nef is associated with virion particles and therefore merely entry of the viral particles and hence Nef could activate Akt. We infected PBLs with wild type HIV-1 (HIV-WT) and an isogenic mutant infectious HIV-1 clone deleted for the *nef* gene (HIVΔNef) and compared the level of Akt activation. Wild-type HIV-1 infection was associated with an increased Akt phosphorylation in contrast to PBLs infected with HIVΔNef and the effect was dose dependent (Fig. 1G, upper panel). Since the effect of Nef on Akt activation would be more accurately analyzed in the fraction of infected cells, we infected PBLs with different amount of viral particles derived from wild type and Nef deleted mutant (based on their p24 quantitation) and measured the levels of pAkt at early time points only in the p24-positive cells (Fig. 1G lower panel, Fig. 1H). The gating strategy is shown in Fig. 1H, left panel. We found that with equal amount of virus particle, levels of pAkt were higher in WT at post 3 h of infection as compared to Nef-deleted mutant in p24-positive cells ($p=0.04$ WT vs. Nef-deleted mutant) (Fig. 1G lower panel). In addition, we also observed relatively higher amount of p24 detection in PBLs infected with wild type HIV-1 as compared to delta Nef mutant post 24h of infection in unstimulated condition further confirms the decreased infectivity of delta Nef HIV-1 (Supplementary Fig. 4)

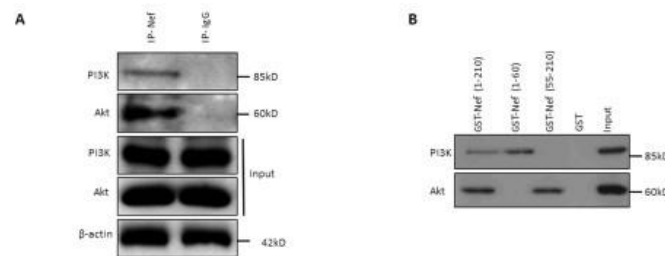


Figure 2. HIV-1 Nef interacts with Akt and PI3K. (A), Nef interacts with Akt and PI3K in PBLs treated with rNef as detected by co-immunoprecipitation. Five million PBLs isolated from healthy donor were left untreated or treated with HIV-1 rNef (100 ng/ml) and subjected to immunoprecipitation with anti-HIV Nef-Ab or isotype control antibody followed by western blot using indicated antibodies ($n = 3$). (B), Nef interacts with Akt and PI3K via its C-terminus and N-terminus respectively, as measured by pull-down assays. Representative western blot analysis of pull down assay using 1500 μg of PBLs lysates with 20 μg of different HIV-Nef constructs [N-terminus, 1-60aa, C-terminus 55-210aa and full length Nef, 1-210 aa] expressed in BL21DE as indicated. Input corresponds to 10% of the material used for pull down. Presence of PI3K and Akt was detected by respective antibodies mentioned in Material and Method section ($n = 3$).

HIV-1 Nef interacts with Akt and PI3K via its C and N terminal extremity respectively. We tested the interaction between Nef and Akt as well as between Nef and PI3K, an upstream kinase involved in Akt phosphorylation and known to interact with Nef^{10,20}. We observed that endogenous Akt and endogenous PI3K co-immunoprecipitated with HIV-1 Nef in peripheral blood lymphocytes (PBLs) isolated from healthy donors treated with recombinant Nef (rNef) (Fig. 2A). To determine the domain of HIV-1 Nef that binds Akt or PI3K, we expressed the full-length HIV-1 Nef and Nef proteins truncated at their N-terminal or C-terminal extremity as GST fusion proteins in *E. coli* and tested their ability to interact with Akt and PI3K present in PBL lysates (Fig. 2B). Our GST pull-down assays indicated that HIV-1 Nef interacts with Akt via its C-terminal region (residues 55–210), while HIV-1 Nef interaction with PI3K occurs via Nef N-terminal region (residues 1–60) (Fig. 2B).

Nef triggers the activation of NF- κ B and CD28RE via lipid raft mediated Akt signaling. PI3K activity and consequently Akt activation contribute to T-cell receptor (TCR) signaling as key regulators of T-cell activation, differentiation and proliferation^{28–30}. Therefore, we tested the effect of rNef on Akt phosphorylation in the context of TCR stimulation using treatment with anti-CD3 and/or anti-CD28 monoclonal antibodies. Recombinant Nef-mediated Akt phosphorylation was increased following TCR stimulation, compared to that observed upon rNef treatment alone (Fig. 3A). Our results indicate that rNef, alone or in conjunction with TCR stimulation is able to significantly activate Akt in PBLs from healthy donors. Since both proteins have been reported to translocate to lipid rafts^{13,32}, we questioned whether the HIV-1 Nef/Akt interaction involves lipid rafts. We observed that rNef treatment alone or combined with TCR stimulation indeed induced the translocation of activated Akt to lipid rafts in parallel to its own localization into lipid rafts (Fig. 3B,C). In addition, we observed the colocalization of rNef and pAkt at the cell margins further suggesting lipid rafts as a possible site of Nef and Akt interaction (Fig. 1C,D).

TCR/CD28 co-stimulation activates CD28 response element (CD28RE) and nuclear factor-kappa B (NF- κ B) that can recruit the histone acetyltransferase PCAF (p300/CREB-associated factor) to the *IL-2* promoter³³. Moreover, Nef by itself has been reported to activate NF- κ B in T cells²³. Here, we observed that rNef activated CD28RE and NF- κ B (Fig. 3D). This activation was increased by TCR triggering and blocked by the Akt inhibitor VIII (Fig. 3D), suggesting that Nef participates to NF- κ B and CD28RE activation via Akt phosphorylation. Recombinant Nef together with TCR stimulation, but not rNef alone, stimulated IL-2 production ($p = 0.05$, anti-CD3 alone vs. anti-CD3 + rNef) (Fig. 4A,B) and T-cell proliferation ($p = 0.006$, anti-CD3 alone vs. anti-CD3 + rNef) (Fig. 4C) that were significantly inhibited by the Akt inhibitor VIII (IL2 production: $p = 0.05$ at 25 μM ; $p = 0.01$ at 50 μM and T cell proliferation: $p = 0.001$ at 25 μM ; $p = 0.002$ at 50 μM) and the PI3K inhibitor LY294002 (IL2 production: $p = 0.008$ at 25 μM ; $p = 0.008$ at 50 μM and T cell proliferation: $p = 0.003$ at 25 μM ; $p = 0.001$ at 50 μM) (Fig. 4D,E). The enhancement of proliferation and IL-2 production in PBLs was Nef-specific, since it was totally blocked by a neutralizing anti-Nef antibody (Fig. 4B,C). Altogether, our results show that *in vitro* exogenous Nef activates Akt especially in TCR-stimulated PBLs resulting in increased IL-2 production and T-cell proliferation (Fig. 4). In addition, we found a significant difference in the levels of IL-2 and T cell proliferation in CD3 alone stimulated cells and cells stimulated with CD3 in addition to rNef treatment (Fig. 4D,E).

Protease inhibitors (PI) block Akt activation in PBLs treated with rNef *in vitro*. Since HIV-1 protease inhibitors (PI), but not reverse transcriptase inhibitors (RTI), have been reported to block Akt activation in several cell types such as peripheral blood mononuclear cells (PBMCs) and monocytes/macrophages^{34–37}, we assessed the potential role of PIs as immunomodulators in addition to their antiviral effect in HIV-1 infection. *In vitro*, we observed that PIs such as atazanavir and lopinavir/ritonavir inhibited Akt hyperactivation in a

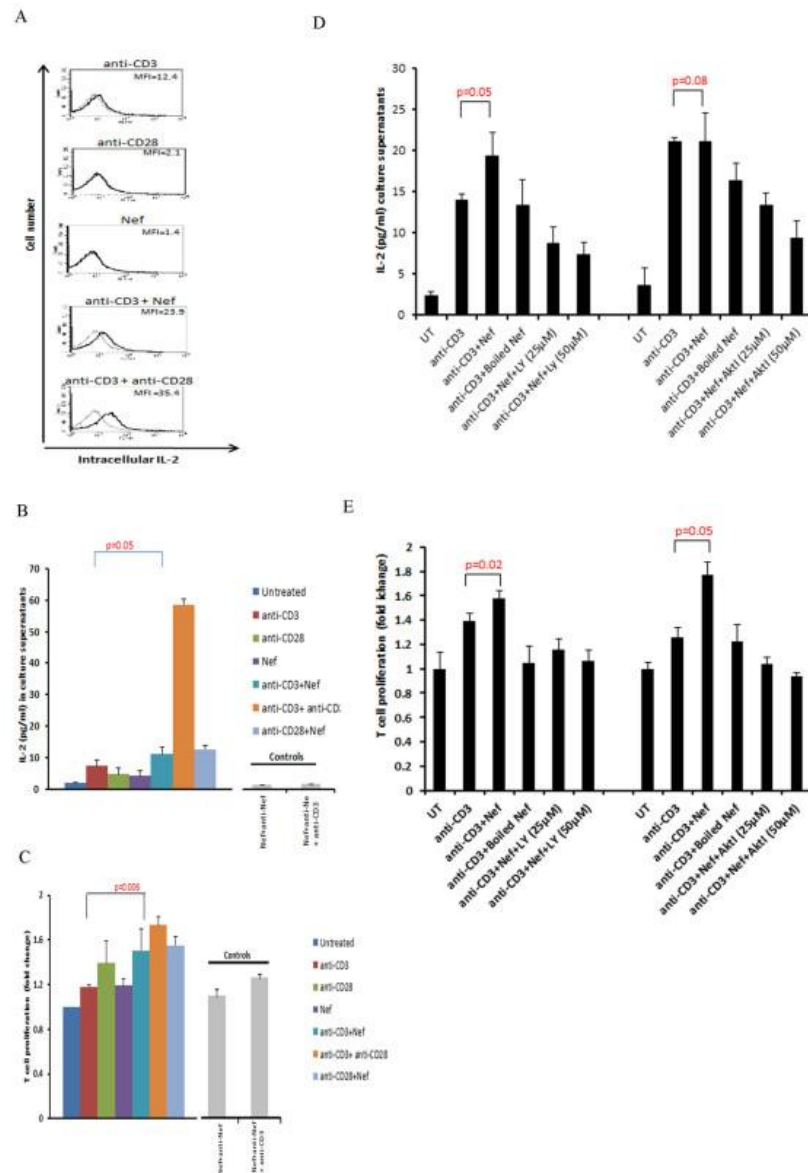


Figure 4. *In vitro* rNef-mediated Akt-dependent hyperactivation of T cells favors increased IL-2 production and T-cell proliferation. (A,B) Exogenous HIV-1 Nef triggers IL-2 production in TCR-stimulated T-cells. (A), Five million PBLs were stimulated with anti-CD3-mAb (5 µg/ml), anti-CD3-mAb + rNef, anti-CD28-mAb (5 µg/ml), anti-CD28-mAb + anti-CD3-mAb or rNef (100 ng/ml) alone for 24 h. Post treatment cells were fixed, permeabilized and expression of intracellular IL-2 was determined by flow cytometry. Flow cytometric data indicating the mean fluorescence intensity (MFI) for intracellular IL-2 production. Data are representative of 5 independent experiments. (B), Five million PBLs were either left untreated or treated with rNef (100 ng/ml), anti-CD3-mAb (5 µg/ml), anti-CD28-mAb (5 µg/ml), anti-CD3-mAb + rNef, anti-CD28-mAb + rNef, and antiCD3-mAb + antiCD28-mAb for 24 h. Post 24 h supernatants were collected and

IL-2 production was measured in supernatants by ELISA. Means \pm s.d. ($n = 3$). (C), Exogenous HIV-1 Nef triggers cell proliferation in TCR-stimulated T cells. Five million PBLs were either left untreated or treated with rNef (100 ng/ml), anti-CD3-mAb (5 μ g/ml), anti-CD28-mAb (5 μ g/ml), anti-CD3-mAb + rNef, anti-CD28-mAb + rNef and antiCD3-mAb+ antiCD28-mAb in the absence of Akt 1 and PI3K inhibitor for 24h. Cell proliferation was measured using MTT cell proliferation assay. Means \pm s.d. ($n = 3$). (D,E), Exogenous HIV-1 Nef triggers IL-2 production and T cell proliferation in CD3 stimulated cells in PI3K/Akt dependent manner. Five million PBLs were either left untreated or treated with Akt 1 (25 μ M, 50 μ M) and PI3K inhibitor LY294002 (25 μ M, 50 μ M) for 2 h followed by treatment with rNef (100 ng/ml), heat denatured rNef (100 ng/ml), anti-CD3-mAb (5 μ g/ml) and anti-CD3-mAb + rNef, for 24 h. After 24h supernatants were collected and IL-2 production and T cell proliferation was determined by ELISA and MTT assay respectively. Means \pm s.d. ($n = 3$). LY: LY294002; Akt1: Akt inhibitor VIII.

lower in PBLs isolated from HIV-1 patients treated with PI in comparison to PBLs isolated from HIV-1 patients treated with RTI ($p = 0.018$ and $p = 0.036$, respectively) (Fig. 6A,B).

Protease inhibitors limit HIV-1 reactivation from T lymphoid cells containing stably integrated HIV-1 or HIV-1 LTR constructs and stimulated with several stimuli including Nef via blocking Akt pathway. Our results showing decreased IL-2 production and T-cell proliferation in PI-treated patients compared to RTI-treated patients suggest that controlling the Akt pathway using PI participates to a lower activation of T cells and thereby could have an impact on HIV-1 recovery from latently-infected T cells^{38–43}.

Therefore, we assessed the respective role of PI or RTI treatment on HIV-1 reactivation from latency by measuring viral production in TCR-stimulated latently-infected J-Lat 8.4 cells treated with rNef. J-Lat 8.4 cells have full length HIV-1 minus env and nef genes. We observed that viral reactivation was significantly blocked by treatment with PI, but not with RTI (Fig. 7A). Similarly PI, but not RTI, blocked the activation of viral production by co-stimulation with anti-CD3 + anti-CD28 (Fig. 7B) and by SAHA, a histone deacetylase inhibitor well-known as PI3K/Akt activator^{44,45} (Fig. 7C).

We then tested the impact of rNef treatment alone on the activation of HIV-1 5'LTR in 1G5 cells, a Jurkat derivative T cell latent model cell line containing stably integrated luciferase gene under the control of the 5'LTR promoter. We found rNef treatment resulted in almost two-fold increase in the expression of luciferase activity as compared to mock treated cells which was subsequently blocked by protease inhibitor (Fig. 8 upper panel). We observed that the Nef-mediated reactivation of HIV-1 from latency in the 1G5 cells was blocked by PI parallel to decreased Akt activation (Fig. 8 lower panel). Our results indicate that PI-mediated blockade of Akt activation could impact the HIV-1 reservoir especially when driven by viral proteins such as Nef.

Discussion

HIV-1 Nef a pleiotropic early protein has been demonstrated to activate several key signaling molecules including Akt, MAPK and ERK^{46–48}. To further add to the previous findings we have demonstrated that HIV-1 Nef acts as a transient adaptor molecule for PI3K and Akt and induces phosphorylation of Akt and subsequent T cell proliferation in *in vitro* and *ex vivo* settings. In addition, we also found that protease inhibitors but not reverse transcriptase inhibitors block Nef-induced Akt phosphorylation resulting in lesser IL-2 production and T-cell proliferation. Most importantly, our results indicate that PI rather than RTI block HIV-1 reactivation from latently infected T-cells especially when stimulated by Nef.

For the first time to our knowledge, we showed that HIV-1 Nef is internalized by CD4+ T cells (Fig. 1C–E) and binds to both Akt and PI3K, suggesting a critical role for Nef as an adaptor protein in the PI3K/Akt pathway (Fig. 2). Our results are consistent with the previously published study where an interaction of Nef and PI3K has been shown¹⁹. We also demonstrated that Nef colocalizes with Akt in PBLs treated with rNef with the concentration of Nef usually reported in patients (1–10 ng/ml)⁴⁹ (Fig. 1C,D, Supplementary Figs 5 and 6). Nef localization was observed mostly at the cell margins and in the cytoplasm (Fig. 1C,D). On the other hand, expression of Akt was majorly concentrated at plasma membrane and also diffused throughout the cell including nucleus⁵⁰ (Fig. 1C). The mode of internalization and secretion of Nef is still a conundrum. Some studies suggest the involvement of exosomes in intercellular transfer of Nef from infected cells to the bystander cells^{51–53}. On the other hand, a recently published study suggests the cell to cell contact rather than exosomes mediated intercellular transfer of Nef⁵⁴. In our study, we found the direct internalization of rNef by the PBLs (Fig. 1E).

Since it has been described that Nef could induce apoptosis in PBLs^{49,51}, we quantitated the metabolic activity of rNef treated PBLs at various time points and concentration (1–100 ng/ml) using MTT assay and/or WST-1 assay and monitored cell viability using trypan blue staining (Supplementary Fig. 1A,B). We did not observe any significant decrease in cell viability post rNef treatment. Since results obtained with 100 ng/ml of rNef were more pronounced without significant toxicity (Fig. 1A), we have therefore used this concentration throughout in our experiments. However, we also demonstrated that rNef can induce similar effects at concentration usually reported in the serum of patients (1–10 ng/ml) (Fig. 1A–D and Supplementary Figs 5 and 6)⁴⁴.

The PI3K/Akt pathway is modulated by several pathogenic viruses¹⁵. We observed that both exogenous as well as endogenous Nef were able to activate Akt in PBLs in both dose dependent (Fig. 1A,C,D,G,H) and time dependent manner (Fig. 1B). Interestingly, we also observed some increase in Akt phosphorylation with increasing dose of HIV Δ Nef (Fig. 1G). Therefore, we cannot rule out the role of other viral protein(s) contributing to Akt phosphorylation, e.g. the Tat protein (Fig. 1G)⁵⁵.

The role of lipid rafts in intracellular trafficking and signal transduction has been established by several independent studies. Nef interacts with a number of signaling partners including p21-activated kinase-2 in lipid

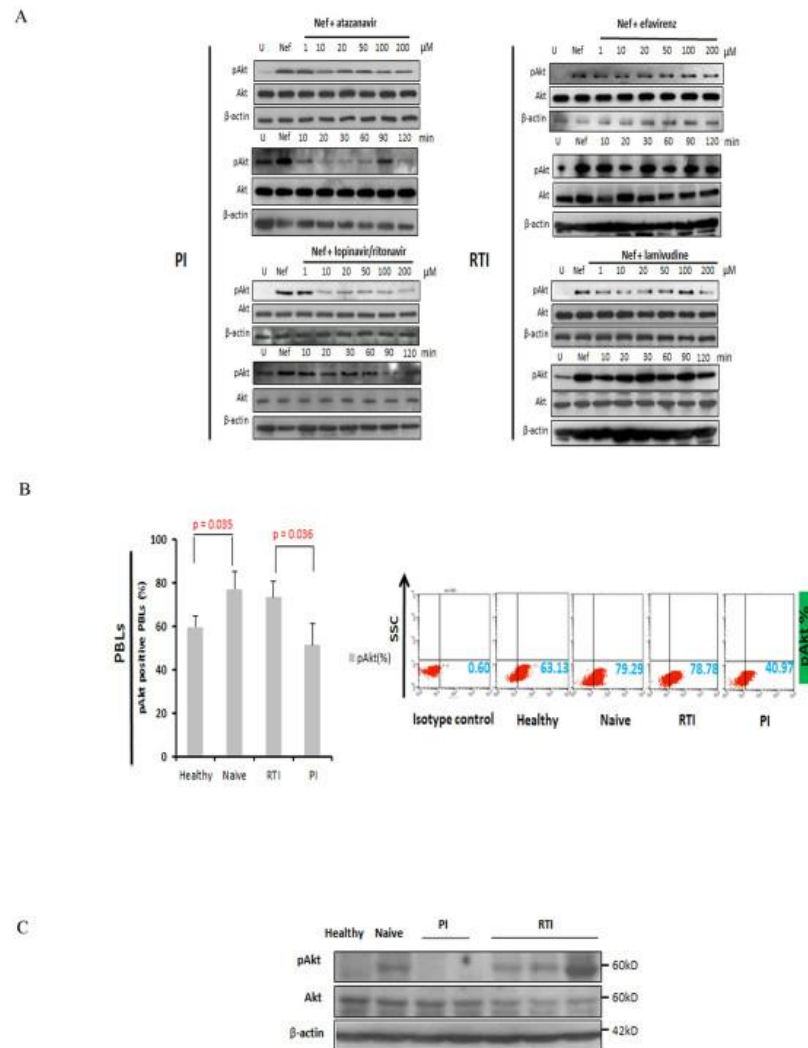


Figure 5. HIV-1 protease inhibitors block Akt activation in rNef-treated PBLs *in vitro* and *ex vivo* in PBLs isolated from cART-treated patients. (A), HIV protease inhibitors, but not RTIs, inhibit pAkt(Ser473) activation in a dose dependent manner in PBLs treated with rNef as determined by western blot. Five million PBLs were treated with rNef (100 ng/ml) for 30 min. Expression of pAkt(Ser473), total Akt and β -actin was determined by western blotting (n = 8). (B), Decreased Akt activation in PBLs from PI-treated patients compared to RTI-treated and cART naive patients as measured by flow cytometric analysis. Mean values \pm s.d. (n = 5). (C), Decreased Akt activation in PBLs from PI-treated patients compared to RTI-treated and cART naive patients as measured by western-blotting.

rafts^{32,36}. Calay *et al.* demonstrated that disruption of lipid rafts negatively influences the activation of Akt at regulatory sites Ser(473) and Thr(308)³⁷. In agreement with previous findings, our data show for the first time that Nef and Akt interaction involves lipid rafts (Fig. 3B,C). In addition, we observed also the localization of rNef and pAkt at the cell margins of the PBLs suggesting lipid rafts as a possible site of Nef and Akt interaction

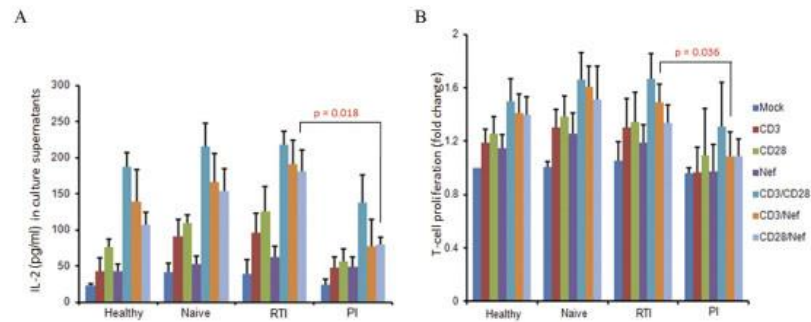


Figure 6. Reduced IL-2 production and T-cell proliferation in PBLs isolated from PI-treated patients. (A,B), Lower IL-2 production (A) and decreased proliferation of PBLs (B) in PI-treated patients compared to RTI-treated and cART naive patients, respectively. PBLs were isolated from healthy, naive and HIV-1 infected individuals under PI and RTI treatment. Five million PBLs were either left untreated or treated with rNef (100 ng/ml), anti-CD3-mAb (5 μ g/ml), anti-CD28-mAb (5 μ g/ml), anti-CD3-mAb + rNef, anti-CD28-mAb + rNef, and anti-CD3-mAb + anti-CD28-mAb for 24 h. IL-2 production (A) and cell proliferation (B) was measured using ELISA and MTT cell proliferation assay respectively. Means \pm s.d. (n = 5).

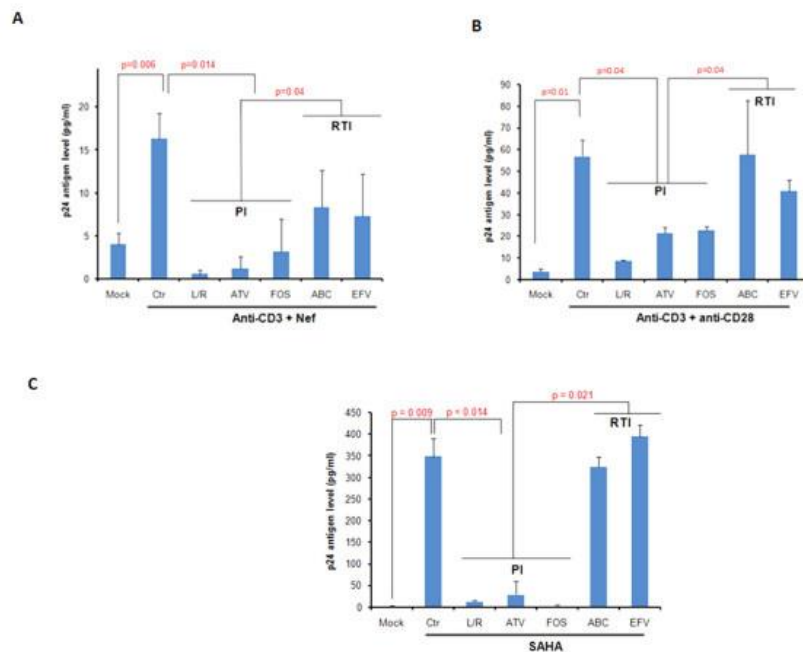


Figure 7. HIV-1 protease inhibitors block HIV-1 recovery from latently infected J-Lat 8.4 cells. (A–C), J-Lat cells were either left untreated or treated with anti-CD3-mAb (2 μ g/ml) + rNef (100 ng/ml) (A), anti-CD28-mAb (2.67 μ g/ml) + anti-CD3-mAb (2 μ g/ml) (B) and SAHA (2.5 μ M) (C) in the absence or presence of different PI and RTI compounds as indicated. The viral production was determined by measuring the level of p24 antigen using ELISA. Means \pm s.d. (n = 6). Ctr: Control; L/R, lopinavir/ritonavir; ATV: atazanavir; FOS: fosamprenavir; ABC: abacavir; EFV: efavirenz.

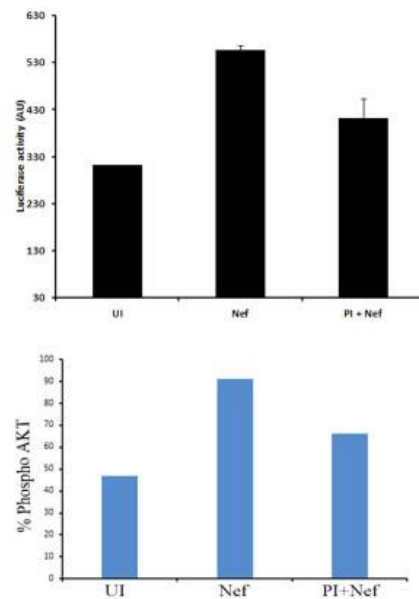


Figure 8. rNef induces HIV-1 expression in a latent model cell line 1G5 and protease inhibitor can subsequently block it. Five million 1G5 cells were either left untreated or treated with rNef in the presence or absence of protease inhibitor. Post 24h of treatment cells, the expression of luciferase was determined (upper panel) and expression of pAkt was determined by flow cytometry (lower panel). The data shown are representative of two independent experiments.

(Fig. 1C,D). Several aspects of T cell biology including differentiation and proliferation are modulated by activation of signaling pathways by T cell receptor⁵⁸. In addition, lipid rafts and Akt signaling play a critical role in TCR signaling⁵⁸. We found that in response to stimulation of TCR by anti-CD3-mAb and/or anti-CD28-mAb there was an increase in the activation of Akt. We also observed the synergistic effect of Nef with anti-CD3-mAb and anti-CD28-mAb (Fig. 3A). It seems that Nef can reduce the threshold of CD3/CD28 required for T cell stimulation as shown previously⁵⁹. *In vivo*, exogenous Nef secreted by infected cells can activate uninfected CD4+ T cells present in the vicinity and make them susceptible for infection⁶⁰. In this way, Nef could expand the viral reservoir in infected individual.

Production of IL-2, a cytokine considered as a hallmark of CD4+ T-cell activation, is mediated by the stimulation of NF- κ B, NFAT and AP-1⁶¹. Nef has been shown to induce IL-2 production in CD4+ T cells in NFAT and NF- κ B dependent manners. Further extending the previous study, we found that rNef activates CD28 responsive element and NF- κ B via the Akt pathway which in turn could influence IL-2 production⁴⁸ (Figs 3D and 4A). In addition, Nef exhibited synergism with TCR/CD28 co-stimulation in terms of cell proliferation and IL-2 production mediated by PI3K/Akt pathway (Fig. 4A-E). Our data are in agreement with previous findings⁶²⁻⁶⁵. In addition to the previous reports, we found these effects were mediated by Akt/PI3K signaling pathway and were Nef-specific. We observed that, although Nef/anti-CD3 and Nef/anti-CD28 costimulation resulted in higher levels of T-cell proliferation and IL-2 production than treatment with anti-CD3 or anti-CD28 alone, however it was less potent than treatment with anti-CD3/anti-CD28 costimulation (Fig. 4A,B).

Several studies suggest the central role of PI3K/Akt pathway in modulating HIV-1 latent reservoir. For instance, disulfiram is known to deplete phosphatase and tensin homolog (PTEN) protein levels in U1 cells and in resting CD4+ T cells from HIV-negative donors resulting in hyperphosphorylation of Akt and subsequently activation of HIV-1 expression⁶⁶. In another instance, 57704, an agonist of PI3K, reactivates HIV-1 in several latent cell lines⁶⁶. In addition, the role of PI but not RTI in inhibiting Akt activation has been shown in several cell types³⁴⁻³⁷.

We demonstrated that Nef induced Akt hyperactivation and T-cell proliferation could be effectively inhibited by protease inhibitors but not by reverse transcriptase inhibitors *in vitro* and *ex vivo* (Figs 5 and 6). We found that PI could block Akt activation in both time and dose dependent manners (Fig. 5A,B). The PI nelfinavir has been shown to inhibit Akt phosphorylation by inhibiting proteasomal activity and inducing the unfolded protein response in SQ20B cells⁶⁷.

The choice of treatment (PI versus RTI) has been reported to influence several aspects of HIV-1 pathogenesis⁶⁸. Besides inhibiting polyprotein processing, studies suggest that PI can influence apoptosis⁶⁸ and can block cell to cell dissemination of virions between T cells⁶⁹.

Latency represents the major obstacle in the complete cure of HIV-1^{70,71}. Several agents targeting various signaling cascades are known to reactivate latent HIV-1 and are termed as latency-reversing-agents (LRAs)^{72,73} including activator of PI3K/Akt pathway. In addition, several strategies have been employed in identifying cellular factors involved in viral replication⁷⁴. Indeed Akt inhibitors are suggested as a potential therapeutic molecule against HIV-1⁷⁵. We found that protease inhibitors could effectively inhibit the viral reactivation from chronically infected J-Lat cells stimulated with various stimuli (Fig. 7A–C). As indirect evidence we stimulated J-Lat cells with the HDAC inhibitor SAHA which is a potent activator of Akt pathway. We found that PI but not RTI can block the SAHA dependent activation of HIV-1 in latently infected J-Lat cells (Fig. 7C).

To have more direct evidence of the effect of PI on Nef-mediated Akt activation and subsequently on HIV-1 reactivation from latency, we treated Jurkat 1G5 cells, containing an integrated HIV LTR luciferase construct, with protease inhibitor in the presence or absence of rNef. We found that rNef treatment resulted in almost two fold induction in luciferase activity which was subsequently blocked by protease inhibitor (Fig. 8). A two-fold increase in HIV-1 reactivation from 1G5 cells treated with rNef, is in agreement with previously described two-fold increase in NF- κ B-mediated LTR activation in U937 cells transfected with LTR-Luc and also two-fold increase of p24 levels in supernatants of chronically HIV-infected U1 cells treated with rNef⁶⁰. Since decreased HIV reactivation in Nef-stimulated 1G5 cells treated with PI parallels decreased Akt activation (Fig. 8, lower panel), our results indicate that PIs have a negative impact on HIV reactivation from latency triggered by Nef protein and Akt activation. Altogether our findings suggest the role of Nef in regulation of HIV-1 latency at physiological level which cannot be compared with strong latency reversal agents such as HDAC inhibitors. In addition, we have determined the levels of pAkt in the blood of naïve, PI and RTI treated patients. We found low levels of pAkt in the patients treated with PI as compared to RTI (Supplementary Fig. 5) further support our findings.

Conclusion

Our study shows that exposure of T cells to the viral protein Nef leads to Akt activation, which is a critical phenomenon that favors IL-2 production and cellular proliferation. Importantly, we observed that the immunomodulatory effects of PI, by blocking Akt activation especially triggered by Nef, limit HIV-1 recovery from latently-infected T cells. Altogether, our results support the need to further assess the therapeutic use of HIV-1 protease inhibitors and its impact on persistence of HIV-1 reservoirs.

Materials and Methods

Study Approval. Ethical approval was granted by the human subject ethics committees of the Saint-Pierre University Hospital and the Besançon University Hospital (CPP EST-2), and written informed consent was obtained from the patients. In addition, all experiments were carried out in accordance with the approved guidelines and regulations (Declaration of Helsinki).

Isolation and culture of PBMCs and PBLs. Human PBMCs and purified PBLs were prepared from the peripheral blood of healthy donors and cultured as described previously⁷⁶.

Recombinant Nef treatment. PBLs (5×10^6 cells) were treated with recombinant myristoylated Nef protein (rNef) from the SF2 HIV-1 strain (100 ng/ml) (cat # PR-382, Jena Bioscience, Jena, Germany). Cell pellets were collected at various times after rNef treatment, washed extensively and either lysed before western blot analysis or fixed with BD Cytotfix (BD Biosciences, San Jose, CA) for 20 min before flow cytometric analysis. Some PBLs were treated with the PI3K inhibitors LY294002 (0, 25 μ M, 50 μ M) or Wortmannin (0, 0.5 μ M, 1 μ M) (Cell Signaling Technology, Beverly, MA), or with the Akt inhibitor VIII (cat # sc-202048A) (0, 25, 50 μ M) (Santa Cruz Biotechnology, Santa Cruz, CA) for two hours before addition of rNef (100 ng/ml) for 30 min.

Confocal microscopy. Five million PBLs cells were incubated with the varying concentration of rNef (1–10 ng/ml) for a period of 30 min. Purity of PBLs cells were assessed by flow cytometry. Cells were washed three times with 1X PBS containing 2% fetal bovine serum followed by fixation in 4% PFA prepared in 1X PBS for 15 min at room temperature. Cells were then washed and permeabilized in 0.1% triton-100 in PBS for 10 min at room temperature followed incubation in primary antibodies: mouse anti-HIV-1 Nef (cat# 65905, Santa Cruz Biotechnology, TX, USA), rabbit anti-Akt1 (cat# 1618, Santa Cruz Biotechnology, TX, USA), rabbit anti-pAKT(Ser473) (cat# 4060, Cell Signaling Technologies, MA, USA) for 1 h at room temperature. Cells were washed and incubated in secondary antibodies: anti-Mouse FITC (cat# 50-010, Argene, FR), anti-Rabbit TRITC (cat# 6718, Abcam, UK) for 1 h at room temperature. Cell pellet was finally dissolved in 10 μ l of 1X PBS containing DAPI (Sigma-Aldrich, USA) and placed on slide and mounted with fluorescent mounting media (cat# S3023, DAKO). Cells were observed under Olympus confocal microscope using 405 nm, 488 nm and 543 nm excitation lasers.

Immunoprecipitation assay. PBLs were either left untreated or were treated with HIV-1 rNef (100 ng/ml) for 30 min. The cell lysates were pre-cleared by adding 50 μ l of protein A magnetic beads (Millipore SAS, Molsheim, France) for 1 h at 4 °C. The cleared supernatants were removed, combined with 10 μ g/ml anti HIV-1 Nef-Ab or isotype control antibody (Chemicon/Millipore SAS, Molsheim, France) and incubated overnight at 4 °C. The lysates were further incubated with 50 μ l protein A magnetic beads (Millipore) at 4 °C for 2 h. Immune complex were washed in the presence of protease inhibitors (Roche, Meylan, France) and bound proteins were eluted with sample buffer and run on 10% SDS-PAGE gels.

Pull-down assay. HIV-1 Nef (1–210), N-terminus (1–60) and C-terminus (55–210) were cloned in pGEX4T-1 (kindly provided by Dr. Fackler, University of Heidelberg, Germany). For pull-down assays, each construct was transformed into bacteria (BL21DE from Novagen/Millipore SAS, Molsheim, France). A single colony

was inoculated in 5 ml broth containing 50 µg/ml ampicillin at 37 °C, 250 rpm in a shaker overnight. A 300 µl aliquot of overnight bacterial culture was used to inoculate 300 ml LB broth containing 50 µg/ml ampicillin and grown for 2.5 h at 37 °C in a shaker (until $OD_{600} = 0.5$). IPTG was added at a final concentration of 0.1 mM and the culture was grown for an additional 3 h at 37 °C at the shaker. The bacteria were pelleted, washed with ice cold TBS and lysed in bacterial protein extraction reagent (Thermo Fisher Scientific, Rochester, NY). The expressed proteins were immobilized on glutathione agarose beads (Thermo Fisher Scientific) and washed five times with $1 \times$ PBS. Twenty-five micrograms were incubated overnight at 4 °C with 1500 µg of PBL lysate. The suspension was then washed three times in PBS, denatured and analyzed by SDS-PAGE/autoradiography. Input corresponds to 10% of the material used for pull-down.

Western blot to detect Nef-induced signaling. Cellular extracts from rNef-treated PBLs or PBLs isolated from the peripheral blood of HIV-1 infected subjects were used to examine Akt, PI3K and Nef proteins expression by western blotting as described previously⁶⁰. Anti-Akt-mAb (cat # 2967S, Cell Signaling Technologies, St Quentin, France), anti-PI3K p85 mAb (Cell Signaling Technology, cat # 4292S), anti-Nef-mAb (cat # sc-65905, Santa Cruz Biotechnology), anti-pAkt (Thr308) mAb (cat # 2965S, Cell Signaling Technology), anti-pAkt (Ser473) mAb (cat # 4060S, Cell Signaling Technology), anti-flotillin (Sigma-Aldrich, St. Louis, MO) and anti- β -actin (Sigma-Aldrich) were used. Antiretroviral drugs (atazanavir, lopinavir/ritonavir, efavirenz, lamivudine) were obtained from the hospital pharmacy of CHRU Besançon. PBLs were treated for different periods of time with these drugs at a final concentration of 50 µM. The level of pAkt/Akt was determined by immunoblotting.

For the detection of Akt, pAkt and Nef proteins in lipid rafts, PBLs were incubated with rNef (100 ng/ml) for various periods of time, then washed extensively and treated with a lysis buffer containing 1% Triton X-100. Cell lysates were ultra-centrifuged over a sucrose gradient ($100.000 \times g$) for 16 hours at 4 °C. Lipid rafts are localized in the light fractions (1–3) as described previously⁷⁷. SDS-PAGE was performed using 20 µl of the pooled fractions (1–3). Proteins were then transferred to nitrocellulose membranes that were blocked and revealed with antibodies directed against Akt, pAkt, Nef or flotillin-1-enriched lipid rafts.

Electrophoretic mobility shift assay. Following stimulation of PBLs with CD3 mAb (5 µg/ml) (cat# 555336), CD28 mAb (5 µg/ml) (cat# 555725) (BD Pharmingen, San Diego, CA) and/or rNef protein (100 ng/ml) for 30 minutes, electrophoretic mobility shift assays (EMSA) were carried out to measure CD28 responsive element (CD28RE) and NF- κ B activation as described previously⁶⁰. The complexes were analyzed on a 6% native polyacrylamide gel.

Cell culture and transfection assays. 293T cells were grown in DMEM supplemented with 10% fetal bovine serum (FBS), 100 IU of penicillin/ml and 100 µg of streptomycin/ml (Invitrogen, Carlsbad, CA). Wild-type and nef-deleted proviral infectious clones of NL4-3 virus isolates (HIV-1_{pNL4-3} WT and HIV-1_{pNL4-3}ΔNef) were kindly provided by Dr Serge Benichou (Institut Cochin, Paris France). Replication competent viruses were produced in 293T cells by the calcium phosphate transfection method (OZ Biosciences, Marseille, France). 5×10^6 PBLs were infected various doses of p24 of either wild-type or ΔNef virus infectious clones. Cell lysates were prepared and levels of Akt and pAkt were determined by immunoblotting and flow cytometry.

IL-2 production and T-cell proliferation. PBLs were either left untreated or treated with Akt inhibitor VIII (25 µM, 50 µM) or PI3K inhibitor LY294002 (25 µM, 50 µM) for two hours followed by treatment with rNef (100 ng/ml), anti-CD3 mAb (5 µg/ml), anti-CD28 mAbs (5 µg/ml) for 24 h and levels of intracellular IL-2 and IL-2 production in culture supernatants were measured using flow cytometric analysis (FACSCalibur flow cytometer, BD Biosciences, San Jose, CA) and ELISA (Bender MedSystems cat # BMS221HS, Paris, France) and cell proliferation was measured using the MTT cell proliferation assay kit (cat# 10009365, Cayman Chemical, Ann Arbor, MI).

FACS analysis. PBLs were fixed and permeabilized (BD Cytofix/Cytoperm kit) (BD Biosciences). Briefly, 2.5×10^6 PBLs were thoroughly resuspended in 250 µl of BD Cytofix/Cytoperm solution for 20 min at 4 °C. Cells were washed two times in 1X BD Perm/Wash solution. The cells were pelleted by centrifugation. The PBLs were thoroughly resuspended in 500 µl of 1X BD Perm/Wash containing BD PhosflowPE-conjugated anti-pAkt (cat# 558275)/anti-Akt (cat# 560049) antibodies (BD Biosciences) or isotype control antibody (cat# 551436) (BD Pharmingen). The cells were incubated at 4 °C for 30 min in the dark. The cells were washed three times in 1X BD Perm/Wash solution and resuspended in 1X PBS prior to flow cytometric analysis.

MTT cell assay. Cell viability was measured using the MTT assay kit (Cayman Chemical). PBLs were seeded at a density of 0.1×10^6 /well in triplicates in 96-well plates in a final volume of 100 µl medium containing antiretroviral drugs (50 µM). After 0-2 hrs, 10 µl MTT reagent was added to each well and the plates were incubated for another 4 h in the cell culture incubator at 37 °C. The plate was centrifuged at 500g for 10 min. The cell culture media was aspirated, 100 µl crystal dissolving solution was added to each well and the absorbance was measured at 570 nm using Multiskan Ex (Thermo Electron Corporation, Cergy-Pontoise, France).

Reactivation from latency in J-Lat 8.4 cells. The T-lymphoid cell line J-Lat 8.4 was obtained from the AIDS Research and Reference Reagent Program (National Institute of Allergy and Infectious Disease [NIAID], National Institute of Health [NIH]). J-Lat 8.4 cells were mock-treated or treated with anti-CD3 (2 µg/ml), anti-CD28 (2.67 µg/ml) and/or rNef (100 ng/ml), or with SAHA (2.5 µM) in the absence or presence of the different PI and RTI compounds. At 24 h or 48 h post treatment, culture supernatants were harvested and HIV-1

production was measured by determining p24 antigen concentration by ELISA (Innogenetics)⁴⁵. A WST 1 assay (Roche) was performed to assess the viability of treated cells.

Luciferase assay. Five million 1G5 cells were either left untreated or treated with protease inhibitor and/or rNef for 24 h. After 24 h cells were collected and luciferase expression was determined using promega luciferase assay kit as per manufactures instructions.

Statistical analyses. The figures show the means and standard deviations for independent experiments. Statistical comparison was performed using the Mann-Whitney U tests (SPSS Inc.; Chicago; USA) or student t test. The level of significance was set at $p < 0.05$.

References

- Fayard, E., Xue, G., Parcellier, A., Bozulic, L. & Hemmings, B. A. Protein kinase B (PKB/Akt), a key mediator of the PI3K signaling pathway. *Curr. Top. Microbiol. Immunol.* **346**, 31–56 (2010).
- Malim, M. H. & Emerman, M. HIV-1 accessory proteins—ensuring viral survival in a hostile environment. *Cell Host. Microbe* **3**, 388–398 (2008).
- Maartens, G., Celum, C. & Lewin, S. R. HIV infection: epidemiology, pathogenesis, treatment, and prevention. *Lancet* **384**, 258–271 (2014).
- Deng, K. & Siliciano, R. F. HIV: Early treatment may not be early enough. *Nature* **512**, 35–36 (2014).
- Liu, X. & Kumar, A. Differential signaling mechanism for HIV-1 Nef-mediated production of IL-6 and IL-8 in human astrocytes. *Sci Rep.* **5**, 9867 (2015).
- Iordanskiy, S., Santos, S. & Bukrinsky, M. Nature, nurture and HIV: The effect of producer cell on viral physiology. *Virology* **443**, 208–213 (2013).
- Gorry, P. R. *et al.* Pathogenicity and immunogenicity of attenuated, nef-deleted HIV-1 strains *in vivo*. *Retrovirology* **4**, 66 (2007).
- Renkema, G. H. & Saksela, K. Interactions of HIV-1 NEF with cellular signal transducing proteins. *Front Biosci.* **5**, D268–D283 (2000).
- Chavez, L., Calvanese, V. & Verdin, E. HIV Latency is established directly and early in both resting and activated primary CD4 T cells. *Mos Pathog.* **11**, e1004955 (2015).
- Wolf, D. *et al.* HIV-1 Nef associated PAK and PI3-kinases stimulate Akt-independent Bad-phosphorylation to induce anti-apoptotic signals. *Nat. Med.* **7**, 1217–1224 (2001).
- Huse, M. The T-cell-receptor signaling network. *J. Cell Sci.* **122**, 1269–1273 (2009).
- Churchill, M. J. *et al.* Transcriptional activity of blood- and cerebrospinal fluid-derived nef/long-terminal repeat sequences isolated from a slow progressor infected with nef-deleted human immunodeficiency virus type 1 (HIV-1) who developed HIV-associated dementia. *J. Neurovirol.* **12**, 219–228 (2006).
- Muratori, C. *et al.* Massive secretion by T cells is caused by HIV Nef in infected cells and by Nef transfer to bystander cells. *Cell Host. Microbe* **6**, 218–230 (2009).
- Qiao, X. *et al.* Human immunodeficiency virus 1 Nef suppresses CD40-dependent immunoglobulin class switching in bystander B cells. *Nat. Immunol.* **7**, 302–310 (2006).
- Diehl, N. & Schaal, H. Make yourself at home: viral hijacking of the PI3K/Akt signaling pathway. *Viruses.* **5**, 3192–212 (2013).
- Ben-Romano, R. *et al.* Nelfinavir-induced insulin resistance is associated with impaired plasma membrane recruitment of the PI 3-kinase effectors Akt/PKB and PKC-zeta. *Diabetologia* **47**, 1107–17 (2004).
- Srirangam, A. *et al.* Effects of HIV protease inhibitor ritonavir on Akt-regulated cell proliferation in breast cancer. *Clin Cancer Res.* **12**, 1883–96 (2006).
- Rajan, A. A Phase I Trial of Nelfinavir (Viracept) in Adults With Solid Tumors. 2011, (Date of access: 30/09/2011). Available from <https://clinicaltrials.gov/ct2/show/record/NCT01445106>.
- Plastaras, J. P. *et al.* Validation and toxicity of PI3K/Akt pathway inhibition by HIV protease inhibitors in humans. *Cancer Biol Ther* **7**, 628–35 (2008).
- Blagoveshchenskaya, A. D., Thomas, L., Feliciangeli, S. E., Hung, C. H. & Thomas, G. HIV-1 Nef downregulates MHC-1 by a PACS-1- and PI3K-regulated ARF6 endocytic pathway. *Cell* **111**, 853–866 (2002).
- Sarbasov, D. D., Guertin, D. A., Ali, S. M. & Sabatini, D. M. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* **307**, 1098–1101 (2005).
- Lakadamyali, M., Rust, M. J. & Zhuang, X. Endocytosis of influenza viruses. *Microbes Infect* **6**, 929–36 (2004).
- Henriques, C. M., Rino, J., Nibbs, R. J., Graham, G. J. & Barata, J. T. IL-7 induces rapid clathrin-mediated internalization and JAK3-dependent degradation of IL-7Ralpha in T cells. *Blood.* **115**, 3269–77 (2010).
- Kotov, A., Zhou, J., Flicker, P. & Aiken, C. Association of Nef with the human immunodeficiency virus type 1 core. *J. Virol.* **73**, 8824–30 (1999).
- Welker, R., Harris, M., Cardel, B. & Kräusslich, H. G. Virion incorporation of human immunodeficiency virus type 1 Nef is mediated by a bipartite membrane-targeting signal: analysis of its role in enhancement of viral infectivity. *J. Virol.* **72**, 8833–40 (1998).
- Rosa, A. *et al.* HIV-1 Nef promotes infection by excluding SERINC5 from virion incorporation. *Nature* **526**, 212–7 (2015).
- Usami, Y., Wu, Y. & Göttlinger, H. G. SERINC3 and SERINC5 restrict HIV-1 infectivity and are counteracted by Nef. *Nature* **526**, 218–23 (2015).
- Stephens, L. *et al.* Protein kinase B kinases that mediate phosphatidylinositol 3,4,5-trisphosphate-dependent activation of protein kinase B. *Science* **279**, 710–714 (1998).
- Navarro, M. N. & Cantrell, D. A. Serine-threonine kinases in TCR signaling. *Nat. Immunol.* **15**, 808–814 (2014).
- Parry, R. V., Riley, J. L. & Ward, S. G. Signaling to suit function: tailoring phosphoinositide 3-kinase during T-cell activation. *Trends Immunol.* **28**, 161–168 (2007).
- Wang, J. K., Kiyokawa, E., Verdin, E. & Trono, D. The Nef protein of HIV-1 associates with rafts and primes T cells for activation. *Proc. Natl. Acad. Sci. USA* **97**, 394–399 (2000).
- Fenard, D. *et al.* Nef is physically recruited into the immunological synapse and potentiates T cell activation early after TCR engagement. *J. Immunol.* **175**, 6050–6057 (2005).
- Thomas, R. M., Gao, L. & Wells, A. D. Signals from CD28 induce stable epigenetic modification of the IL-2 promoter. *J. Immunol.* **174**, 4639–4646 (2005).
- Gantt, S., Casper, C. & Ambinder, R. E. Insights into the broad cellular effects of nelfinavir and the HIV protease inhibitors supporting their role in cancer treatment and prevention. *Curr. Opin. Oncol.* **25**, 495–502 (2013).
- Gupta, A. K., Cerniglia, G. J., Mick, R., McKenna, W. G. & Muschel, R. J. HIV protease inhibitors block Akt signaling and radiosensitize tumor cells both *in vitro* and *in vivo*. *Cancer Res.* **65**, 8256–8265 (2005).
- Bernstein, W. B. & Dennis, P. A. Repositioning HIV protease inhibitors as cancer therapeutics. *Curr Opin HIV AIDS* **3**, 666–75 (2008).

37. Wallet, M. A. *et al.* The HIV-1 protease inhibitor nelfinavir activates PP2 and inhibits MAPK signaling in macrophages: a pathway to reduce inflammation. *J. Leukoc. Biol.* **92**, 795–805 (2012).
38. Wagner, T. A. *et al.* HIV latency. Proliferation of cells with HIV integrated into cancer genes contributes to persistent infection. *Science* **345**, 570–573 (2014).
39. Van Lint, C., Bouchat, S. & Marcello, A. HIV-1 transcription and latency: an update. *Retrovirology* **10**, 67 (2013).
40. Hill, A. L., Rosenbloom, D. I., Fu, F., Nowak, M. A. & Siliciano, R. F. Predicting the outcomes of treatment to eradicate the latent reservoir for HIV-1. *Proc. Natl. Acad. Sci. USA* **111**, 13475–13480 (2014).
41. Ho, Y. C. *et al.* Replication-competent noninduced proviruses in the latent reservoir increase barrier to HIV-1 cure. *Cell* **155**, 540–551 (2013).
42. Deeks, S. G. *et al.* Towards an HIV cure: a global scientific strategy. *Nat. Rev. Immunol.* **12**, 607–614 (2012).
43. Kumar, A., Abbas, W. & Herbein, G. HIV-1 latency in monocytes/macrophages. *Viruses* **6**, 1837–1860 (2014).
44. Contreras, X. *et al.* Suberoylanilide hydroxamic acid reactivates HIV from latently infected cells. *J. Biol. Chem.* **284**, 6782–6789 (2009).
45. Reuse, S. *et al.* Synergistic activation of HIV-1 expression by deacetylase inhibitors and prostratin: implications for treatment of latent infection. *PLoS One* **4**, e6093 (2009).
46. Schragar, J. A., Der Minasian, V. & Marsh, J. W. HIV Nef increases T cell ERK MAP kinase activity. *J. Biol. Chem.* **277**, 6137–42 (2002).
47. Xue, M. *et al.* HIV-1 Nef and KSHV oncogene K1 synergistically promote angiogenesis by inducing cellular miR-718 to regulate the PTEN/AKT/mTOR signaling pathway. *Nucleic Acids Res.* **42**, 9862–79 (2014).
48. Neri, E., Giolo, G., Potesta, M., Petrini, S. & Doria, M. The HIV-1 Nef protein has a dual role in T cell receptor signaling in infected CD4+ T lymphocytes. *Virology* **410**, 316–326 (2011).
49. James, C. O. *et al.* Extracellular Nef protein targets CD4+ T cells for apoptosis by interacting with CXCR4 surface receptors. *J. Virol.* **78**, 3099–3109 (2004).
50. Martelli, A. M. *et al.* The emerging multiple roles of nuclear Akt. *Biochim. Biophys. Acta* **1823**, 2168–2178 (2012).
51. Lenassi, M. *et al.* HIV Nef is secreted in exosomes and triggers apoptosis in bystander CD4+ T cells. *Traffic* **11**, 110–122 (2010).
52. de Carvalho, J. V. *et al.* Nef neutralizes the ability of exosomes from CD4+ T cells to act as decoys during HIV-1 infection. *PLoS One* **9**, e113691 (2014).
53. Arenaccio, C. *et al.* Exosomes from human immunodeficiency virus type 1 (HIV-1)-infected cells license quiescent CD4+ T lymphocytes to replicate HIV-1 through a Nef- and ADAMI7-dependent mechanism. *J. Virol.* **88**, 11529–11539 (2014).
54. Luo, X., Fan, Y., Park, I. W. & He, J. J. Exosomes are unlikely involved in intercellular Nef transfer. *PLoS One* **10**, e0124436 (2015).
55. Deregius, M. C. *et al.* HIV-1-Tat protein activates phosphatidylinositol 3-kinase/ AKT-dependent survival pathways in Kaposi's sarcoma cells. *J. Biol. Chem.* **277**, 25195–25202 (2002).
56. Alexander, M., Bor, Y. C., Ravichandran, K. S., Hammarskjöld, M. L. & Rekosh, D. Human immunodeficiency virus type 1 Nef associates with lipid rafts to downmodulate cell surface CD4 and class I major histocompatibility complex expression and to increase viral infectivity. *J. Virol.* **78**, 1685–1696 (2004).
57. Calay, D. *et al.* Inhibition of Akt signaling by exclusion from lipid rafts in normal and transformed epidermal keratinocytes. *J. Invest. Dermatol.* **130**, 1136–1145 (2010).
58. Honejš, V. & Hrdinka, M. Membrane microdomains in immunoreceptor signaling. *FEBS Lett.* **588**, 2392–7 (2014).
59. Keppeler, O. T., Tibroni, N., Venzke, S., Rauch, S. & Fackler, O. T. Modulation of specific surface receptors and activation sensitization in primary resting CD4+ T lymphocytes by the Nef protein of HIV-1. *J. Leukoc. Biol.* **79**, 616–627 (2006).
60. Varin, A. *et al.* Exogenous Nef protein activates NF- κ B, AP-1, and c-Jun N-terminal kinase and stimulates HIV transcription in promonocytic cells. Role in AIDS pathogenesis. *J. Biol. Chem.* **278**, 2219–2227 (2003).
61. Markle, T. J., Philip, M. & Brockman, M. A. HIV-1 Nef and T-cell activation: a history of contradictions. *Future. Virol.* **8** (2013).
62. Liu, X. *et al.* HIV-1 Nef induces C/CL5 production in astrocytes through p38-MAPK and PI3K/Akt pathway and utilizes NF- κ B, C/EBP and AP-1 transcription factors. *Sci Rep.* **4**, 4450 (2014).
63. Schragar, J. A. & Marsh, J. W. HIV-1 Nef increases T cell activation in a stimulus-dependent manner. *Proc. Natl. Acad. Sci. USA* **96**, 8167–8172 (1999).
64. Park, I. W. & He, J. J. HIV-1 Nef-mediated T-cell activation and chemotaxis are decoupled using a HIV-1/SIVpb1.9. chimeric nef variant. *Arch. Virol.* **158**, 845–852 (2013).
65. Fortin, J. E., Barat, C., Beausejour, Y., Barbeau, B. & Tremblay, M. J. Hyper-responsiveness to stimulation of human immunodeficiency virus-infected CD4+ T cells requires Nef and Tat virus gene products and results from higher NEAT, NF- κ B, and AP-1 induction. *J. Biol. Chem.* **279**, 39520–39531 (2004).
66. Doyon, G., Zerbato, J., Mellors, J. W. & Sluis-Cremer, N. Disulfiram reactivates latent HIV-1 expression through depletion of the phosphatase and tensin homolog. *AIDS* **27**, F7–F11 (2013).
67. Gupta, A. K. *et al.* The HIV protease inhibitor nelfinavir downregulates Akt phosphorylation by inhibiting proteasomal activity and inducing the unfolded protein response. *Neoplasia* **9**, 271–278 (2007).
68. Cummins, N. W., Sainki, A. M., Natesampillai, S., Bren, G. D. & Badley, A. D. Choice of antiretroviral therapy differentially impacts survival of HIV-infected CD4 T cells. *Mol. Cell Ther.* **2**, 1 (2014).
69. Titanji, B. K., Aasa-Chapman, M., Pillay, D. & Jolly, C. Protease inhibitors effectively block cell-to-cell spread of HIV-1 between T cells. *Retrovirology* **10**, 161 (2013).
70. Tyagi, M. & Bukrinsky, M. Human immunodeficiency virus (HIV) latency: the major hurdle in HIV eradication. *Mol. Med.* **18**, 1096–1108 (2012).
71. Matsuda, Y., Kobayashi-Ishihara, M., Fujikawa, D., Ishida, T., Watanabe, T. & Yamagishi, M. Epigenetic heterogeneity in HIV-1 latency establishment. *Sci Rep.* **5**, 7701 (2015).
72. Darcis, G. *et al.* An In-Depth Comparison of Latency-Reversing Agent Combinations in Various *In Vitro* and *Ex Vivo* HIV-1 Latency Models Identified Bryostatins-1 + JQ1 and Ingenol-B + JQ1 to Potently Reactivate Viral Gene Expression. *PLoS Pathog.* **11**, e1005063 (2015).
73. Kumar, A., Darcis, G., Van Lint, C. & Herbein, G. Epigenetic control of HIV-1 post integration latency: implications for therapy. *Clinical Epigenetics* **7**, 103 (2015).
74. König, R. & Stertz, S. Recent strategies and progress in identifying host factors involved in virus replication. *Curr Opin Microbiol.* **26**, 79–88 (2015).
75. Chugh, P. *et al.* Akt inhibitors as an HIV-1 infected macrophage-specific anti-viral therapy. *Retrovirology* **5**, 11 (2008).
76. Herbein, G. *et al.* Apoptosis of CD8+ T cells is mediated by macrophages through interaction of HIV gp120 with chemokine receptor CXCR4. *Nature* **395**, 189–194 (1998).
77. Popik, W., Alce, T. M. & Au, W. C. Human immunodeficiency virus type 1 uses lipid raft-coloalized CD4 and chemokine receptors for productive entry into CD4+ T cells. *J. Virol.* **76**, 4709–4722 (2002).

Acknowledgements

We thank the HIV-1-infected patients for their willingness to participate in this study and Elodie Goudeseune, Joelle Cailleau and Annick Caestecker who cared for the patients. We thank Christelle Cardona for excellent

technical assistance. This work was supported by grants from the University of Franche-Comté, the Région Franche-Comté (RECH-FON12-000013), the Agence Nationale de Recherche sur le SIDA (ANRS, n°13543 and 13544) and HIVERA 2013 (EURECA project) (to GH), from the Belgian Fund for Scientific Research (FRS-FNRS, Belgium), the NEAT program (European AIDS treatment network), the "Fondation Roi Baudouin" (Belgium) and the ANRS (France) (to CVL), and the "Institut Universitaire de France (IUF)" (to OR) and by HIVERA:EURECA, ERANET 01K11307A (to R.K.). W.A. and K.A.K. are recipients of doctoral scholarships from the Higher Education Commission, Pakistan. A.K. is a recipient of a postdoctoral grant of Région Franche-Comté (N° 201 2C-06102). L.C. is Postdoctoral Researcher of the FRS-FNRS. S.B. is a recipient of fellowship from the Belgian 'Fonds pour la Recherche dans l'Industrie et l'Agriculture' (FRIA). J.S.G. was a fellow of the Excellence program 'Cibles'. C.V.L. is Research Director of the FRS-FNRS.

Author Contributions

G.H., C.V.L., R.K., C.R. and T.F. conceived and designed the project. W.A., A.K., L.C., K.A.K., S.B., A.V., A.L., J.S.G., C.V., N.D., L.C., S.P. and A.B. performed experiments. G.H., C.V.L., O.R., K.K., N.C., S.D.W. and C.R. participated to the enrolment of HIV-infected patients and to the study of the viral reservoir. G.H., C.V.L., A.K. and L.C. wrote the manuscript.

Additional Information

Supplementary information accompanies this paper at <http://www.nature.com/srep>

Competing financial interests: G.H. and O.R. are editorial board members. The authors declare no other conflict of interest.

How to cite this article: Kumar, A. *et al.* Tuning of AKT-pathway by Nef and its blockade by protease inhibitors results in limited recovery in latently HIV infected T-cell line. *Sci. Rep.* **6**, 24090; doi:10.1038/srep24090 (2016).



This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>

8. Etude de l'impact des inhibiteurs de protéase sur les réservoirs de latence virale et l'activation d'Akt dans l'infection par le VIH.

8.1. Impact du type de traitement antiviral sur l'infection par VIH-1

Nous avons évalué l'effet des thérapies antirétrovirales combinées (combinaison AntiRetroviral Therapy, cART) sur la taille des réservoirs de latence du VIH-1.

Le réservoir viral a été quantifié par qPCR (ADN proviral) dans les lymphocytes T CD4+ quiescents et les monocytes isolés de patients infectés par le VIH (essai clinique HIVERA-EURECA, numéro NCT02858414). Les patients ont été classés en trois groupes selon le type de traitement reçu : patients sous inhibiteurs de protéases virales (groupe PI), patients sous inhibiteurs non nucléosidiques de transcriptase inverse (NNRTI), patients naïfs de traitement (groupe N).

Les mesures ont montré une diminution significative de la quantité d'ADN proviral présent dans les monocytes et dans les lymphocytes T CD4+ quiescents sous traitements cART par rapports aux patients naïfs de traitement.

Nous avons également observé une quantité d'ADN proviral plus faible dans les monocytes que dans les lymphocytes T CD4+ quiescents autologues, chez les patients naïfs de traitement et chez les patients traités par cART.

Nous n'avons pas observé de différence significative dans la mesure de l'ADN proviral entre les patients traités par NNRTI et les patients traités par PI.

8.2. Effet sur l'activation d'Akt par la protéine Nef du VIH

La protéine Nef du VIH-1 augmente l'expression d'Akt dans les macrophages, ainsi que son activation, *in vitro*. L'utilisation de Nef recombinante sur des macrophages dérivés de monocytes entraîne une augmentation de l'expression d'Akt au niveau transcriptionnel, mesuré par RT-qPCR et au niveau protéique, mesuré par western blot. L'utilisation de Nef recombinante sur des macrophages entraîne également une augmentation de l'activation d'Akt, observable par l'augmentation de la phosphorylation sur le résidu Sérine 473, mesuré par Western blot.

Les inhibiteurs de protéase entraînent une réduction de l'activation d'Akt. Le traitement des macrophages par l'inhibiteur de protéase Lopinavir/Ritonavir bloque l'activation d'Akt par Nef recombinante *in vitro*. Une réduction de l'activation d'Akt, observable par la phosphorylation du résidu Sérine 473, est mesurée dans les macrophages traités par Lopinavir/Ritonavir et rNef par cytométrie en flux, par rapport aux macrophages traités par rNef uniquement. Cette réduction de l'activation d'Akt a également été vérifiée par Western blot.

8.3. Blocage de la réactivation du VIH *in vitro*

La réactivation du VIH latent dans les cellules de la lignée monocytoïde U1 infectées de manière chronique par le TNF-alpha est bloquée lors du traitement de ces cellules par l'inhibiteur de protéase Lopinavir/Ritonavir. Le traitement des cellules par l'inhibiteur de transcriptase inverse Névirapine ne réduit pas la réactivation par TNF-alpha, quantifiée par mesure de la protéine virale p24, par rapport à la réactivation par TNF-alpha seul. Le traitement par Lopinavir/Ritonavir bloque la réactivation, à un niveau similaire à l'absence de réactivation. Le traitement par inhibiteur d'Akt réduit également la réactivation. Le traitement par Lopinavir/Ritonavir réduit également l'activation d'Akt, mesurée par Western blot, mais pas le traitement par Névirapine. Les PI et les inhibiteurs d'Akt bloquent la réactivation virale *in vitro*, ce qui n'est pas le cas des NNRTI.

8.4. Réduction de l'activation d'Akt ex vivo.

Le niveau d'activation d'Akt a été mesuré par cytométrie en flux par mesure du rapport entre le marquage phosphoAkt et le marquage Akt total sur les lymphocytes de sang périphérique (PBLs), les lymphocytes T CD4+ quiescents et les monocytes de patients infectés par le VIH. Nous avons observé une réduction de l'activation d'Akt dans les monocytes par rapport aux lymphocytes T CD4+ quiescents autologues. Nous avons également observé une diminution de l'activation d'Akt chez les patients sous inhibiteurs de protéases virale (PI) par rapport aux patients sous inhibiteurs de transcriptase inverse (NNRTI), dans les lymphocytes T CD4+ quiescents et dans les monocytes.

8.5. Discussion

Nous avons montré que les PI réduisent la taille du réservoir de latence virale dans les monocytes de patients infectés par le VIH par rapport aux NNRTI. Globalement, la taille du réservoir de latence dans les monocytes est inférieure au réservoir présent dans les lymphocytes T CD4+, ce qui correspond aux observations réalisés par ailleurs, ainsi que chez les macaques infectés par le SIV [317].

Nous avons montré une réduction de l'activation d'Akt dans les monocytes des patients sous PI par rapport aux patients sous NNRTI. Cette observation est similaire aux données connues pour les lymphocytes T CD4+. Nous avons également observé une réduction de la réactivation virale lors du traitement par PI. L'activation de la voie de signalisation PI3K/Akt a été montrée comme pouvant induire une réactivation de l'expression du VIH. Le blocage de l'activation d'Akt et de la réactivation par les PI pourrait être l'objet d'une modification des approches thérapeutiques, notamment dans le cadre d'une stratégie « block and lock ». Ces inhibiteurs de protéases pourraient également être associés à des inhibiteurs d'Akt. Cela permettrait une inhibition de la réactivation et une réduction de la taille des réservoirs de latence par l'effet pro-apoptotique de l'inhibition d'Akt [318].

Le rôle cellulaire central d'Akt, qui participe notamment de manière importante à la régulation de la survie cellulaire, en fait une cible thérapeutique d'intérêt. Le ciblage d'Akt par des inhibiteurs de protéase et des inhibiteurs spécifiques d'Akt pourrait permettre une réduction de la survie cellulaire des cellules infectées par le VIH, accélérant ainsi la clairance des réservoirs de latence virale.

La difficulté de la mise en place de nouvelles stratégies thérapeutiques ciblant les monocytes/macrophages, notamment avec des inhibiteurs de protéase, tient dans la résistance de ces cellules aux traitements qui est importante. La pénétration cellulaire des traitements est réduite

dans les macrophages, notamment pour les inhibiteurs de protéases [200]. De plus, la longue durée de vie et leur résistance aux effets cytopathiques rendent le réservoir viral des monocytes/macrophages stable [319].

Cette étude a fait l'objet d'une publication dans la revue Viruses en 2018.

8.6. Article paru dans la revue Viruses en 2018 « Counteracting Akt Activation by HIV Protease Inhibitors in Monocytes/Macrophages »



Article

Counteracting Akt Activation by HIV Protease Inhibitors in Monocytes/Macrophages

Sébastien Pasquereau ^{1,†}, Amit Kumar ^{1,†} , Wasim Abbas ¹ and Georges Herbein ^{1,2,*}

¹ Pathogens & Inflammation/EPILAB Laboratory, UPRES EA4266, University of Franche-Comté, COMUE Bourgogne Franche-Comté University, 25030 Besançon, France; sebastien.pasquereau@univ-fcomte.fr (S.P.); amit.aiims2005@gmail.com (A.K.); wazim_cemb@hotmail.com (W.A.)

² Department of Virology, CHRU Besançon, 25030 Besançon, France

* Correspondence: georges.herbein@univ-fcomte.fr; Tel.: +33-381-665-552

† These authors contributed equally to this work.

Received: 16 February 2018; Accepted: 11 April 2018; Published: 13 April 2018



Abstract: Akt signaling plays a central role in many biological processes that are key players in human immunodeficiency virus 1 (HIV-1) pathogenesis. The persistence of latent reservoirs in successfully treated patients, mainly located in macrophages and latently infected resting CD4+ T cells, remains a major obstacle in HIV-1 eradication. We assessed the *in vitro* effects of an HIV protease inhibitor (PI) and a non-nucleoside reverse transcriptase inhibitor (NNRTI) on HIV-1 Nef-induced Akt activation in macrophages and on HIV-1 reactivation in U1 monocytoïd cells. *Ex vivo*, we investigated the impact of combination antiretroviral therapy (cART) on Akt activation, as measured by flow cytometry, and on the viral reservoir size, quantified by qPCR, in monocytes and autologous resting CD4+ T cells from HIV-infected individuals (Trial registration: NCT02858414). We found that, in myeloid cells, both Akt activation and HIV-1 reactivation were inhibited by PI but not by NNRTI *in vitro*. Our results indicate that cART decreases Akt activation and reduces the size of the HIV reservoir in both monocytes and resting CD4+ T cells. Our study indicates that Akt activation could play a role in HIV reservoir formation, indicating that drugs which target Akt could be efficient for limiting its size in aviremic chronically infected patients.

Keywords: Akt; HIV-1; monocyte; resting CD4+ T cells; cART; protease inhibitor; NNRTI; reservoir

1. Introduction

The serine/threonine kinase Akt, also known as protein kinase B, plays important roles in the cellular processes involved in HIV-1 pathogenesis. As a key regulator in the phosphoinositide 3-kinase (PI3K) pathway, it impacts cell survival, metabolism, growth, and proliferation [1]. The HIV-1 infection progression is fuelled by immune hyperactivation, which constitutes a hallmark of the disease. In infected individuals, the tumor necrosis factor (TNF) alpha is one of the proinflammatory cytokines that could be critical for the formation of HIV-1 reservoirs [2–5].

HIV-1 Nef is an accessory viral protein known to be abundantly expressed in the early stages of infection. Several aspects of the viral pathogenesis have been showed to be affected by this multifunctional protein, which plays an important role despite lacking enzymatic activity [6,7]. Besides endogenously produced Nef, extracellular soluble Nef and/or vesicle-associated Nef are found in the serum or extracellular environment of infected individuals and display immunomodulatory effects such as the suppression of immunoglobulin class switching in bystander B cells and neurotoxicity [8–11]. The PI3K/Akt pathway, a key pathway of cell survival, is known to be manipulated by a plethora of viral pathogens, including among others human cytomegalovirus, Epstein Barr virus, influenza A virus, and HIV-1 [12]. Several studies have also reported the impact of

protease inhibitors (PIs) on Akt signaling in several cell types and in clinical trials [13–19]. We observed previously a critical role for Nef in the hyperactivation of T cells through Akt activation and that blocking Akt activation especially by HIV-1 protease inhibitors (PIs) could limit HIV-1 recovery from latently infected T cells [20,21].

The use of combination anti-retroviral therapy (cART) has allowed for the reduction of the viral load of infected individuals to undetectable levels [22]. However, in the majority of patients that are considered successfully treated by cART, low levels of active viral replication can be detected by the use of new ultrasensitive assays [23]. A major obstacle to HIV-1 eradication is indeed the persistence of replication-competent proviruses [24,25]. These latent reservoirs are established early during acute viral infection and include, among others, macrophages and latently infected resting CD4+ T cells, these latently infected resting CD4+ T cells being the main viral reservoir [26–29]. Recently, cART intensification was assessed and did not reduce residual HIV-1 viremia in patients on cART, indicating that its potential to eradicate the virus appears to be limited [30]. In addition, immune hyperactivation, especially TNF production, still persists in aviremic patients under cART and could explain the limited effect of cART on the eradication of HIV reservoirs [31,32].

Initiation of cART during primary HIV infection may limit the establishment of viral reservoirs, and very early cART limits the seeding of the HIV reservoir in long-lived central memory CD4+ T cells [26,33,34]. By contrast, the HIV reservoir in monocytes/macrophages has been much less studied and the impact of cART on the monocyte/macrophage HIV-1 reservoir in aviremic chronically infected patients is so far unknown [29,35,36].

We report here a study indicating that Akt activation by Nef in Monocyte-Derived Macrophages (MDMs) can be blocked by the PI lopinavir/ritonavir, but not by the NNRTI nevirapine. We observed the important role of Akt activation in HIV-1 reactivation from latently infected U1 monocytoïd cells *in vitro*, which could be blocked by PI but not by NNRTI. Finally, *in vivo* cART-blocked Akt activation in monocytes, but also in resting CD4+ T cells, meanwhile limited the size of the viral reservoir in both cell types isolated from aviremic chronically HIV-1 infected patients.

2. Materials and Methods

2.1. Isolation and Culture of Peripheral Blood Mononuclear Cells (PBMCs), Peripheral Blood Lymphocytes (PBLs), MDMs, and Resting CD4+ T Cells

Human PBMCs, purified PBLs, and MDMs were prepared from the peripheral blood of healthy donors and cultured as described previously [37]. Purified CD25⁻, CD69⁻, and HLA-DR⁻ CD4+ T cells (resting CD4+ T cells) were obtained from the peripheral blood of HIV⁺ patients using a negative selection assay using magnetic beads (LD columns, Miltenyi Biotec, Bergisch Gladbach, Germany) as described previously [38]. PBMCs were isolated from the peripheral blood of HIV⁺ patients by Ficoll gradient centrifugation as described previously [37] and cultured in Roswell Park Memorial Institute (RPMI) medium supplemented with 10% (*v/v*) fetal calf serum. PBLs in suspension and adherent monocytes were collected after 2 h as described previously [37].

2.2. Recombinant Nef Treatment

MDMs (5×10^6 cells) were treated with recombinant myristoylated Nef protein (rNef) from the SF2 HIV-1 strain (1–100 ng/mL) (cat # PR-382, Jena Bioscience, Jena, Germany). Cell pellets were collected at various times after rNef treatment, washed extensively, and either lysed before Western blot analysis or fixed with BD Cytotfix (BD Biosciences, San Jose, CA, USA) for 20 min before flow cytometric analysis. MDMs were treated with the Akt inhibitor VIII (cat # sc-202048A) (0, 25, 50 μ M) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 2 h before addition of rNef (100 ng/mL) for 30 min.

2.3. Western Blot

Cellular extracts from MDMs treated with rNef were used to examine Akt and pAkt expression by Western blotting as described previously [39]. Anti-Akt-mAb (cat # 2967S, Cell Signaling Technologies, St Quentin, France), anti-pAkt (Thr308) mAb (cat # 2965S, Cell Signaling Technology), anti-pAkt (Ser473) mAb (cat # 4060S, Cell Signaling Technology), and anti- β -actin (Sigma-Aldrich, St. Louis, MO, USA) were used. Antiretroviral drugs (lopinavir/ritonavir and nevirapine) were obtained from the hospital pharmacy of the Centre Hospitalier Régional Universitaire (CHRU) Besançon. MDMs were treated for different periods of time with these drugs at indicated concentrations. The level of pAkt/Akt was determined by immunoblotting.

2.4. RT-PCR Assay

For the detection of Akt mRNA using an RT-PCR assay, total RNA was extracted from MDMs either left untreated or treated with increasing concentrations of rNef (1–25 ng/mL) for 30 min using an RNeasy mini kit (Qiagen, Hilden, Germany). A total of 2 μ g of RNA was reverse transcribed into cDNA with Superscript IV RT (Life Technologies, Carlsbad, CA, USA) using oligo (dT) primers. The 5 μ L of reverse transcription reaction product was amplified using primers against Akt (sense, 5'-ATCCCCTCAACAACCTTCTCAGT-3'; antisense, 5'-CTTCCGTCCACTCTTCTCTTTC-3'). The beta-globin gene was amplified as an internal control (sense, 5'-TCCCCTCTACCCCTACTTTCTA-3'; antisense, 5'-TGCCTGGACTAATCTGCAAGAG-3'). The PCR product was electrophoresed on a 2% agarose gel containing SYBR Green I Stain (Lonza, Rockland, ME, USA).

2.5. FACS Analysis

PBLs, monocytes, and resting CD4+ T cells were fixed and permeabilized (BD Cytotfix/Cytoperm kit) (BD Biosciences, San Jose, CA, USA). Briefly, 10^6 cells were thoroughly resuspended in 250 μ L of BD Cytotfix/Cytoperm solution for 20 min at 4 °C. Cells were washed two times in 1X BD Perm/Wash solution. The cells were pelleted by centrifugation. The cells were thoroughly resuspended in 500 μ L of 1X BD Perm/Wash containing PE-conjugated anti-pAkt (cat# 558275)/anti-Akt (cat# 560049) antibodies (BD Biosciences) or isotype control antibody (cat# 551436) (BD Pharmingen). The cells were incubated at 4 °C for 30 min in the dark. The cells were washed three times in 1X BD Perm/Wash solution and resuspended in 1X PBS prior to flow cytometric analysis.

2.6. MTT Cell Assay

Cell viability was measured using the MTT assay kit (Cayman Chemical, Ann Arbor, MI, USA). Where specified, MDMs and monocytoïd U1 cells stimulated or not with TNF were seeded at a density of 0.1×10^6 /well in triplicates in 96-well plates in a final volume of 100 μ L medium containing lopinavir/ritonavir, nevirapine, and Akt inhibitor VIII at 50 μ M for 0–2 h. MDMs were left untreated or treated with rNef (1–100 ng/mL) for 30 min. After 0–2 h, 10 μ L MTT reagent was added to each well and the plates were incubated for another 4 h in the cell culture incubator at 37 °C. The plate was centrifuged at 500 g for 10 min. The cell culture media was aspirated, 100 μ L of crystal-dissolving solution was added to each well, and the absorbance was measured at 570 nm using Multiskan Ex (Thermo Electron Corporation, Cergy-Pontoise, France).

2.7. Reactivation from Latency in U1 Cells

The monocytoïd U1 cell line was obtained from the AIDS Research and Reference Reagent Program (National Institute of Allergy and Infectious Disease (NIAID), National Institute of Health (NIH)). Cells were mock-treated or treated with TNF 10 ng/mL in the absence or presence of lopinavir/ritonavir, nevirapine, and Akt inhibitor VIII at 50 μ M. At 24 h post treatment, culture supernatants of U1 cells were harvested and HIV-1 production was measured by determining

p24 antigen concentration by ELISA (Innogenetics, Ghent, Belgium) [40]. A WST 1 assay (Roche, Basel, Switzerland) was performed to assess the viability of treated cells.

2.8. Quantification of HIV-1 Proviral DNA

DNA was extracted from 10^6 cells monocytes and resting CD4+ T cells using a QIAamp DNA Blood kit (Qiagen, Hilden, Germany) as per the manufacturer's instructions. HIV-1 proviral DNA was quantified using a Biocentric Generic HIV DNA Cell kit (Biocentric, Bandol, France) on an MX3005p real-time PCR instrument (Stratagene, San Diego, CA, USA) as per the manufacturers' instructions.

2.9. Patients

We enrolled 31 chronically HIV-1-infected individuals at the Besançon University Hospital (Besançon, France). The patients were treated with cART (treatment range: 1.4–27.2 years) and had undetectable plasma HIV-1 RNA levels (<40 copies/mL) for at least 1 year. These 31 patients treated with cART (PI, $n = 8$; NNRIL, $n = 23$) were studied for levels of Akt activation and HIV-1 proviral DNA in monocytes and autologous resting CD4+ T cells. In addition, we enrolled four HIV-1-infected individuals at the Besançon University Hospital (Besançon, France). These patients were naïve from cART treatment and were studied for HIV-1 proviral DNA in monocytes and autologous resting CD4+ T cells.

2.10. Statistical Analyses

The figures show the means and standard deviations for independent experiments. Plotting and statistical analysis were performed using Excel. Results from *in vitro* reactivation studies and HIV proviral DNA using patient cell cultures of monocytes and resting CD4+ T cells are shown as medians and quartiles. Data sets were analyzed using an unpaired nonparametric *t*-test (Mann–Whitney test) or Wilcoxon *t* test. Differences were considered significant at a value of $p < 0.05$.

2.11. Ethics Approval and Consent to Participate

All of the patients who were enrolled at the Besançon University Hospital (France) gave their written informed consent to participate in the study according to the Helsinki declaration. The Human Protection Committee East Area II (CPP EST-2) from France was consulted and approved the study (CPP14/455) (Trial registration number: NCT02858414; Name of registry: Exploratory Study of Cellular Reservoirs in Blood From HIV Infected Patients (EURECA); URL of registry: clinicaltrials.gov; Date of registration: 29 July 2016; Date of enrolment of the first participant to the trial: 9 June 2015; “Retrospectively registered”). This study did not rely solely on medical records. The authors did not have any contact with the study subjects and performed tests on patient blood samples that were part of routine care. The blood samples were anonymized before being used by the authors.

3. Results

3.1. Recombinant Nef Increases Akt Expression and Phosphorylation in MDMs *In Vitro*

We studied the impact of Nef on both Akt expression and activation in MDMs. We observed that treatment of MDMs with rNef led to enhanced Akt expression in a dose-dependent manner (Figure 1A, upper panel). We performed an RT-PCR assay in order to evaluate mRNA Akt expression in rNef-treated MDMs. We observed enhanced mRNA Akt levels in rNef-treated MDMs compared to untreated MDMs, indicating that the increase in Akt expression after Nef treatment is transcriptional (Figure 1A, lower panel). Akt is activated by its phosphorylation on Ser473 and Thr308 residues [41]. We found that Akt became phosphorylated on serine⁴⁷³ and threonine³⁰⁸ in MDM treated with rNef as determined by Western blotting and flow cytometry (Figures 1B and 2A). The increased expression of total Akt measured in rNef-treated MDM was dose-dependent (Figure 1C). We did not find any

significant toxicity of rNef (1–100 ng/mL) for as long as 30 min as determined by a cell viability assay (Figure 1D).

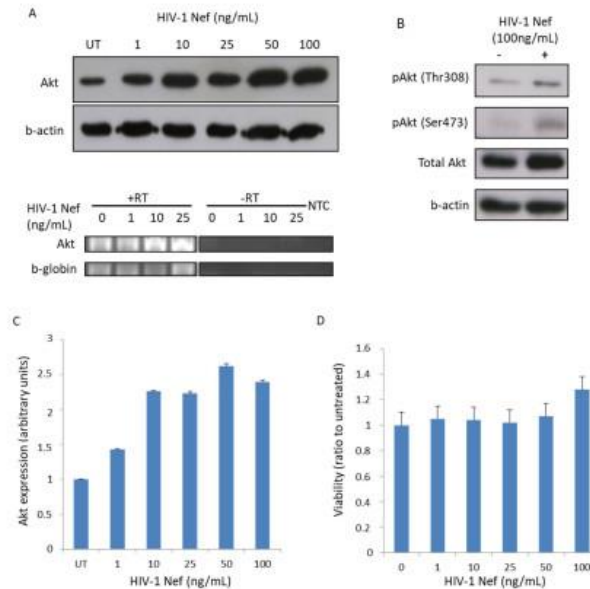


Figure 1. HIV-1 Nef enhances Akt expression and activation in MDMs in vitro. (A) Monocyte-Derived Macrophages (MDMs) were either left untreated or treated with increasing concentrations of rNef (1–100 ng/mL) for 30 min. After incubation, protein lysates and RNA extracts were made. *Upper panel.* Expression of total Akt and β -actin was determined by Western blotting. *Lower panel.* Akt mRNA expression was measured by an RT-PCR assay on a 2% agarose gel. Beta-globin was used as an internal control. (B) MDMs were either left untreated or treated with rNef (100 ng/mL) for 30 min. After incubation, protein lysates were made and expression of pAkt(Ser473), pAkt(Thr308), and total Akt and β -actin were determined by Western blotting ($n = 3$). (C) The histogram shows the enhanced expression of total Akt in MDMs treated with increasing concentrations of Nef for 30 min. UT, untreated. (D) No significant toxicity of rNef (1–100 ng/mL) was observed in MDM treated for 30 min as measured by a cell viability assay.

3.2. The Protease Inhibitor (PI) Lopinavir/Ritonavir Blocks Akt Activation in MDMs Treated with rNef in Vitro

Since HIV-1 protease inhibitors (PI), but not reverse transcriptase inhibitors (RTI), have been reported to block Akt activation in several cell types, such as peripheral blood mononuclear cells (PBMCs) and monocytes/macrophages [13,15–19], we assessed the potential role of PIs as immunomodulators in addition to their antiviral effect in HIV-1 infection. In vitro, we observed that the PI lopinavir/ritonavir, but not the non-nucleoside reverse transcriptase inhibitor (NNRTI) nevirapine, inhibited Akt hyperactivation in MDMs stimulated with rNef using flow cytometric analysis (Figure 2A,B). The inhibition of Akt activation by lopinavir/ritonavir was dose-dependent (Figure 2C upper panel and Figure 2D). We observed a slight inhibition of Akt activation by lopinavir/ritonavir in the absence of Nef treatment (Figure 2C lower panel). At the concentrations of anti-HIV drugs used, more than 80–90% of treated cells were viable after two hours of treatment.

Viruses 2018, 10, 190

6 of 18

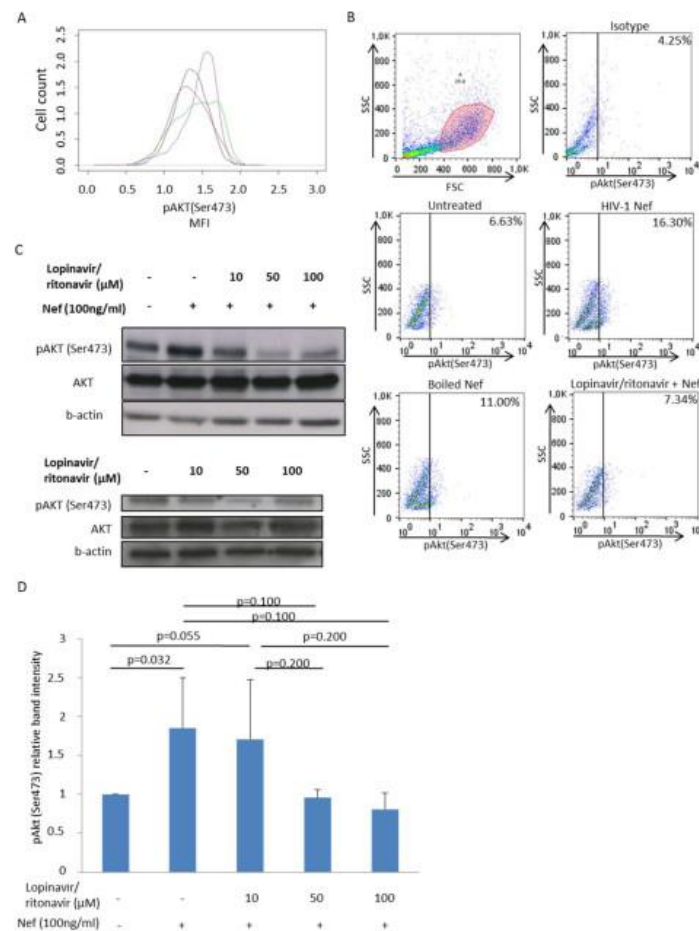


Figure 2. The protease inhibitor (PI) lopinavir/ritonavir blocks Akt activation in MDMs treated with Nef. (A) Activation of Akt (pAkt) in MDMs treated with rNef (100 ng/mL) for 30 min is blocked by the PI lopinavir/ritonavir (50 μM) but not by the non-nucleoside reverse transcriptase inhibitor (NNRTI) nevirapine (50 μM) as measured by flow cytometric analysis. Red: untreated MDM, green: MDM treated with Nef, blue: MDM treated with Nef + lopinavir/ritonavir; purple: MDM treated with Nef + nevirapine. Results are representative of three independent experiments. (B) The PI lopinavir/ritonavir inhibits Akt activation (pAktSer473) in MDMs treated with rNef as determined by flow cytometric analysis. One million MDMs were treated with rNef (100 ng/mL) for 30 min in the absence or presence of lopinavir/ritonavir (50 μM). Boiled Nef was used as a control. Results are representative of three independent experiments. (C) Measurement of Akt activation in MDMs stimulated with rNef (100 ng/mL) or not and treated with increasing concentrations of the PI lopinavir/ritonavir (0–100 μM) as measured by Western blotting. *Upper panel.* Decreased Akt activation in MDMs stimulated with rNef (100 ng/mL) and treated with increasing concentrations of the PI lopinavir/ritonavir (0–100 μM). Beta-actin was used as an internal control. *Lower panel.* Akt activation is slightly inhibited by lopinavir/ritonavir in the absence of Nef treatment. Beta-actin was used as an internal control. Results are representative of three independent experiments. (D) Decreased Akt activation in Nef-stimulated MDMs treated with lopinavir/ritonavir is dose-dependent. Histogram represents means (\pm s.d.) of three independent experiments.

3.3. The Protease Inhibitor Lopinavir/Ritonavir Limits HIV-1 Reactivation from Chronically Infected U1 Monocytoid Cells Stimulated with TNF via Blocking the Akt Pathway

Since Akt activation participates in immune activation and favours resistance to apoptosis, controlling the Akt pathway using PIs could have an impact on HIV-1 recovery from latently infected monocytoid cells [42,43]. Therefore, we assessed the role of PI treatment on HIV-1 reactivation from latency by measuring viral production in latently infected monocytoid cells U1 treated with TNF. U1 monocytoid cells contain two copies of integrated HIV-1 provirus [44]. In U1 cells stimulated with TNF (10 ng/mL), we observed increased Akt phosphorylation on Ser473 using Western blotting (Figure 3A, left panel). The TNF-induced Akt activation in U1 cells was blocked mostly by treatment with lopinavir/ritonavir (Figure 3A, right panel). In addition, viral reactivation induced by TNF in U1 cells was significantly blocked by treatment with the PI lopinavir/ritonavir and the Akt inhibitor VIII, but not by the NNRTI nevirapine, as measured by p24 detection using ELISA (Figure 3B). Our results indicate that a PI-mediated blockade of Akt activation could limit HIV-1 reactivation of integrated provirus from U1 monocytoid cells especially when driven by TNF.

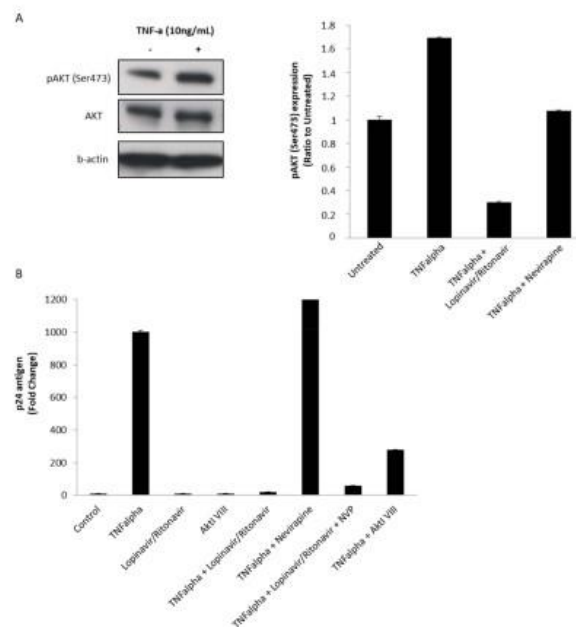


Figure 3. Enhanced Akt activation in monocytoid U1 cells stimulated with Tumor Necrosis Factor (TNF) alpha and blockade of HIV-1 recovery from U1 cells stimulated with TNF and treated with the PI lopinavir/ritonavir. (A) *Left panel.* Enhanced Akt activation (pAkt Ser473) in U1 cells treated with TNF (10 ng/mL) for 30 min as measured by Western blot. Beta-actin was used as an internal control. *Right panel.* The histogram shows that increased pAkt levels observed in TNF-stimulated U1 cells are blocked mostly with lopinavir/ritonavir treatment using Western-blot quantification by ImageJ software. Results are representative of three independent experiments. (B) U1 cells were either left untreated or treated with TNF(10 ng/mL) in the absence or presence of the PI lopinavir/ritonavir, the NNRTI nevirapine, and the Akt inhibitor VIII (all at 50 μ M). The viral production was determined by measuring the level of p24 antigen at 24 h post treatment using ELISA. Means (\pm s.d.) of three independent experiments are shown.

3.4. Decreased Akt Activation in Monocytes from Aviremic Patients under PI-Cart

To further demonstrate the critical role of Akt in HIV-1 pathogenesis and the potential interest of Akt blockade by PI *in vivo*, we measured Akt activation levels in monocytes and autologous resting CD4+ T cells isolated from HIV-1-positive patients. Thirty-one patients with chronic HIV-1 infection treated with cART (treatment range: 1.4–27.2 years) and with undetectable plasma HIV-1 RNA levels (<40 copies/mL) for at least 1 year were included in the study between 2015 and 2017. Of these thirty-one patients (mean age 47.6 years; range 28–69 years) treated with cART, twenty-three were treated with NNRTI-based cART and eight with PI-based cART (as their most recent treatment) and for more than one year (Table 1). We did not observe significant differences for nadir median CD4 counts (255×10^6 versus 288×10^6 cells/L, $p = 0.734$), median CD4 counts at the initiation of treatment (361×10^6 versus 332×10^6 cells/L, $p = 0.407$), median CD4 counts at the last point (614×10^6 versus 761×10^6 cells/L, $p = 0.288$), and median HIV RNA load at zenith (5.10 versus 4.98 log/mL, $p = 0.982$) between PI-treated and NNRTI-treated patients (Table 1). We did not find significant differences between PI-treated and NNRTI-treated patients for the number of failures of treatment (1.5 versus 1, $p = 0.694$) and the duration of therapy (11.6 versus 9 years, $p = 0.498$). The duration with undetectable plasma HIV-1 RNA levels was not significantly different in the PI-treated arm compared to the NNRTI-treated arm (5.8 versus 6.5 years, $p = 0.804$) (Table 1).

Table 1. Biological and virological characteristics of HIV-1-positive patients under PI-cART and NNRTI-cART.

	PI	NNRTI	
Biological Characteristics	n = 8	n = 23	p-Value
Age (years), mean (\pm s.d.)	42.2 (10.5)	49 (7.3)	0.136
CD4+ T cell counts at nadir, absolute (cells per μ L), median (IQR)	255.5 (185.7–368.8)	288.8 (94.6–362.6)	0.734
CD4+ T cell counts at initiation of treatment, absolute (cells per μ L), median (IQR)	361.8 (300.5–530.5)	332.3 (128.5–425.4)	0.407
CD4+ T cell counts at the last point, absolute (cells per μ L), median (IQR)	614 (552.5–664.1)	761.6 (492–934.4)	0.288
pVL at zenith (log copies per mL), median (IQR)	5.10 (4.40–5.26)	4.98 (4.26–5.53)	0.982
Previous treatment failure, median (IQR)	1.5 (0–6)	1 (0–2.5)	0.694
Time with therapy (years), mean \pm s.d.	11.6 (8)	9 (4.7)	0.498
Time with undetectable pVL (years), mean \pm s.d.	5.8 (3.2)	6.5 (3.8)	0.804
Akt Activation Assay			
Resting CD4+ T Cells			
% of pAkt(Ser473) positive cells, mean \pm s.d.	22.8 (23.8)	52.2 (43.5)	0.449
Monocytes			
% of pAkt(Ser473) positive cells, mean \pm s.d.	13.5 (18.0)	32.1 (33.0)	0.220
p-value, monocytes versus resting CD4+ T cells	0.295	0.442	
Proviral HIV DNA quantification Assay			
Resting CD4+ T Cells			
Proviral HIV-1 DNA (log copies per 10^6 cells), median (IQR)	2.58 (2.45–2.81)	2.53 (2.04–2.79)	0.730
Monocytes			
Proviral HIV-1 DNA (log copies per 10^6 cells), median (IQR)	2.47 (2.43–2.55)	2.12 (1.43–2.53)	0.163
p-value, monocytes vs resting CD4+ T cells	0.462	0.129	

IQR = interquartile range.

In HIV-1-positive patients under cART, Akt activation was lower in monocytes compared to autologous resting CD4+ T cells with the lowest activation in the bulk of PBLs (Figures 4 and 5A) (Table 1). The lowest Akt activation was observed in aviremic patients under PI-cART rather than under NNRTI-cART, and primarily in monocytes and PBLs rather than in autologous resting CD4+ T cells (Figure 5B) (Table 1). PI-cART was more efficient for decreasing Akt activation than NNRTI-cART irrespective of the cell type tested (Figure 5C) (Table 1). Altogether, our results indicate that PI-cART treatment is the most potent to decrease Akt activation especially in monocytes isolated from aviremic patients.

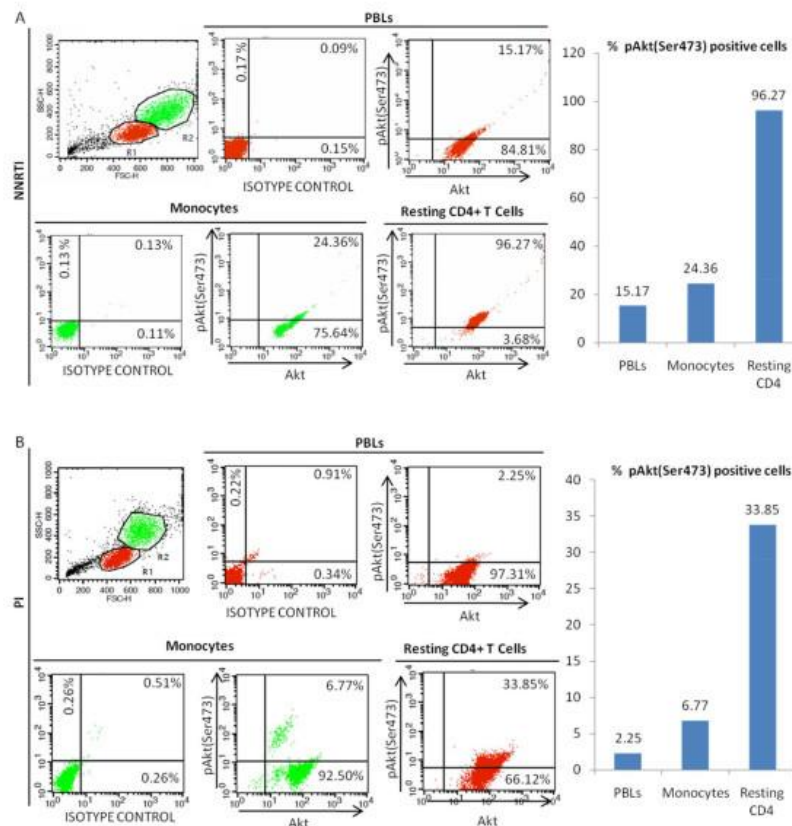


Figure 4. Levels of Akt activation in peripheral blood lymphocytes (PBLs), monocytes, and resting CD4+ T cells from aviremic HIV-1 patients under cART. A,B. Akt activation was lower in PBLs and monocytes than in resting CD4+ T cells isolated from (A) a NNRTI-treated patient and (B) a PI-treated patient as measured by flow cytometric analysis. Results are representative of 23 independent experiments.

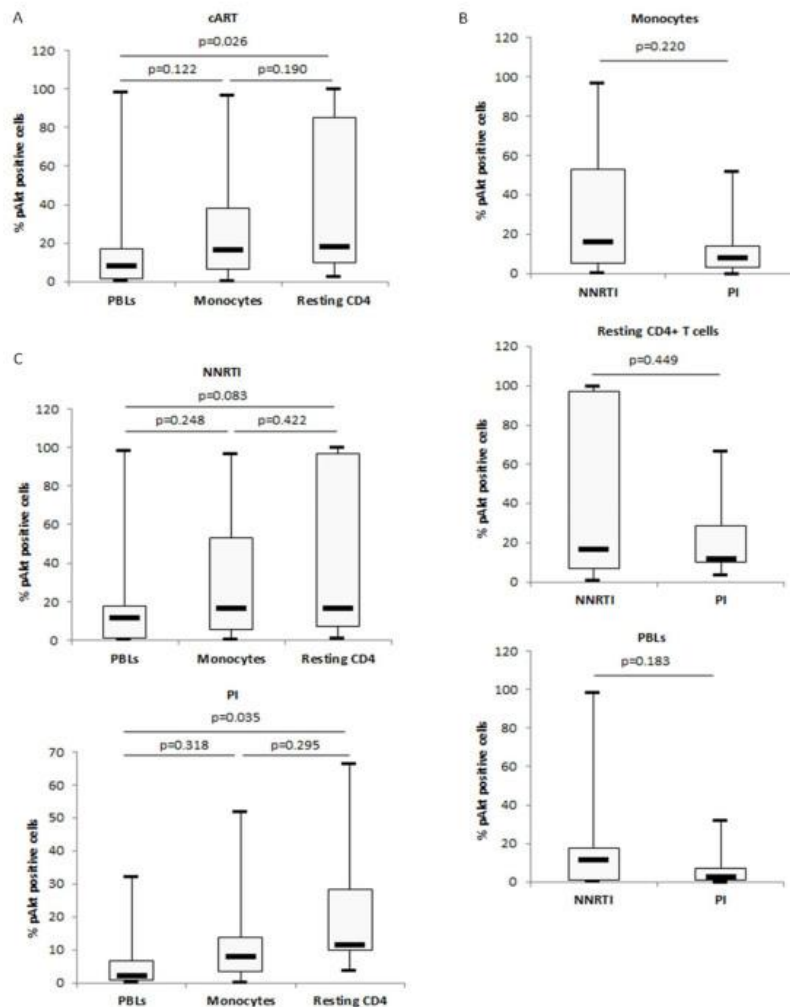


Figure 5. Impact of cART, PI-, and NNRTI-treatment on Akt activation in monocytes, resting CD4+ T cells, and PBLs isolated from aviremic HIV-1-infected patients. (A) Percentage of P-Akt positive cells in PBLs, monocytes, and autologous resting CD4+ T cells isolated from cART-treated patients ($n = 23$) as measured by flow cytometric analysis. The histogram shows median and quartiles. (B) Percentage of P-Akt positive cells depends on the cell type tested and on the cART treatment used (PI versus NNRTI) as measured by flow cytometric analysis. The histogram shows median and quartiles ($n = 23$). (C) Respective percentages of P-Akt positive PBLs, monocytes, and resting CD4+ T cells from aviremic patients under PI-cART and NNRTI-cART as measured by flow cytometric analysis. The histogram shows median and quartiles ($n = 23$).

3.5. Limited Amounts of HIV-1 Proviral DNA in Monocytes from Aviremic Patients under Cart

Besides resting CD4+ T cells, latently infected monocytes/macrophages that harbour integrated replication-competent viral DNA represent a primary long-lived source of persistent HIV-1 in patients

under cART [27–29]. Therefore, we decided to analyze the respective sizes of the HIV-1 reservoirs in monocytes and resting CD4+ T cells from patients naïve of treatment and the impact of the cART regimen on the viral reservoir in monocytes and autologous resting CD4+ T cells by quantifying total proviral HIV-1 DNA. The amount of HIV-1 proviral DNA was lower in monocytes compared to autologous resting CD4+ T cells isolated from cART naïve patients (3.2 log versus 3.7 log, $p = 0.114$) (Figure 6A). A similar lower amount of HIV-1 proviral DNA was measured in monocytes compared to resting CD4+ T cells from cART-treated patients (2.2 log versus 2.4 log, $p = 0.095$) (Figure 6B). Under cART, the amount of HIV-1 proviral DNA present in monocytes and resting CD4+ T cells was decreased significantly compared to cART-naïve patients ($p = 0.0056$ and $p = 0.00017$, respectively) (Figure 6C,D). In PI-treated patients but also in NNRTI-treated patients, significantly lower amounts of proviral DNA were measured in monocytes ($p = 0.0081$ and $p = 0.0094$, respectively) and resting CD4+ T cells ($p = 0.0040$ and $p = 0.0054$, respectively) compared to cART-naïve patients (Figure 7A,B). We did not find significant difference for the amount of proviral DNA present in monocytes and resting CD4+ T cells between PI-cART- and NNRTI-cART-treated patients (Figure 7A,B) (Table 1).

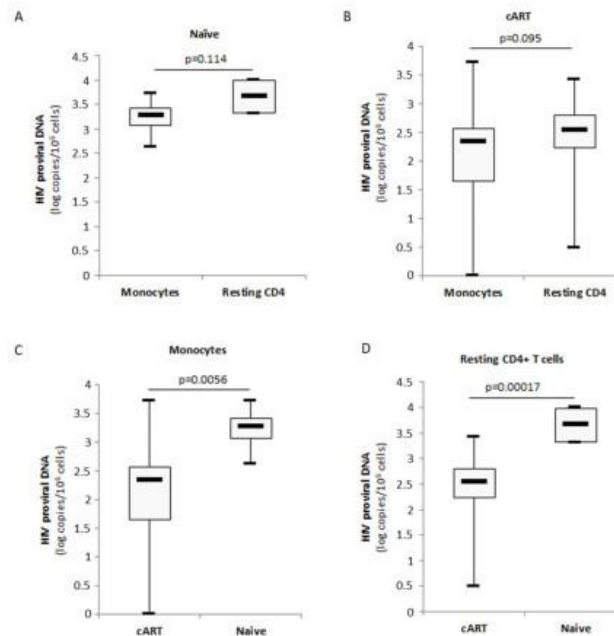


Figure 6. Lower amounts of proviral DNA in monocytes and resting CD4+ T cells from cART-treated patients compared to cART-naïve patients. HIV-1 proviral DNA was measured in monocytes and autologous resting CD4+ T cells isolated from cART-treated patients and cART-naïve patients using a Biocentric Generic HIV DNA Cell kit. (A) Amounts of HIV-1 proviral DNA in monocytes compared to autologous resting CD4+ T cells isolated from cART-naïve patients ($n = 4$). The histogram shows median and quartiles. (B) Amounts of HIV-1 proviral DNA in monocytes compared to autologous resting CD4+ T cells isolated from cART-treated patients ($n = 31$). The histogram shows median and quartiles. (C) Lower amounts of HIV-1 proviral DNA in monocytes from cART-treated patients compared to cART-naïve patients ($n = 31$). The histogram shows median and quartiles. (D) Lower amounts of HIV-1 proviral DNA in resting CD4+ T cells from cART-treated patients compared to cART-naïve patients. The histogram shows median and quartiles ($n = 31$).

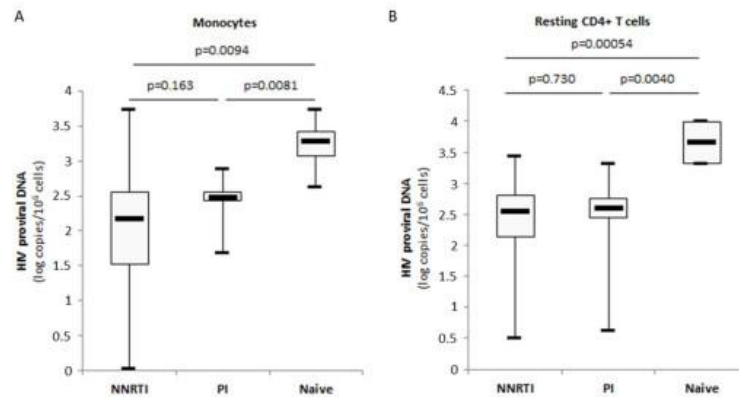


Figure 7. cART reduces the amount of HIV proviral DNA in monocytes and resting CD4+ T cells from aviremic patients irrespective of the PI or NNRTI treatment. (A) Lower amounts of HIV-1 proviral DNA in monocytes from patients under PI-cART or NNRTI-cART compared to cART-naïve patients. The histogram shows median and quartiles ($n = 31$). (B) Lower amounts of HIV-1 proviral DNA in resting CD4+ T cells from patients under PI-cART or NNRTI-cART compared to cART-naïve patients. The histogram shows median and quartiles ($n = 34$).

4. Discussion

HIV-1 Nef, a pleiotropic early viral protein, has been demonstrated to activate several key signaling molecules, including Akt, MAPK, and ERK [45,46]. In the present study, we have demonstrated that Nef upregulates Akt expression and induces Akt phosphorylation in MDMs *in vitro*, which was blocked by the HIV PI lopinavir/ritonavir but not by the NNRTI nevirapine. Both increased Akt activation and enhanced HIV-1 reactivation were observed in U1 monocytoïd cells stimulated with TNF. The HIV-1 reactivation in U1 cells stimulated by TNF was inhibited by the Akt inhibitor VIII and the PI lopinavir/ritonavir, but not by the NNRTI nevirapine. Most importantly, our results indicate that the PIs, but not the NNRTIs, decrease Akt hyperactivation *in vitro* and *in vivo*. Both PI-cART and NNRTI-cART limit the size of the HIV-1 reservoir in latently infected monocytes from aviremic chronically infected patients.

Exogenous HIV-1 Nef is internalized by MDMs as reported previously for CD4+T cells [20,47]. The mode of internalization and secretion of Nef is still a conundrum. Some studies suggest the involvement of exosomes in intercellular transfer of Nef from infected cells to the bystander cells [48,49]. On the other hand, a recently published study suggests the cell-to-cell contact rather than exosomes-mediated intercellular transfer of Nef [50]. *In vivo*, exogenous Nef and TNF secreted by infected cells as soluble proteins and/or as exosomes can activate uninfected CD4+ T cells and MDMs present in the vicinity and make them susceptible to infection [31,39,51]. Following Nef treatment, a 2-fold increase in NF- κ B-mediated LTR activation in U937 cells transfected with LTR-Luc and also a 2-fold increase of p24 levels in supernatants of chronically HIV-infected U1 cells have been previously described [39,52,53]. In U1 cells treated with TNF, both NF- κ B activation and HIV-1 reactivation from latency are observed [40,53]. Thus, both Nef and TNF could activate monocytes/macrophages and thereby expand the viral reservoir in infected individuals.

Latency represents the major obstacle in the complete cure of HIV-1 [25,54–56]. Several agents targeting various signaling cascades are known to reactivate latent HIV-1 and are termed latency-reversing agents (LRAs) [57,58]. Among LRAs, activators of the PI3K/Akt pathway could be used to reactivate the HIV-1 latent reservoir in both lymphoid and myeloid cells. For instance, disulfiram is known to deplete phosphatase and tensin homolog (PTEN) protein levels in U1 cells

and in resting CD4+ T cells from HIV-negative donors, resulting in hyperphosphorylation of Akt and subsequently activation of HIV-1 expression [59]. In another instance, 57,704, an agonist of PI3K, reactivates HIV-1 in several latent cell lines [59]. LRAs expose latently infected cells to the host immune system and to cART, namely the “shock and kill” strategy. This strategy faces multiple barriers which prevent the complete eradication of replication-competent viruses and must therefore be optimized. Recently, a “block and lock” strategy has been hypothesized which focuses on suppressing residual levels of HIV-1 transcription to lock the virus in a deep latency state to prevent viral reactivation [25]. In view of the “block and lock” strategy, Akt inhibitors could be suggested as potential therapeutic molecules against HIV-1 especially in infected monocytes/macrophages [60]. The role of PIs, but not RTIs, in inhibiting Akt activation has been shown in several cell types [15,18,60–62]. We observed that PIs could block Nef-mediated Akt activation in MDMs in a dose-dependent manner (Figure 2). Furthermore, PIs inhibit the viral reactivation from chronically infected monocytoïd U1 cells stimulated with TNF similar to Akt inhibitor VIII (Figure 3). Thus, the inhibition of viral reactivation by PIs could participate in the “block and lock” strategy and thereby curtail the HIV-1 reservoir especially in myeloid cells.

Although the use of cART has been extensively studied in regard to residual viremia, only little is known on their impact on cellular reservoirs of HIV-1 especially in monocytes isolated from patients. We determined the levels of Akt activation and HIV-1 proviral DNA in monocytes and autologous resting CD4+ T cells from cART-treated patients. In cART-naïve patients, we observed that the amount of HIV-1 proviral DNA was 0.5 log smaller in monocytes than in resting CD4+ T cells (Figure 6). In agreement with our results, the number of productively infected monocytes is nearly 10-fold lower than the number of productively infected CD4+ T cells in the blood of Simian Immunodeficiency Virus-infected macaques [63]. In agreement with decreased Akt activation in resting CD4+ T cells from PI-treated patients [21], we observed low levels of Akt activation in monocytes isolated from PI-treated patients. By using the proviral HIV DNA assay that allows for evaluating in a quantitative manner the amount of intracellular provirus, we observed that cART was efficient for limiting the size of the HIV-1 reservoir in monocytes and resting CD4+ T cells compared to cART-naïve patients (Figure 6). To diminish the size of the viral reservoir, several studies have shown that an early treatment during HIV-1 primo-infection could be beneficial [33,34], and on-demand pre-exposure prophylaxis has been shown to be highly efficient to prevent HIV transmission [64]. In addition, PIs have been reported to block the cell-to-cell spread of HIV-1 [65]. Since myeloid cells are a main target of macrophage-tropic HIV-1 strains following HIV exposure, future studies will have to assess the effect of on-demand pre-exposure prophylaxis not only on CD4+ T cells but also on the monocyte/macrophage population subset.

In addition to its role in HIV-1 reactivation and latency, Akt activation is critical for cell survival [1]. Therefore, the blockade of Akt activation by PIs in latently infected resting monocytes/macrophages could trigger an accelerated cell death and thereby favour the clearance of the HIV reservoir in myeloid cells [19]. In fact, HIV- and SIV-infected macrophages are not efficiently killed by CD8+ T cells like infected CD4+ T cells are [66,67]. Also, resident tissue macrophages remain in tissues long term, are relatively resistant to the cytopathic effects of HIV infection compared to CD4+ T cells, and may serve as stable viral reservoirs [68]. In addition, the relative potency of PIs in chronically infected monocytes/macrophages could be a limiting factor for the clearance of the virus in myeloid cells [69–71]. Therefore, besides their antiviral activity, PIs rather than NNRTIs could, by targeting Akt, trigger apoptosis in HIV-infected monocytes/macrophages and neighbouring CD4+ T cells [72,73] and thereby participate in the clearance of both myeloid and lymphoid infected cells.

5. Conclusions

Our study shows that exposure of MDMs to HIV-1 Nef leads to enhanced Akt expression and activation. Importantly, we observed that the immunomodulatory effects of PIs, by blocking Akt activation especially triggered by Nef and TNF, limit HIV-1 recovery from latently infected

monocytoid U1 cells in vitro. Finally, in aviremic chronically HIV-1-infected patients, our study indicates that PI-based cART decreases Akt activation and limits the size of the monocyte reservoir. Altogether, our results support the need to further assess the therapeutic use of HIV-1 protease inhibitors in view of their impact on persistence of myeloid HIV-1 reservoirs and might provide and/or accompany new therapeutic strategies for a remission.

Acknowledgments: We thank the HIV-1-infected patients for their willingness to participate in this study and Aurélie Proust, Catherine Chirouze, Jean-François Faucher, Laurent Hustache-Mathieu, Adeline Folzer, Marie-Christine Drobacheff-Thiebaut who cared for the patients. This work was supported by grants from the University of Franche-Comté, the Agence Nationale de Recherche sur le SIDA (ANRS, No 13543 and 13544) and HIVERA.EURECA, ERANET 01K11307A (to Georges Herbein). Wasim Abbas is a recipient of doctoral scholarships from the Higher Education Commission, Pakistan. Amit Kumar is a recipient of a postdoctoral grant of Région Franche-Comté (No 2012C-06102). The funders had no role in the data collection, analysis, patient recruitment, or decision to publish.

Author Contributions: Georges Herbein conceived and designed the project. Wasim Abbas, Amit Kumar and Sébastien Pasquereau performed experiments. Georges Herbein and Sébastien Pasquereau wrote the manuscript.

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

References

1. Fayard, E.; Xue, G.; Parcellier, A.; Bozulic, L.; Hemmings, B.A. Protein kinase B (PKB/Akt), a key mediator of the PI3K signaling pathway. *Curr. Top. Microbiol. Immunol.* **2010**, *346*, 31–56. [[CrossRef](#)] [[PubMed](#)]
2. Maartens, G.; Celum, C.; Lewin, S.R. HIV infection: Epidemiology, pathogenesis, treatment, and prevention. *Lancet* **2014**, *384*, 258–271. [[CrossRef](#)]
3. Deng, K.; Siliciano, R.F. HIV: Early treatment may not be early enough. *Nature* **2014**, *512*, 35. [[CrossRef](#)] [[PubMed](#)]
4. Chun, T.-W.; Moir, S.; Fauci, A.S. HIV reservoirs as obstacles and opportunities for an HIV cure. *Nat. Immunol.* **2015**, *16*, 584–589. [[CrossRef](#)] [[PubMed](#)]
5. Pasquereau, S.; Kumar, A.; Herbein, G. Targeting TNF and TNF Receptor Pathway in HIV-1 Infection: From Immune Activation to Viral Reservoirs. *Viruses* **2017**, *9*. [[CrossRef](#)] [[PubMed](#)]
6. Malim, M.H.; Emerman, M. HIV-1 accessory proteins—ensuring viral survival in a hostile environment. *Cell Host Microbe* **2008**, *3*, 388–398. [[CrossRef](#)] [[PubMed](#)]
7. Liu, X.; Kumar, A. Differential signaling mechanism for HIV-1 Nef-mediated production of IL-6 and IL-8 in human astrocytes. *Sci. Rep.* **2015**, *5*, 9867. [[CrossRef](#)] [[PubMed](#)]
8. Muratori, C.; Cavallin, L.E.; Krätzel, K.; Tinari, A.; De Milito, A.; Fais, S.; D’Aloja, P.; Federico, M.; Vullo, V.; Fomina, A.; et al. Massive secretion by T cells is caused by HIV Nef in infected cells and by Nef transfer to bystander cells. *Cell Host Microbe* **2009**, *6*, 218–230. [[CrossRef](#)] [[PubMed](#)]
9. Qiao, X.; He, B.; Chiu, A.; Knowles, D.M.; Chadburn, A.; Cerutti, A. Human immunodeficiency virus 1 Nef suppresses CD40-dependent immunoglobulin class switching in bystander B cells. *Nat. Immunol.* **2006**, *7*, 302–310. [[CrossRef](#)] [[PubMed](#)]
10. Lee, J.-H.; Schierer, S.; Blume, K.; Dindorf, J.; Wittki, S.; Xiang, W.; Ostalecki, C.; Koliha, N.; Wild, S.; Schuler, G.; et al. HIV-Nef and ADAM17-Containing Plasma Extracellular Vesicles Induce and Correlate with Immune Pathogenesis in Chronic HIV Infection. *EBioMedicine* **2016**, *6*, 103–113. [[CrossRef](#)] [[PubMed](#)]
11. Sami Saribas, A.; Cicalese, S.; Ahooyi, T.M.; Khalili, K.; Amini, S.; Sariyer, I.K. HIV-1 Nef is released in extracellular vesicles derived from astrocytes: Evidence for Nef-mediated neurotoxicity. *Cell Death Dis.* **2017**, *8*, e2542. [[CrossRef](#)] [[PubMed](#)]
12. Diehl, N.; Schaal, H. Make Yourself at Home: Viral Hijacking of the PI3K/Akt Signaling Pathway. *Viruses* **2013**, *5*, 3192–3212. [[CrossRef](#)] [[PubMed](#)]
13. Srirangam, A.; Mitra, R.; Wang, M.; Gorski, J.C.; Badve, S.; Baldrige, L.A.; Hamilton, J.; Kishimoto, H.; Hawes, J.; Li, L.; et al. Effects of HIV Protease Inhibitor Ritonavir on Akt-Regulated Cell Proliferation in Breast Cancer. *Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res.* **2006**, *12*, 1883–1896. [[CrossRef](#)] [[PubMed](#)]

14. Plastaras, J.P.; Vapiwala, N.; Ahmed, M.S.; Gudonis, D.; Cerniglia, G.J.; Feldman, M.D.; Frank, I.; Gupta, A.K. Validation and toxicity of PI3K/Akt pathway inhibition by HIV protease inhibitors in humans. *Cancer Biol. Ther.* **2008**, *7*, 628–635. [[CrossRef](#)] [[PubMed](#)]
15. Gantt, S.; Casper, C.; Ambinder, R.F. Insights into the broad cellular effects of nelfinavir and the HIV protease inhibitors supporting their role in cancer treatment and prevention. *Curr. Opin. Oncol.* **2013**, *25*, 495–502. [[CrossRef](#)] [[PubMed](#)]
16. Gupta, A.K.; Cerniglia, G.J.; Mick, R.; McKenna, W.G.; Muschel, R.J. HIV protease inhibitors block Akt signaling and radiosensitize tumor cells both in vitro and in vivo. *Cancer Res.* **2005**, *65*, 8256–8265. [[CrossRef](#)] [[PubMed](#)]
17. Bernstein, W.B.; Dennis, P.A. Repositioning HIV protease inhibitors as cancer therapeutics. *Curr. Opin. HIV AIDS* **2008**, *3*, 666–675. [[CrossRef](#)] [[PubMed](#)]
18. Wallet, M.A.; Reist, C.M.; Williams, J.C.; Appelberg, S.; Guiulfo, G.L.; Gardner, B.; Sleasman, J.W.; Goodenow, M.M. The HIV-1 protease inhibitor nelfinavir activates PP2 and inhibits MAPK signaling in macrophages: A pathway to reduce inflammation. *J. Leukoc. Biol.* **2012**, *92*, 795–805. [[CrossRef](#)] [[PubMed](#)]
19. Bacigalupo, I.; Palladino, C.; Leone, P.; Toschi, E.; Sgadari, C.; Ensoli, B.; Barillari, G. Inhibition of MMP-9 expression by ritonavir or saquinavir is associated with inactivation of the AKT/Fra-1 pathway in cervical intraepithelial neoplasia cells. *Oncol. Lett.* **2017**, *13*, 2903–2908. [[CrossRef](#)] [[PubMed](#)]
20. Kumar, A.; Abbas, W.; Colin, L.; Khan, K.A.; Bouchat, S.; Varin, A.; Larbi, A.; Gatot, J.-S.; Kabeya, K.; Vanhulle, C.; et al. Tuning of AKT-pathway by Nef and its blockade by protease inhibitors results in limited recovery in latently HIV infected T-cell line. *Sci. Rep.* **2016**, *6*, 24090. [[CrossRef](#)] [[PubMed](#)]
21. Kumar, A.; Abbas, W.; Bouchat, S.; Gatot, J.-S.; Pasquereau, S.; Kabeya, K.; Clumeck, N.; De Wit, S.; Van Lint, C.; Herbein, G. Limited HIV-1 Reactivation in Resting CD4(+) T cells from Aviremic Patients under Protease Inhibitors. *Sci. Rep.* **2016**, *6*, 38313. [[CrossRef](#)] [[PubMed](#)]
22. Perelson, A.S.; Essunger, P.; Cao, Y.; Vesanan, M.; Hurley, A.; Saksela, K.; Markowitz, M.; Ho, D.D. Decay characteristics of HIV-1-infected compartments during combination therapy. *Nature* **1997**, *387*, 188. [[CrossRef](#)] [[PubMed](#)]
23. Palmer, S.; Maldarelli, F.; Wiegand, A.; Bernstein, B.; Hanna, G.J.; Brun, S.C.; Kempf, D.J.; Mellors, J.W.; Coffin, J.M.; King, M.S. Low-level viremia persists for at least 7 years in patients on suppressive antiretroviral therapy. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 3879–3884. [[CrossRef](#)] [[PubMed](#)]
24. Ho, Y.-C.; Shan, L.; Hosmane, N.N.; Wang, J.; Laskey, S.B.; Rosenbloom, D.I.S.; Lai, J.; Blankson, J.N.; Siliciano, J.D.; Siliciano, R.F. Replication-competent noninduced proviruses in the latent reservoir increase barrier to HIV-1 cure. *Cell* **2013**, *155*, 540–551. [[CrossRef](#)] [[PubMed](#)]
25. Darcis, G.; Van Driessche, B.; Van Lint, C. HIV Latency: Should We Shock or Lock? *Trends Immunol.* **2017**, *38*, 217–228. [[CrossRef](#)] [[PubMed](#)]
26. Siliciano, R.F. Opening Fronts in HIV Vaccine Development: Targeting reservoirs to clear and cure. *Nat. Med.* **2014**, *20*, 480–481. [[CrossRef](#)] [[PubMed](#)]
27. Le Douce, V.; Herbein, G.; Rohr, O.; Schwartz, C. Molecular mechanisms of HIV-1 persistence in the monocyte-macrophage lineage. *Retrovirology* **2010**, *7*, 32. [[CrossRef](#)] [[PubMed](#)]
28. Kumar, A.; Abbas, W.; Herbein, G. HIV-1 Latency in Monocytes/Macrophages. *Viruses* **2014**, *6*, 1837–1860. [[CrossRef](#)] [[PubMed](#)]
29. Abbas, W.; Tariq, M.; Iqbal, M.; Kumar, A.; Herbein, G. Eradication of HIV-1 from the macrophage reservoir: An uncertain goal? *Viruses* **2015**, *7*, 1578–1598. [[CrossRef](#)] [[PubMed](#)]
30. Dinoso, J.B.; Kim, S.Y.; Wiegand, A.M.; Palmer, S.E.; Gange, S.J.; Cranmer, L.; O'Shea, A.; Callender, M.; Spivak, A.; Brennan, T.; et al. Treatment intensification does not reduce residual HIV-1 viremia in patients on highly active antiretroviral therapy. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 9403–9408. [[CrossRef](#)] [[PubMed](#)]
31. Herbein, G. TNF and HIV-1 Nef: An Intimate Interplay. *EBioMedicine* **2016**, *13*, 25–26. [[CrossRef](#)] [[PubMed](#)]
32. Siliciano, J.D.; Siliciano, R.F. Recent developments in the effort to cure HIV infection: Going beyond N = 1. *J. Clin. Investig.* **2016**, *126*, 409–414. [[CrossRef](#)] [[PubMed](#)]
33. Sáez-Cirion, A.; Bacchus, C.; Hocqueloux, L.; Avettand-Fenoel, V.; Girault, I.; Lecuroux, C.; Potard, V.; Versmisse, P.; Melard, A.; Prazuck, T.; et al. Post-treatment HIV-1 controllers with a long-term virological remission after the interruption of early initiated antiretroviral therapy ANRS VISCONTI Study. *PLoS Pathog.* **2013**, *9*, e1003211. [[CrossRef](#)] [[PubMed](#)]

34. Buzón, M.J.; Massanella, M.; Llibre, J.M.; Esteve, A.; Dahl, V.; Puertas, M.C.; Gatell, J.M.; Domingo, P.; Paredes, R.; Sharkey, M.; et al. HIV-1 replication and immune dynamics are affected by raltegravir intensification of HAART-suppressed subjects. *Nat. Med.* **2010**, *16*, 460–465. [[CrossRef](#)] [[PubMed](#)]
35. DiNapoli, S.R.; Hirsch, V.M.; Brenchley, J.M. Macrophages in Progressive Human Immunodeficiency Virus/Simian Immunodeficiency Virus Infections. *J. Virol.* **2016**, *90*, 7596–7606. [[CrossRef](#)] [[PubMed](#)]
36. Clayton, K.L.; Garcia, J.V.; Clements, J.E.; Walker, B.D. HIV Infection of Macrophages: Implications for Pathogenesis and Cure. *Pathog. Immun.* **2017**, *2*, 179–192. [[CrossRef](#)] [[PubMed](#)]
37. Herbein, G.; Mahlknecht, U.; Batliwalla, F.; Gregersen, P.; Pappas, T.; Butler, J.; O'Brien, W.A.; Verdin, E. Apoptosis of CD8+ T cells is mediated by macrophages through interaction of HIV gp120 with chemokine receptor CXCR4. *Nature* **1998**, *395*, 189–194. [[CrossRef](#)] [[PubMed](#)]
38. Bouchat, S.; Gatot, J.-S.; Kabeya, K.; Cardona, C.; Colin, L.; Herbein, G.; De Wit, S.; Clumeck, N.; Lambotte, O.; Rouzioux, C.; et al. Histone methyltransferase inhibitors induce HIV-1 recovery in resting CD4(+) T cells from HIV-1-infected HAART-treated patients. *AIDS* **2012**, *26*, 1473–1482. [[CrossRef](#)] [[PubMed](#)]
39. Varin, A.; Manna, S.K.; Quivy, V.; Decrion, A.-Z.; Van Lint, C.; Herbein, G.; Aggarwal, B.B. Exogenous Nef protein activates NF-kappa B, AP-1, and c-Jun N-terminal kinase and stimulates HIV transcription in promonocytic cells. Role in AIDS pathogenesis. *J. Biol. Chem.* **2003**, *278*, 2219–2227. [[CrossRef](#)] [[PubMed](#)]
40. Reuse, S.; Calao, M.; Kabeya, K.; Guiguen, A.; Gatot, J.-S.; Quivy, V.; Vanhulle, C.; Lamine, A.; Vaira, D.; Demonte, D.; et al. Synergistic activation of HIV-1 expression by deacetylase inhibitors and prostratin: Implications for treatment of latent infection. *PLoS ONE* **2009**, *4*, e6093. [[CrossRef](#)] [[PubMed](#)]
41. Sarbassov, D.D.; Guertin, D.A.; Ali, S.M.; Sabatini, D.M. Phosphorylation and Regulation of Akt/PKB by the Rictor-mTOR Complex. *Science* **2005**, *307*, 1098–1101. [[CrossRef](#)] [[PubMed](#)]
42. Wagner, T.A.; McLaughlin, S.; Garg, K.; Cheung, C.Y.K.; Larsen, B.B.; Styrchak, S.; Huang, H.C.; Edlefsen, P.T.; Mullins, J.I.; Frenkel, L.M. HIV latency. Proliferation of cells with HIV integrated into cancer genes contributes to persistent infection. *Science* **2014**, *345*, 570–573. [[CrossRef](#)] [[PubMed](#)]
43. Hill, A.L.; Rosenbloom, D.I.S.; Fu, F.; Nowak, M.A.; Siliciano, R.F. Predicting the outcomes of treatment to eradicate the latent reservoir for HIV-1. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 13475–13480. [[CrossRef](#)] [[PubMed](#)]
44. Folks, T.M.; Justement, J.; Kinter, A.; Dinarello, C.A.; Fauci, A.S. Cytokine-induced expression of HIV-1 in a chronically infected promonocyte cell line. *Science* **1987**, *238*, 800–802. [[CrossRef](#)] [[PubMed](#)]
45. Schragar, J.A.; Der Minassian, V.; Marsh, J.W. HIV Nef increases T cell ERK MAP kinase activity. *J. Biol. Chem.* **2002**, *277*, 6137–6142. [[CrossRef](#)] [[PubMed](#)]
46. Xue, M.; Yao, S.; Hu, M.; Li, W.; Hao, T.; Zhou, F.; Zhu, X.; Lu, H.; Qin, D.; Yan, Q.; et al. HIV-1 Nef and KSHV oncogene K1 synergistically promote angiogenesis by inducing cellular miR-718 to regulate the PTEN/AKT/mTOR signaling pathway. *Nucleic Acids Res.* **2014**, *42*, 9862–9879. [[CrossRef](#)] [[PubMed](#)]
47. Alessandrini, L.; Santarcangelo, A.C.; Olivetta, E.; Ferrantelli, F.; d'Aloja, P.; Pugliese, K.; Pelosi, E.; Chelucci, C.; Mattia, G.; Peschle, C.; et al. T-tropic human immunodeficiency virus (HIV) type 1 Nef protein enters human monocyte-macrophages and induces resistance to HIV replication: A possible mechanism of HIV T-tropic emergence in AIDS. *J. Gen. Virol.* **2000**, *81*, 2905–2917. [[CrossRef](#)] [[PubMed](#)]
48. James, C.O.; Huang, M.-B.; Khan, M.; Garcia-Barrio, M.; Powell, M.D.; Bond, V.C. Extracellular Nef protein targets CD4+ T cells for apoptosis by interacting with CXCR4 surface receptors. *J. Virol.* **2004**, *78*, 3099–3109. [[CrossRef](#)] [[PubMed](#)]
49. Arenaccio, C.; Chiozzini, C.; Columba-Cabezas, S.; Manfredi, F.; Federico, M. Cell activation and HIV-1 replication in unstimulated CD4+ T lymphocytes ingesting exosomes from cells expressing defective HIV-1. *Retrovirology* **2014**, *11*, 46. [[CrossRef](#)] [[PubMed](#)]
50. Luo, X.; Fan, Y.; Park, I.-W.; He, J.J. Exosomes are unlikely involved in intercellular Nef transfer. *PLoS ONE* **2015**, *10*, e0124436. [[CrossRef](#)] [[PubMed](#)]
51. Ostalecki, C.; Wittki, S.; Lee, J.-H.; Geist, M.M.; Tibroni, N.; Harrer, T.; Schuler, G.; Fackler, O.T.; Baur, A.S. HIV Nef- and Notch1-dependent Endocytosis of ADAM17 Induces Vesicular TNF Secretion in Chronic HIV Infection. *EBioMedicine* **2016**, *13*, 294–304. [[CrossRef](#)] [[PubMed](#)]
52. Nidai Ozes, O.; Mayo, L.D.; Gustin, J.A.; Pfeffer, S.R.; Pfeffer, L.M.; Donner, D.B. NF-κB activation by tumour necrosis factor requires the Akt serine-threonine kinase. *Nature* **1999**, *401*, 82–85. [[CrossRef](#)] [[PubMed](#)]

53. Herbein, G.; Varin, A.; Larbi, A.; Fortin, C.; Mähknecht, U.; Fulop, T.; Aggarwal, B. Nef and TNF α are Coplayers that Favor HIV-1 Replication in Monocytic Cells and Primary Macrophages. *Curr. HIV Res.* **2008**, *6*, 117–129. [[CrossRef](#)] [[PubMed](#)]
54. Tyagi, M.; Bukrinsky, M. Human immunodeficiency virus (HIV) latency: The major hurdle in HIV eradication. *Mol. Med.* **2012**, *18*, 1096–1108. [[CrossRef](#)] [[PubMed](#)]
55. Matsuda, Y.; Kobayashi-Ishihara, M.; Fujikawa, D.; Ishida, T.; Watanabe, T.; Yamagishi, M. Epigenetic heterogeneity in HIV-1 latency establishment. *Sci. Rep.* **2015**, *5*, 7701. [[CrossRef](#)] [[PubMed](#)]
56. Kim, Y.; Anderson, J.L.; Lewin, S.R. Getting the “Kill” into “Shock and Kill”: Strategies to Eliminate Latent HIV. *Cell Host Microbe* **2018**, *23*, 14–26. [[CrossRef](#)] [[PubMed](#)]
57. Darcis, G.; Kula, A.; Bouchat, S.; Fujinaga, K.; Corazza, F.; Ait-Ammar, A.; Delacourt, N.; Melard, A.; Kabeya, K.; Vanhulle, C.; et al. An In-Depth Comparison of Latency-Reversing Agent Combinations in Various In Vitro and Ex Vivo HIV-1 Latency Models Identified Bryostatins-1+JQ1 and Ingenol-B+JQ1 to Potently Reactivate Viral Gene Expression. *PLoS Pathog.* **2015**, *11*, e1005063. [[CrossRef](#)] [[PubMed](#)]
58. Kumar, A.; Darcis, G.; Van Lint, C.; Herbein, G. Epigenetic control of HIV-1 post integration latency: Implications for therapy. *Clin. Epigenet.* **2015**, *7*, 103. [[CrossRef](#)] [[PubMed](#)]
59. Doyon, G.; Zerbato, J.; Mellors, J.W.; Sluis-Cremer, N. Disulfiram reactivates latent HIV-1 expression through depletion of the phosphatase and tensin homolog. *AIDS* **2013**, *27*, F7–F11. [[CrossRef](#)] [[PubMed](#)]
60. Chugh, P.; Bradel-Tretheway, B.; Monteiro-Filho, C.M.R.; Planelles, V.; Maggior, S.B.; Dewhurst, S.; Kim, B. Akt inhibitors as an HIV-1 infected macrophage-specific anti-viral therapy. *Retrovirology* **2008**, *5*, 11. [[CrossRef](#)] [[PubMed](#)]
61. Gupta, A.K.; Li, B.; Cemiglia, G.J.; Ahmed, M.S.; Hahn, S.M.; Maity, A. The HIV protease inhibitor nelfinavir downregulates Akt phosphorylation by inhibiting proteasomal activity and inducing the unfolded protein response. *Neoplasia* **2007**, *9*, 271–278. [[CrossRef](#)] [[PubMed](#)]
62. Kourjian, G.; Rucevic, M.; Berberich, M.J.; Dinter, J.; Wambua, D.; Boucau, J.; Le Gall, S. HIV Protease Inhibitor-Induced Cathepsin Modulation Alters Antigen Processing and Cross-Presentation. *J. Immunol.* **2016**, *196*, 3595–3607. [[CrossRef](#)] [[PubMed](#)]
63. Avalos, C.R.; Price, S.L.; Forsyth, E.R.; Pin, J.N.; Shirk, E.N.; Bullock, B.T.; Queen, S.E.; Li, M.; Gellerup, D.; O'Connor, S.L.; et al. Quantitation of Productively Infected Monocytes and Macrophages of Simian Immunodeficiency Virus-Infected Macaques. *J. Virol.* **2016**, *90*, 5643–5656. [[CrossRef](#)] [[PubMed](#)]
64. Molina, J.-M.; Capitant, C.; Spire, B.; Pialoux, G.; Cotte, L.; Charreau, I.; Tremblay, C.; Le Gall, J.-M.; Cua, E.; Pasquet, A.; et al. On-Demand Preexposure Prophylaxis in Men at High Risk for HIV-1 Infection. *N. Engl. J. Med.* **2015**, *373*, 2237–2246. [[CrossRef](#)] [[PubMed](#)]
65. Titanji, B.K.; Aasa-Chapman, M.; Pillay, D.; Jolly, C. Protease inhibitors effectively block cell-to-cell spread of HIV-1 between T cells. *Retrovirology* **2013**, *10*, 161. [[CrossRef](#)] [[PubMed](#)]
66. Walker-Sperling, V.E.K.; Buckheit, R.W.; Blankson, J.N. Comparative Analysis of the Capacity of Elite Suppressor CD4+ and CD8+ T Cells To Inhibit HIV-1 Replication in Monocyte-Derived Macrophages. *J. Virol.* **2014**, *88*, 9789–9798. [[CrossRef](#)] [[PubMed](#)]
67. Vojnov, L.; Martins, M.A.; Bean, A.T.; Veloso de Santana, M.G.; Sacha, J.B.; Wilson, N.A.; Bonaldo, M.C.; Galler, R.; Stevenson, M.; Watkins, D.I. The majority of freshly sorted simian immunodeficiency virus (SIV)-specific CD8(+) T cells cannot suppress viral replication in SIV-infected macrophages. *J. Virol.* **2012**, *86*, 4682–4687. [[CrossRef](#)] [[PubMed](#)]
68. Ginhoux, F.; Jung, S. Monocytes and macrophages: Developmental pathways and tissue homeostasis. *Nat. Rev. Immunol.* **2014**, *14*, 392–404. [[CrossRef](#)] [[PubMed](#)]
69. Perno, C.-F.; Newcomb, F.M.; Davis, D.A.; Aquaro, S.; Humphrey, R.W.; Calìò, R.; Yarchoan, R. Relative Potency of Protease Inhibitors in Monocytes/Macrophages Acutely and Chronically Infected with Human Immunodeficiency Virus. *J. Infect. Dis.* **1998**, *178*, 413–422. [[CrossRef](#)] [[PubMed](#)]
70. Perno, C.-F.; Balestra, E.; Francesconi, M.; Abdelahad, D.; Calìò, R.; Balzarini, J.; Aquaro, S. Antiviral profile of HIV inhibitors in macrophages: Implications for therapy. *Curr. Top. Med. Chem.* **2004**, *4*, 1009–1015. [[CrossRef](#)] [[PubMed](#)]
71. Aquaro, S.; Svicher, V.; Schols, D.; Pollicita, M.; Antinori, A.; Balzarini, J.; Perno, C.F. Mechanisms underlying activity of antiretroviral drugs in HIV-1-infected macrophages: New therapeutic strategies. *J. Leukoc. Biol.* **2006**, *80*, 1103–1110. [[CrossRef](#)] [[PubMed](#)]

72. Cummins, N.W.; Sainski, A.M.; Natesampillai, S.; Bren, G.D.; Badley, A.D. Choice of antiretroviral therapy differentially impacts survival of HIV-infected CD4 T cells. *Mol. Cell. Ther.* **2014**, *2*, 1. [[CrossRef](#)] [[PubMed](#)]
73. Mahlknecht, U.; Herbein, G. Macrophages and T-cell apoptosis in HIV infection: A leading role for accessory cells? *Trends Immunol.* **2001**, *22*, 256–260. [[CrossRef](#)]



© 2018 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).

III. Evaluation de l'efficacité de traitements anti-coronavirus : recherche d'un traitement antiviral contre le SARS-CoV-2

9. Etude de la réutilisation de traitements existants sur l'infection par les coronavirus.

9.1. Evaluation de la toxicité des traitements sélectionnés.

Nous avons évalué l'effet de plusieurs traitements précédemment autorisés pour d'autres pathologies sur l'infection par les coronavirus. Parmi les traitements testés, quatre composés, l'acide valproïque, le disulfiram, la rapamycine et le lopéramide, ont précédemment montré un effet sur les coronavirus [320]. La toxicité des traitements a été évaluée sur des cellules MRC5 traitées par doses croissantes des différents composés. La mesure de cytotoxicité par test MTT a montré une forte toxicité de l'acide valproic (VPA), du disulfiram, du lopéramide et de la rapamycine. La forte toxicité observée avec ces quatre traitements ne permet pas de mesurer avec fiabilité l'effet antiviral sur des cellules MRC5 infectées avec le coronavirus HCoV-229E. La mesure de toxicité réalisée pour le traitement des cellules par Lopinavir/Ritonavir, Chloroquine et Resveratrol montre une faible toxicité pour ces trois traitements.

9.2. Evaluation de l'effet sur la réplication du HCoV-229E.

La mesure de l'effet antiviral sur des cellules MRC5 infectées avec HCoV-229E a été réalisée. Le resveratrol et le lopinavir/ritonavir montre la plus forte réduction de la réplication virale, la chloroquine montrant également une réduction de la réplication virale.

L'effet antiviral de ces trois traitements a été mesuré seul ou en combinaison. L'utilisation de traitements combinés n'a pas montré une augmentation de l'effet antiviral par rapport aux traitements seuls. Il ne semble donc pas y avoir d'effet synergique de ces traitements. Nous avons évalué l'impact du moment du traitement sur l'effet antiviral de ces trois traitements. Nous n'avons pas observé de différence significative lorsque les cellules sont pré-traitées ou lorsque les cellules sont traitées après l'infection. L'effet antiviral semble donc affecter la réplication virale mais pas la pénétration virale.

9.3. Evaluation de l'effet sur la réplication du SARS-CoV-2.

Ces résultats obtenus sur HCoV-229E ont permis de valider ces trois traitements pour une évaluation de leur effet sur SARS-CoV-2. L'effet antiviral observé sur HCoV-229E du lopinavir/ritonavir et de la chloroquine est cohérent avec les travaux publiés précédemment par d'autres équipes [321].

La mesure de toxicité sur les cellules Vero E6, utilisées pour l'étude du SARS-CoV-2, a été réalisée avec des doses croissantes de lopinavir/Ritonavir, Chloroquine et Resveratrol. Nous avons observé une forte toxicité du lopinavir/ritonavir et de la chloroquine. Le resveratrol présente une toxicité réduite. La mesure de l'effet antiviral du lopinavir/ritonavir et de la chloroquine sur des cellules Vero E6 infectées par SARS-CoV-2 n'a pas pu être réalisée de manière fiable en raison de la toxicité observée pour ces molécules. La mesure de l'activité antivirale du resveratrol montre une forte inhibition de la réplication du SARS-CoV-2 dans les cellules Vero E6.

9.4. Discussion

Nous avons montré l'effet antiviral sur le coronavirus HCoV-229E de trois composés, lopinavir/ritonavir, chloroquine et resveratrol. Le resveratrol est le composé le plus efficace, avec un indice de spécificité de 45. Nous avons également montré un effet antiviral du resveratrol sur le SARS-CoV-2. Le lopinavir/ritonavir et la chloroquine ont montré une toxicité cellulaire élevée sur les cellules Vero E6, ce qui ne permet pas d'observer un effet antiviral significatif.

La toxicité observée pour le lopinavir/ritonavir et la chloroquine est similaire à celle observée par d'autres équipes [321]. Bien que ces deux molécules aient un potentiel antiviral intéressant, les différents essais réalisés n'ont pas montré d'effet antiviral significatif chez les patients atteints de la Covid-19. Aucune amélioration clinique, réduction de la mortalité ou réduction de la charge virale n'ont été montrés pour ces deux molécules [285,286,322].

Le resveratrol semble être une molécule d'intérêt pour le traitement des infections par les coronavirus. L'inhibition de la réplication du SARS-CoV-2 par le resveratrol que nous avons montré est similaire aux résultats obtenus par d'autres équipes [323]. Le resveratrol a précédemment été montré comme possédant des propriétés anti-inflammatoires, qui renforcent son intérêt dans le cadre du traitement de la Covid-19 [324]. Une limitation de l'utilisation du resveratrol reste cependant sa faible biodisponibilité [325]. L'obtention d'une concentration plasmatique suffisante nécessite l'utilisation d'une forte dose de resveratrol, avec un temps de demi-vie réduit.

Globalement, bien que le resveratrol semble être prometteur, il reste nécessaire de confirmer son efficacité dans un modèle pré-clinique ainsi que de réfléchir à de nouveaux modes d'administration permettant une efficacité optimale.

Cette étude a fait l'objet d'une publication dans la revue Viruses en 2021.

9.5. Article paru dans la revue Viruses en 2021 : « Resveratrol Inhibits HCoV-229E and SARS-CoV-2 Coronavirus Replication In Vitro »



Article

Resveratrol Inhibits HCoV-229E and SARS-CoV-2 Coronavirus Replication In Vitro

Sébastien Pasquereau ¹, Zeina Nehme ^{1,2}, Sandy Haidar Ahmad ^{1,2}, Fadoua Daouad ³, Jeanne Van Assche ³, Clémentine Wallet ³, Christian Schwartz ³, Olivier Rohr ³, Stéphanie Morot-Bizot ⁴ and Georges Herbein ^{1,5,*}

¹ Pathogens & Inflammation/EPILAB Laboratory, EA 4266, Université de Franche-Comté, Université Bourgogne Franche-Comté (UBFC), 25030 Besançon, France; sebastien.pasquereau@univ-fcomte.fr (S.P.); zeina.nehme@univ-fcomte.fr (Z.N.); sandyhaidar@outlook.com (S.H.A.)

² Lebanese University, P.O. Box 6573/14 Badaro Museum, Beirut, Lebanon

³ FMST, EA7292, Université de Strasbourg, IUT Louis Pasteur, 67300 Schiltigheim, France; f.daouad@unistra.fr (F.D.); jvanassche@unistra.fr (J.V.A.); clementine.wallet@unistra.fr (C.W.); schwartz.christian@unistra.fr (C.S.); olivier.rohr@unistra.fr (O.R.)

⁴ Apex Biosolutions, 25000 Besançon, France; smorot@apexlabo.com

⁵ Department of Virology, Besançon University Hospital, 25030 Besançon, France

* Correspondence: georges.herbein@univ-fcomte.fr



Citation: Pasquereau, S.; Nehme, Z.; Haidar Ahmad, S.; Daouad, F.; Van Assche, J.; Wallet, C.; Schwartz, C.; Rohr, O.; Morot-Bizot, S.; Herbein, G. Resveratrol Inhibits HCoV-229E and SARS-CoV-2 Coronavirus Replication In Vitro. *Viruses* **2021**, *13*, 354. <https://doi.org/10.3390/v13020354>

Academic Editor: Graciela Andrei

Received: 21 January 2021

Accepted: 17 February 2021

Published: 23 February 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: A novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged in China at the end of 2019 causing a large global outbreak. As treatments are of the utmost importance, drug repurposing embodies a rich and rapid drug discovery landscape, where candidate drug compounds could be identified and optimized. To this end, we tested seven compounds for their ability to reduce replication of human coronavirus (HCoV)-229E, another member of the coronavirus family. Among these seven drugs tested, four of them, namely rapamycin, disulfiram, loperamide and valproic acid, were highly cytotoxic and did not warrant further testing. In contrast, we observed a reduction of the viral titer by 80% with resveratrol (50% effective concentration (EC₅₀) = 4.6 μM) and lopinavir/ritonavir (EC₅₀ = 8.8 μM) and by 60% with chloroquine (EC₅₀ = 5 μM) with very limited cytotoxicity. Among these three drugs, resveratrol was less cytotoxic (cytotoxic concentration 50 (CC₅₀) = 210 μM) than lopinavir/ritonavir (CC₅₀ = 102 μM) and chloroquine (CC₅₀ = 67 μM). Thus, among the seven drugs tested against HCoV-229E, resveratrol demonstrated the optimal antiviral response with low cytotoxicity with a selectivity index (SI) of 45.65. Similarly, among the three drugs with an anti-HCoV-229E activity, namely lopinavir/ritonavir, chloroquine and resveratrol, only the latter showed a reduction of the viral titer on SARS-CoV-2 with reduced cytotoxicity. This opens the door to further evaluation to fight Covid-19.

Keywords: SARS-CoV-2; HCoV-229E; resveratrol; coronavirus; viral inhibition

1. Introduction

Renowned as rapidly evolving viruses, human coronaviruses (HCoVs) are positive-stranded RNA pathogens that encompass seven different strains divided into two genera: alphacoronavirus (alphaCoV) and betacoronavirus (betaCoV) [1]. Whilst alphaCoV includes the lowly pathogenic HCoV-229E and HCoV-NL63, betaCoV ramifies into the more pathogenic HCoV-OC43 and HCoV-HKU1 of the A lineage and the highly pathogenic severe acute respiratory syndrome coronavirus (SARS-CoV), SARS-CoV-2 and the Middle East respiratory syndrome coronavirus (MERS-CoV) of the B and C lineage, respectively [2,3]. HCoV-229E, HCoV-NL63, HCoV-OC43 and HCoV-HKU1 are endemic in human populations, accounting for 15–30% of mild, self-limiting respiratory upper infections, with a greater incidence of lower respiratory tract infection in immunocompromised individuals [4]. Both SARS-CoV, emerging in 2002 with a mortality rate of 9.6%, and MERS-CoV, responsible for the 2012 outbreak with a death rate of 35.5%, are linked to acute

respiratory distress syndrome (ARDS) potentially engendering a chronic reduction in lung function, arrhythmia or death [5]. Emerging in December 2019 from the city of Wuhan in China, SARS-CoV-2, the etiological agent behind coronavirus disease 2019 (Covid-19), is considered the cause of a major public health emergency after its large global outbreak [6]. Although causing milder symptoms compared with SARS-CoV and MERS-CoV, SARS-CoV-2 can result in severe symptoms with multiple organ complications and hyperinflammation, leading ultimately to death in some individuals [7].

Despite the fact that SARS-CoV-2 vaccines have been quickly developed [8], no definitive therapy has been shown to be effective to date against SARS-CoV-2 and therefore new treatments are actively needed [9]. Despite tremendous efforts to develop new drugs, it is well-known that this process might take years to be clinically approved and reach the market [10]. In this context, repurposing of existing drugs appears to be an effective drug discovery platform that is superior in terms of cost and time compared with de novo drug discovery, as rapid assessment of in vitro and in vivo efficacy is conceivable [11]. This is reinforced by the fact that agents previously shown to inhibit the in vitro replication of other members of the coronavirus family, for instance HCoV-229E, could be potential candidates to inhibit the replication of betacoronaviruses such as SARS-CoV, MERS-CoV and SARS-CoV-2 [12].

In the light of what has been mentioned previously, we screened seven drugs already approved for other pathologies against the alphacoronavirus HCoV-229E. On the assumption that an inhibitory effect against HCoV-229E by one of these drugs will be found, results will be extrapolated and assessed in the context of SARS-CoV-2-infected cell cultures.

Among the seven compounds tested, valproic acid (VPA), disulfiram, rapamycin and loperamide have been previously shown to target coronavirus infection [13]. Briefly, VPA is a branched short-chain fatty acid commonly used in the management of neurological disorders [14]. This histone deacetylase inhibitor was projected as a potential candidate for drug repositioning as the viral SARS-CoV-2 protein NSP5 was found to interact with HDAC2 [15]. Disulfiram, an approved alcohol-aversive drug, was shown to inhibit the papain-like proteases of both MERS-CoV and SARS-CoV [16]. The mTOR inhibitor rapamycin, a drug used as an immunosuppressant in transplant patients, was reported to reduce MERS-CoV infection by over 60% in vitro [17] by suppressing viral protein expression and virion release [18]. Alternatively, the antidiarrheal opioid receptor agonist loperamide showed potential antiviral activity against SARS-CoV-2 [11], as well as MERS-CoV, SARS-CoV and HCoV-229E, although the exact mechanism of action is unknown [19].

Being an aspartate protease inhibitor, lopinavir is an extensively studied antiviral drug used in the management of human immunodeficiency virus (HIV) infection in combination with ritonavir, the latter boosting the effects of lopinavir through potent cytochrome P450 3A4 inhibition [20]. Lopinavir was shown to be an inhibitor of broad-spectrum coronaviruses by blocking the replication of HCoV-229E, SARS-CoV and MERS-CoV with a mean 50% effective concentration (EC₅₀) ranging from 6.6 to 17.1 μ M, in contrast to ritonavir, which showed no selective anti-CoV activity [19]. SARS-CoV patients treated with a combination of lopinavir/ritonavir and ribavirin experienced less ARDS with a lower death rate compared with historical controls who received ribavirin and corticosteroids [21]. Randomized controlled trials were conducted to assess the efficacy of lopinavir/ritonavir with or without ribavirin in SARS-CoV-2 patients [22,23].

Used originally as treatment for or prevention of malaria, the 4-aminoquinoline drug chloroquine has been repurposed and successfully employed to target some immunological and rheumatological diseases, for instance systemic lupus erythematosus and rheumatoid arthritis [24]. In the setting of viral infections, multiple mechanisms underlie the effectiveness of chloroquine, prominently chloroquine-mediated alkalization of phagolysosomes and/or viral entry and protein glycosylation inhibition [25]. Potential therapeutic benefits of chloroquine have been demonstrated in coronavirus infections including HCoV-229E, HCoV-OC43, SARS-CoV and SARS-CoV-2 [26]. Indeed, chloroquine inhibits HCoV-229E

replication in Huh7 cells with an EC₅₀ of 3.3 μM [19]. Moreover, pre and post treatment of Vero E6 cultures with chloroquine demonstrated antiviral activity against SARS-CoV-2 with an EC₅₀ and EC₉₀ of 1.13 μM and 6.90 μM , respectively [27]. In vivo, chloroquine administration was shown to inhibit SARS-CoV-2-related pneumonia, promote a virus negative conversion and reduce the disease course [28]. Based on these encouraging results, more than twenty in vivo clinical trials are in progress to evaluate chloroquine's effectiveness against SARS-CoV-2 [29]. Nevertheless, the efficiency of chloroquine against Covid-19 remains highly controversial [30].

Belonging to the polyphenols stilbenoids group, resveratrol is a well-known nutraceutical with a multitude of biological properties and therapeutic applications [31]. Among the latter, resveratrol exhibits an anti-tumor activity that has been confirmed in in vitro and in vivo studies, where resveratrol modulates a divergent set of transcription factors, upstream kinases and intracellular signaling pathways, thus mediating its inhibitory effect on the initiation, promotion and progression of carcinogenesis [32]. In addition, resveratrol exhibits anti-aging and potent antioxidant properties [33], as well as an anti-inflammatory activity, where resveratrol was shown to reduce the secretion and expression of inflammatory factors [34]. Besides, resveratrol has been reported to exhibit a potent anti-viral activity not only against RNA viruses such as influenza virus [35], rhinovirus [36], rotavirus [37], Zika virus [38] and MERS-CoV [39], but also against a few DNA viruses including poxvirus [40] and polyomavirus [41]. In the context of anti-MERS-CoV activities in vitro, resveratrol was shown to inhibit the viral nucleocapsid protein translation and MERS-CoV-induced caspase 3 cleavage, which can promote cell survival and reduce virus-induced apoptosis [39]. Interestingly, resveratrol increases the expression of ACE2 on the cell surface, which has been previously reported to be beneficial for SARS-CoV-2 patients [42,43]. In fact, low levels of ACE2 are observed in Covid-19 patients with poor prognosis and hyperinflammatory storm [44]. In addition, resveratrol is a known activator of sirtuin 1 (SIRT1), which downregulates the expression of AT1R of the PRR/ACE/AngII/AT1R axis and enhances the ACE2/Ang 1-7/MasR axis [45]. Increase in the ACE2/Ang1-7/MasR axis has been reported to reduce hyperinflammation in Covid-19 patients [44]. In the present study, we assessed the anti-coronavirus activity of the seven abovementioned drugs with special emphasis on the impact of resveratrol on HCoV-229E and SARS-CoV-2 compared to lopinavir/ritonavir and chloroquine.

2. Materials and Methods

2.1. Viruses and Reagents Preparation

HCoV-229E was isolated from nasal and throat swabs collected from a man with mild upper respiratory illness (Human Coronavirus 229E ATCC VR-740) and propagated using MRC5 cells (RD Biotech, Besançon, France). HCoV-229E stock virus (5 log PFU/mL) was prepared in MRC5 cells in DMEM media supplemented with 10% FCS. SARS-CoV-2 strain was provided by the Institut Pasteur, Paris (strain BetaCov/France). Compounds were sourced from Sigma-Aldrich (St-Louis, MO, USA, resveratrol, chloroquine diphosphate), Pfizer, (New York, NY, USA, rapamycin), Sanofi (Paris, France, valproic acid or VPA, disulfiram), Abbott (Chicago, IL, USA, lopinavir/ritonavir) and Sandoz (Holzkirchen, Germany, loperamide). The stocks were prepared with DMSO (200 mM resveratrol, 50 mM lopinavir/ritonavir, 1 mM rapamycin, 2.5 mM VPA, 10 mM loperamide, 10 mM disulfiram) or with DMEM media (1 mM chloroquine diphosphate).

2.2. Viral Replication Inhibition Assay

To evaluate the effect of compounds in vitro, MRC5 cells were treated with compounds diluted in culture media at time of infection by HCoV-229E at multiplicity of infection (MOI) = 0.01 (low titer) or 1 (high titer). Antiviral compounds were maintained with the virus inoculum during the 2 h incubation period. The inoculum was removed after incubation, and the cells were overlaid with culture media containing diluted compounds. After 48 h of incubation at 37 °C, supernatants were collected to quantify viral

loads by plaque forming unit (PFU) assay as previously described [46]. Briefly, MRC5 cells were infected with the supernatant for 2 h and then overlaid with culture media containing agarose (1%). Cells were incubated for 48 h. Plaque formation was observed under a light microscope. Cells were stained with MTT for 1 hour and plaque forming units were counted. Four-parameter logistic regression (IC50 Toolkit IC50.org) was used to fit the dose–response curves and determine the 50% effective concentrations (EC50) of the compounds that inhibit viral replication by 50%. Vero E6 cells were treated with compounds at time of infection by SARS-CoV-2 (BetaCoV/France, Institut Pasteur). The viral inoculum was removed after incubation and the cells were overlaid with fresh media containing the compound. After 48 h of incubation at 37 °C, supernatants were collected to quantify viral loads. Viral RNA was extracted using a guanidinium thiocyanate buffer and magnetic silica beads. Finally, RNA was detected for the SARS-CoV-2 envelop (E) gene using one step qRT-PCR (TaqMan Fast Virus 1-Step Master Mix, Applied Biosystem) and the primers designed by Corman et al. [47]. Briefly, the SARS-CoV-2 envelop gene was amplified using E_Sarbeco_F 5'-ACAGGTACGTTAATAGTTAATAGCGT-3' and E_Sarbeco_R 5'-ATATTGCAGCAGTACGCACACA-3' primers. Detection of amplification was done using a specific SARS-CoV-2 envelop probe (E_Sarbeco_P1) 5'-FAM-ACACTAGCCATCCTTACTGCGCTTCG-BHQ1-3'.

2.3. Cytotoxicity Assays

Cytotoxicity of selected compounds was evaluated in MRC5 cells using MTT assay. Briefly, cells were treated with compounds for 48 h. Culture media was replaced and cells were incubated with MTT (1.2 mM, Life Technologies, Eugene, OR, USA) for 2 h. Formazan crystals were dissolved by addition of acidic isopropanol and cell viability was measured by a spectrophotometer (Bio-Rad, Hercules, CA, USA). Cytotoxicity of compounds on Vero E6 cells was measured using several cytotoxic assays, including a WST-1 assay (Roche, Basel, Switzerland). The cytotoxic concentration 50 (CC50) is the concentration of compound that reduced cell viability by 50%. This was calculated using four-parameter logistic regression (IC50 Toolkit IC50.org).

Selectivity index (SI) was calculated by dividing CC50 by EC50 for each compound and virus tested. The selectivity index is an indicator that measures the window between cytotoxicity and antiviral activity. A high selectivity index reflects a low toxicity at concentrations that show an effective antiviral activity. It also indicates that there is an important range of concentrations showing an effective antiviral effect with minimal toxicity. Conversely, a low selectivity index would indicate an important toxicity for concentrations with effective antiviral activity.

3. Results

VPA, disulfiram, rapamycin and loperamide showed cytotoxicity on MRC5 cells with a CC50 of 4 mM, 8.4 µM, 2.5 µM, and 10.1 µM, respectively (Table 1, Figure 1). In addition, rapamycin and VPA display a narrowed selectivity index (SI) of 1.09 and 2.98, respectively (Table 1, Figure 1). By contrast, lopinavir/ritonavir, chloroquine and resveratrol were found to inhibit HCoV-229E replication in MRC5 cells with low cytotoxicity and an SI ranging from 11.6 to 45.6 (Table 1), and were tested further.

In line with previous reports [19], we observed an EC50 of 8.81 µM for lopinavir on HCoV-229E-treated MRC5 cells. We observed an antiviral effect of 50 µM lopinavir/ritonavir against high titers of HCoV-229E (MOI = 1) with approximately 80% inhibition of viral replication in vitro (Figure 1 and Table 1). Against low titers of HCoV-229E (MOI = 0.01), we observed a 63% inhibition of viral replication in cells treated with 10 µM of lopinavir/ritonavir (Figure 2). As combinational therapy of effective compounds against SARS-CoV-2 virus might convey additional benefits in terms of synergy and inhibitory concentration reduction [48], we therefore tested combinatory treatment of lopinavir/ritonavir with chloroquine and resveratrol. However, we did not observe a significant decrease in HCoV-229E in culture versus lopinavir/ritonavir when used alone (Figure 2B).

Table 1. The 50% effective concentration (EC50), cytotoxic concentration 50 (CC50) and selectivity index (SI) for the seven drugs tested for human coronavirus (HCoV)-229E replication in MRC5 cells.

Drug Tested	CC50 (µM)	EC50 (µM)	SI
Lopinavir/Ritonavir	102.5	8.8163	11.62619
Chloroquine	67.9	5	13.58
Resveratrol	210	4.6	45.65217
Rapamycin	2.5	2.28	1.096491
Disulfiram	8.4	0.1387	60.56236
Loperamide	10.1	0.094732	106.6166
VPA	4 mM	1.339 mM	2.987304

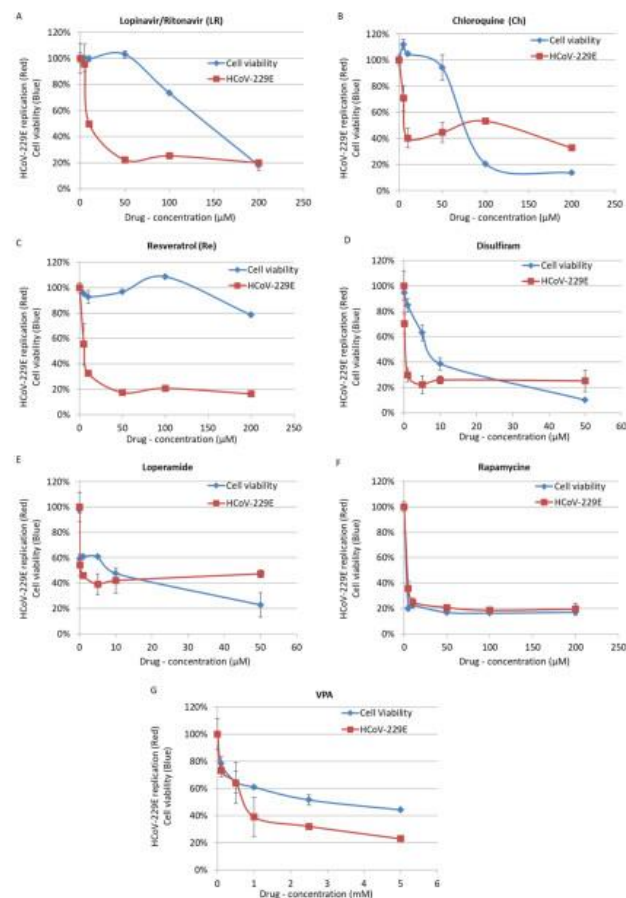


Figure 1. Inhibition of replication of high HCoV-229E viral load (Red), measured by plaque forming unit (PFU) assay, coupled with MTT toxicity assay (Blue) for Lopinavir/Ritonavir (A), Chloroquine (B), Resveratrol (C), Disulfiram (D), Loperamide (E), Rapamycin (F) and valproic acid (VPA) (G). Cells were treated by compounds at the time of infection with HCoV-229E (1 multiplicity of infection (MOI)). PFU and MTT assays were performed after 48 h. Uninfected cells were used for normalization of MTT assay. Untreated cells infected with HCoV-229E were used for normalization of PFU assay.

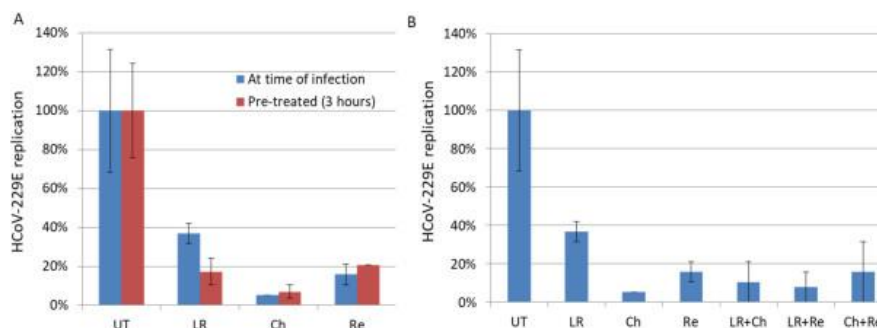


Figure 2. Inhibition of replication of low HCoV-229E viral load (0.01 MOI) by drugs. (A) No pretreatment (Blue) or 3-h pretreatment of cells (Red). (B) Combination of drugs. The cells were treated with Lopinavir/Ritonavir (LR), Chloroquine (Ch), Resveratrol (Re) or left untreated (UT) for 48 h. The viral replication was measured by PFU assay.

As reported previously, we observed that chloroquine inhibits HCoV-229E replication in MRC5 cells when added 3 h before infection but also when added at the time of infection (Figure 2). We also observed that chloroquine inhibits the replication of HCoV-229E in cells infected with a high titer of virus (EC₅₀ of 5 μ M; Figure 1). This is in agreement with the antiviral activity of hydroxychloroquine, a chloroquine derivative, with an EC₉₀ of 5.47 μ M in the context of SARS-CoV-2 infection [49]. We did not observe a synergistic effect of chloroquine used in combinatory treatments with lopinavir/ritonavir or resveratrol (Figure 2b). We also did not observe a significant difference in the effect of chloroquine when cells were treated before or after the infection (Figure 3). We observed some cytotoxicity of chloroquine on treated cells, with a CC₅₀ of 67.9 μ M (Figure 1 and Table 1).

Here, we observed that resveratrol inhibits HCoV-229E replication in MRC5 cells infected with a high viral titer (MOI = 1), with an EC₅₀ of 4.6 μ M (Figure 1 and Table 1). We also observed an inhibition of replication in cells infected with a low viral titer (MOI = 0.01) and treated with resveratrol (Figure 2). We did not observe a significant difference in the effect of resveratrol when cells were treated before or after the infection (Figure 3). We did not observe any synergistic effect of resveratrol used in combination with lopinavir/ritonavir or chloroquine (Figure 2b). Resveratrol displayed a low toxicity in treated cells, with a CC₅₀ of 210 μ M and a selectivity index (SI) equal to 45.65 (Figure 1 and Table 1). Based on the results obtained for HCoV-229E, we performed testing for SARS-CoV-2 inhibition. Treatment of Vero E6 cells with lopinavir/ritonavir and chloroquine was toxic and did not allow for the study of their anti-SARS-CoV-2 effect (Figure 4a). The important cell lysis in culture prevented an accurate measure of SARS-CoV-2 replication, giving partial and unreliable results (Figure 4b). In contrast, the cytotoxicity of resveratrol on Vero E6 cells occurred at concentrations higher than 50 μ M (Figure 4a). We observed that resveratrol inhibited SARS-CoV-2 replication by 3 logs at 25 μ M (Figure 4b). The percentage of inhibition of SARS-CoV-2 ranges from 0 to 99.93% when increasing the concentration of resveratrol from 0 to 25 μ M with an EC₉₀ and EC₅₀ of 11.42 μ M and 10.66 μ M, respectively (Figure 4). A very limited reduction in cell viability was also observed in Vero E6 cells treated with resveratrol at 50 μ M, overall leading to a CC₅₀ = 48.21 μ M and an SI of 4.52. The inhibition of SARS-CoV-2 replication we observed in cells treated with resveratrol did not follow the reduction in cell viability and was a genuine anti-SARS-CoV-2 effect of resveratrol *in vitro*.

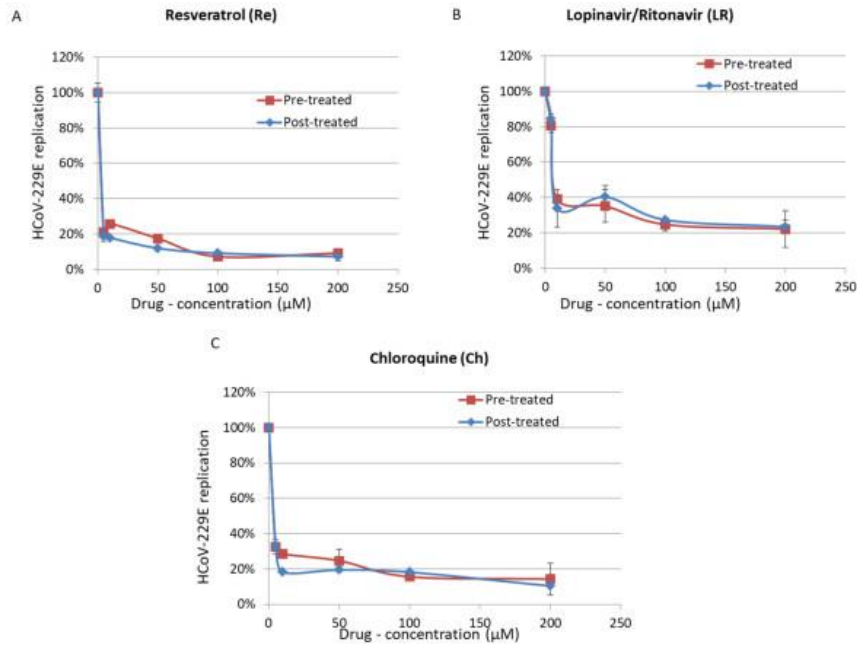


Figure 3. Inhibition of replication of high HCoV-229E viral load (1 MOI) by Resveratrol (A), Lopinavir/Ritonavir (B) and Chloroquine (C) in pre-treated (3 h, Red) or post-treated (4 h, Blue) MRC5 cells. Viral replication was measured by PFU assay after 48 h.

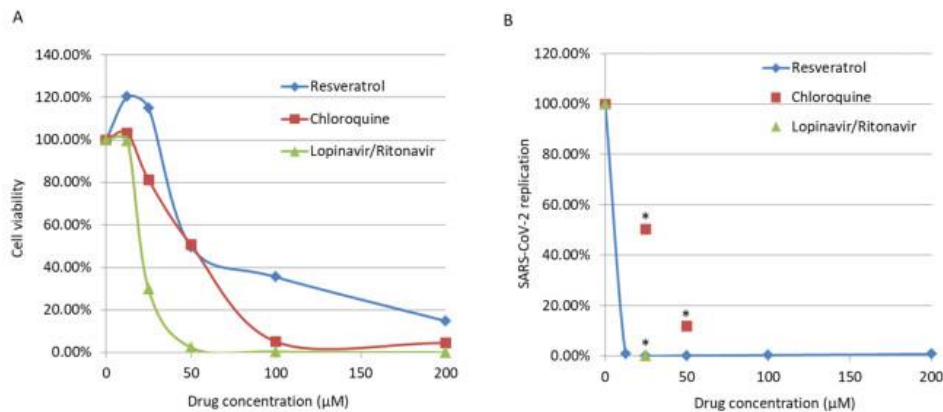


Figure 4. (A) Toxicity assay for Lopinavir/Ritonavir, Chloroquine and Resveratrol in Vero E6 cells, after 48 h. (B) Inhibition of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) replication in Vero E6 cells treated with Resveratrol as measured by qRT-PCR after 48 h. * Cytotoxicity impaired the evaluation of the anti-SARS-CoV-2 effect of Lopinavir/Ritonavir and Chloroquine.

4. Discussion

We confirm the antiviral activity of two compounds (lopinavir/ritonavir and chloroquine) that have been reported previously to inhibit HCoV-229E replication in vitro. In addition, we show the antiviral effect of resveratrol on HCoV-229E and SARS-CoV-2 replication in vitro. In addition, resveratrol is the most powerful compound with an EC50 of 4.6 μ M, a CC50 of 210 μ M and an SI of 45.65, indicating that it could be efficiently used to fight HCoV-229E infection. While their SI is lower, lopinavir/ritonavir and chloroquine might show clinical potential. For instance, it has been observed that HIV patients might reach minimal lopinavir serum concentration at 9.4 μ M (IQR 7.2–12.1 μ M) upon twice daily treatment with 400 mg lopinavir and 100 mg ritonavir [50], which is in the same range of the EC50 against HCoV-229E virus in vitro (8.8 μ M). The previously mentioned dosing regimen has been recommended by the by Chinese National Health Commission in the management of SARS-CoV-2 patients [51]. Nonetheless, no differences in time of clinical improvement, mortality at 28 days and detectable viral loads at various time points were reported between the lopinavir/ritonavir group and the standard-care group in a recent randomized, controlled, open-label trial [22]. Overall, a beneficial effect of lopinavir/ritonavir and chloroquine in Covid-19 patients is still highly debated.

We observed a high cytotoxicity of lopinavir/ritonavir and chloroquine on Vero E6 cells, which precluded the study of their anti-SARS-CoV-2 effect. In fact, several studies have already evaluated the anti-SARS-CoV-2 effect of drugs including lopinavir and chloroquine [48,49]. While most studies showed an antiviral effect in vitro for these two drugs that would warrant further investigation, they also indicated cytotoxicity of these drugs. The cytotoxicity we observed for lopinavir/ritonavir and chloroquine in the present study is in line with these previous reports [48,49].

In contrast to lopinavir/ritonavir and chloroquine, our testing for SARS-CoV-2 inhibition shows a genuine anti-viral effect of resveratrol. We observed a significant decrease in SARS-CoV-2 replication under non-cytotoxic doses of resveratrol up to 25 μ M in vitro. To further confirm the anti-SARS-CoV-2 effect of resveratrol using an in vitro model closer to the in vivo pathophysiology, we will assess in future studies the anti-SARS-CoV-2 effect of resveratrol using organotypic nasal epithelial cultures, which might better reflect the pathophysiology of SARS-CoV-2 infection compared with the renal epithelial Vero E6 cells. During the revision process of our manuscript, new reports that showed the effect of resveratrol on SARS-CoV-2 replication became available. Yang et al. reported the inhibition of SARS-CoV-2 replication by resveratrol, with an EC50 of 4.48 μ M, which is in line with our findings [52]. In a study yet to be published, Ter Ellen and colleagues showed that resveratrol and the structurally related molecule pterostilbene both inhibited the replication of SARS-CoV-2, especially in primary human bronchial epithelium cells [53].

Although resveratrol, which displays both antiviral and anti-inflammatory properties, could be an interesting treatment for Covid-19 patients [54], its use is limited by poor bioavailability. Multiple strategies have been employed to overcome the poor bioavailability of resveratrol in vivo [55], including a soluble formulation of trans-resveratrol [56] and its administration as a nasal spray [57]. Although administered doses vary widely between 10 mg and 5 g [58], previous pharmacokinetic studies showed that patients receiving a single 500 mg oral dose of resveratrol might reach an adequate plasma concentration to promote the pharmacological activities of 71.2 ± 42.4 ng/mL e.g., 0.31 μ M, with a T_{max} of 1.3 h [59]. In addition, nasal administration to expose the lungs to resveratrol reduced tumor volume and tumor multiplicity by 45% and 27%, respectively, in A/J mice with lung cancer [60]. In Covid-19 patients hospitalized in emergency units, the use of naso-tracheal administration of resveratrol might allow for direct delivery of the treatment into the lung and could provide maximum antiviral and anti-inflammatory efficiency.

In conclusion, further testing is needed to confirm whether resveratrol, either alone or in combination therapy, could be of use in a clinical setting. Finally, it is important to note that our results on SARS-CoV-2 inhibition by resveratrol are in vitro data and do not

indicate that resveratrol can be used to treat Covid-19 until double-blinded clinical assays have been successfully performed.

5. Conclusions

We confirm the anti-HCoV-229E activity of two compounds, lopinavir/ritonavir and chloroquine, that have been previously reported to inhibit HCoV-229E replication in vitro. In addition, we show here the antiviral effect of resveratrol on HCoV-229E and SARS-CoV-2 replication in vitro. Resveratrol is the most powerful compound to fight HCoV-229E infection with an EC50 of 4.6 μ M, a CC50 of 210 μ M and an SI of 45.65. In addition, our testing for SARS-CoV-2 inhibition shows an anti-viral effect of resveratrol in vitro, which will be assessed in future studies in organotypic nasal epithelial cultures. This will better reflect the pathophysiology of Covid-19 infection and might lead to its use in a clinical setting, either alone or as part of a combined treatment against Covid-19.

Author Contributions: Conceptualization, G.H.; formal analysis, S.P., S.H.A., Z.N., F.D., J.V.A. and C.W.; investigation, S.P., Z.N., S.H.A., F.D., J.V.A., C.W., C.S. and S.M.-B.; writing—original draft preparation, S.P., Z.N., S.M.-B. and G.H.; writing—review and editing, S.P., Z.N., S.H.A., S.M.-B., F.D., J.V.A., C.W., C.S., O.R. and G.H.; visualization, S.P.; supervision, G.H.; project administration, G.H.; funding acquisition, G.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by University of Franche-Comté and the University of Bourgogne-Franche-Comté.

Data Availability Statement: The data presented in this study is available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Phan, M.V.T.; Ngo Tri, T.; Hong Anh, P.; Baker, S.; Kellam, P.; Cotten, M. Identification and characterization of Coronaviridae genomes from Vietnamese bats and rats based on conserved protein domains. *Virus Evol.* **2018**, *4*, 35. [[CrossRef](#)]
2. Lim, Y.X.; Ng, Y.L.; Tam, J.P.; Liu, D.X. Human Coronaviruses: A Review of Virus–Host Interactions. *Diseases* **2016**, *4*, 26. [[CrossRef](#)]
3. Chan, J.F.-W.; Kok, K.-H.; Zhu, Z.; Chu, H.; To, K.K.-W.; Yuan, S.; Yuen, K.-Y. Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. *Emerg. Microbes Infect.* **2020**, *9*, 221–236. [[CrossRef](#)]
4. Van der Hoek, L. Human coronaviruses: What do they cause? *Antivir. Ther.* **2007**, *12*, 651–658.
5. Fehr, A.R.; Perlman, S. Coronaviruses: An overview of their replication and pathogenesis. *Methods Mol. Biol.* **2015**, *1282*, 1–23.
6. Zheng, J. SARS-CoV-2: An Emerging Coronavirus that Causes a Global Threat. *Int. J. Biol. Sci.* **2020**, *16*, 1678–1685. [[CrossRef](#)]
7. Geng, Y.-J.; Wei, Z.-Y.; Qian, H.-Y.; Huang, J.; Lodato, R.; Castriotta, R.J. Pathophysiological Characteristics and Therapeutic Approaches for Pulmonary Injury and Cardiovascular Complications of Coronavirus Disease 2019. *Cardiovasc. Pathol.* **2020**, *17*, 107228. [[CrossRef](#)]
8. Krammer, F. SARS-CoV-2 vaccines in development. *Nature* **2020**, *586*, 516–527. [[CrossRef](#)]
9. Sanders, J.M.; Monogue, M.L.; Jodlowski, T.Z.; Cutrell, J.B. Pharmacologic Treatments for Coronavirus Disease 2019 (COVID-19): A Review. *JAMA* **2020**, *323*, 1824–1836. [[CrossRef](#)]
10. Chen, W.-H.; Strych, U.; Hotez, P.J.; Bottazzi, M.E. The SARS-CoV-2 Vaccine Pipeline: An Overview. *Curr. Trop. Med. Rep.* **2020**, *3*, 1–4. [[CrossRef](#)]
11. Zhou, Y.; Hou, Y.; Shen, J.; Huang, Y.; Martin, W.; Cheng, F. Network-based drug repurposing for novel coronavirus 2019-nCoV/SARS-CoV-2. *Cell Discov.* **2020**, *6*, 1–18. [[CrossRef](#)]
12. Pyrc, K.; Berkhout, B.; van der Hoek, L. Antiviral strategies against human coronaviruses. *Infect. Disord. Drug Targets* **2007**, *7*, 59–66. [[CrossRef](#)]
13. Li, G.; De Clercq, E. Therapeutic options for the 2019 novel coronavirus (2019-nCoV). *Nat. Rev. Drug Discov.* **2020**, *19*, 149–150. [[CrossRef](#)] [[PubMed](#)]
14. Chateauvieux, S.; Morceau, F.; Dicato, M.; Diederich, M. Molecular and Therapeutic Potential and Toxicity of Valproic Acid. *J. Biomed. Biotechnol.* **2010**, *2010*, 479364. [[CrossRef](#)] [[PubMed](#)]

15. Napolitano, F.; Gambardella, G.; Gao, X.; di Bernardo, D. Computational Drug Repositioning and Elucidation of Mechanism of Action of Compounds against sars-cov-2. *arXiv* **2020**, arXiv:2004.07697.
16. Lin, M.-H.; Moses, D.C.; Hsieh, C.-H.; Cheng, S.-C.; Chen, Y.-H.; Sun, C.-Y.; Chou, C.-Y. Disulfiram can inhibit MERS and SARS coronavirus papain-like proteases via different modes. *Antivir. Res.* **2018**, *150*, 155–163. [[CrossRef](#)] [[PubMed](#)]
17. Dylla, J.; Gross, R.; Kindrachuk, J.; Johnson, R.F.; Olinger, G.G.; Hensley, L.E.; Frieman, M.B.; Jahrling, P.B. Middle East Respiratory Syndrome and Severe Acute Respiratory Syndrome: Current Therapeutic Options and Potential Targets for Novel Therapies. *Drugs* **2017**, *77*, 1935–1966. [[CrossRef](#)] [[PubMed](#)]
18. Jeon, S.; Ko, M.; Lee, J.; Choi, I.; Byun, S.Y.; Park, S.; Shum, D.; Kim, S. Identification of Antiviral Drug Candidates against SARS-CoV-2 from FDA-Approved Drugs. *Antimicrob. Agents Chemother.* **2020**, *64*. [[CrossRef](#)] [[PubMed](#)]
19. De Wilde, A.H.; Jochmans, D.; Posthuma, C.C.; Zevenhoven-Dobbe, J.C.; van Nieuwkoop, S.; Bestebroer, T.M.; van den Hoogen, B.G.; Neyts, J.; Snijder, E.J. Screening of an FDA-approved compound library identifies four small-molecule inhibitors of Middle East respiratory syndrome coronavirus replication in cell culture. *Antimicrob. Agents Chemother.* **2014**, *58*, 4875–4884. [[CrossRef](#)] [[PubMed](#)]
20. Chandwani, A.; Shuter, J. Lopinavir/ritonavir in the treatment of HIV-1 infection: A review. *Ther. Clin. Risk Manag.* **2008**, *4*, 1023–1033. [[PubMed](#)]
21. Chu, C.M.; Cheng, V.C.C.; Hung, I.F.N.; Wong, M.M.L.; Chan, K.H.; Chan, K.S.; Kao, R.Y.T.; Poon, L.L.M.; Wong, C.L.P.; Guan, Y.; et al. Role of lopinavir/ritonavir in the treatment of SARS: Initial virological and clinical findings. *Thorax* **2004**, *59*, 252–256. [[CrossRef](#)] [[PubMed](#)]
22. Cao, B.; Wang, Y.; Wen, D.; Liu, W.; Wang, J.; Fan, G.; Ruan, L.; Song, B.; Cai, Y.; Wei, M.; et al. A Trial of Lopinavir–Ritonavir in Adults Hospitalized with Severe Covid-19. *N. Engl. J. Med.* **2020**, *382*, 1787–1799. [[CrossRef](#)] [[PubMed](#)]
23. ClinicalTrials.gov. Bethesda (MD) National Library of Medicine (US) Identifier NCT00578825, A Multi-centre, Double-Blinded, Randomized, Placebo-Controlled Trial on the Efficacy and Safety of Lopinavir/Ritonavir Plus Ribavirin in the Treatment of Severe Acute Respiratory Syndrome. Available online: <https://clinicaltrials.gov/ct2/show/NCT00578825> (accessed on 11 May 2020).
24. Plantone, D.; Koudriavtseva, T. Current and Future Use of Chloroquine and Hydroxychloroquine in Infectious, Immune, Neoplastic, and Neurological Diseases: A Mini-Review. *Clin. Drug Investig.* **2018**, *38*, 653–671. [[CrossRef](#)]
25. Savarino, A.; Boelaert, J.R.; Cassone, A.; Majori, G.; Cauda, R. Effects of chloroquine on viral infections: An old drug against today's diseases? *Lancet Infect. Dis.* **2003**, *3*, 722–727. [[CrossRef](#)]
26. Devaux, C.A.; Rolain, J.-M.; Colson, P.; Raoult, D. New insights on the antiviral effects of chloroquine against coronavirus: What to expect for COVID-19? *Int. J. Antimicrob. Agents* **2020**, *55*, 105938. [[CrossRef](#)]
27. Wang, M.; Cao, R.; Zhang, L.; Yang, X.; Liu, J.; Xu, M.; Shi, Z.; Hu, Z.; Zhong, W.; Xiao, G. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res.* **2020**, *30*, 269–271. [[CrossRef](#)] [[PubMed](#)]
28. Gao, J.; Tian, Z.; Yang, X. Breakthrough: Chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. *Biosci. Trends* **2020**, *14*, 72–73. [[CrossRef](#)]
29. Lythgoe, M.P.; Middleton, P. Ongoing Clinical Trials for the Management of the COVID-19 Pandemic. *Trends Pharm. Sci.* **2020**, *41*, 363–382. [[CrossRef](#)]
30. Hoffmann, M.; Mösbauer, K.; Hofmann-Winkler, H.; Kaul, A.; Kleine-Weber, H.; Krüger, N.; Gassen, N.C.; Müller, M.A.; Drosten, C.; Pöhlmann, S. Chloroquine does not inhibit infection of human lung cells with SARS-CoV-2. *Nature* **2020**, *585*, 588–590. [[CrossRef](#)] [[PubMed](#)]
31. Salehi, B.; Mishra, A.P.; Nigam, M.; Sener, B.; Kilic, M.; Sharifi-Rad, M.; Fokou, P.V.T.; Martins, N.; Sharifi-Rad, J. Resveratrol: A Double-Edged Sword in Health Benefits. *Biomedicines* **2018**, *6*, 91. [[CrossRef](#)] [[PubMed](#)]
32. Ko, J.-H.; Sethi, G.; Um, J.-Y.; Shanmugam, M.K.; Arfuso, F.; Kumar, A.P.; Bishayee, A.; Ahn, K.S. The Role of Resveratrol in Cancer Therapy. *Int. J. Mol. Sci.* **2017**, *18*, 2589. [[CrossRef](#)]
33. Camins, A.; Junyent, F.; Verdager, E.; Beas-Zarate, C.; Rojas-Mayorquín, A.E.; Ortuño-Sahagún, D.; Pallàs, M. Resveratrol: An Antiaging Drug with Potential Therapeutic Applications in Treating Diseases. *Pharmaceuticals* **2009**, *2*, 194–205. [[CrossRef](#)]
34. Zhou, Z.-X.; Mou, S.-F.; Chen, X.-Q.; Gong, L.-L.; Ge, W.-S. Anti-inflammatory activity of resveratrol prevents inflammation by inhibiting NF- κ B in animal models of acute pharyngitis. *Mol. Med. Rep.* **2018**, *17*, 1269–1274. [[CrossRef](#)]
35. Palamara, A.T.; Nencioni, L.; Aquilano, K.; De Chiara, G.; Hernandez, L.; Cozzolino, F.; Ciriolo, M.R.; Garaci, E. Inhibition of influenza A virus replication by resveratrol. *J. Infect. Dis.* **2005**, *191*, 1719–1729. [[CrossRef](#)]
36. Mastromarino, P.; Capobianco, D.; Cannata, F.; Nardis, C.; Mattia, E.; De Leo, A.; Restignoli, R.; Francioso, A.; Mosca, L. Resveratrol inhibits rhinovirus replication and expression of inflammatory mediators in nasal epithelia. *Antivir. Res.* **2015**, *123*, 15–21. [[CrossRef](#)]
37. Huang, H.; Liao, D.; Zhou, G.; Zhu, Z.; Cui, Y.; Pu, R. Antiviral activities of resveratrol against rotavirus in vitro and in vivo. *Phytomedicine* **2020**, *77*, 153230. [[CrossRef](#)]
38. Mohd, A.; Zainal, N.; Tan, K.-K.; AbuBakar, S. Resveratrol affects Zika virus replication in vitro. *Sci. Rep.* **2019**, *9*, 14336. [[CrossRef](#)] [[PubMed](#)]
39. Lin, S.-C.; Ho, C.-T.; Chuo, W.-H.; Li, S.; Wang, T.T.; Lin, C.-C. Effective inhibition of MERS-CoV infection by resveratrol. *BMC Infect. Dis.* **2017**, *17*, 144. [[CrossRef](#)]
40. Cao, S.; Realegeno, S.; Pant, A.; Satheshkumar, P.S.; Yang, Z. Suppression of Poxvirus Replication by Resveratrol. *Front. Microbiol.* **2017**, *8*, 2196. [[CrossRef](#)]

41. Berardi, V.; Ricci, F.; Castelli, M.; Galati, G.; Risuleo, G. Resveratrol exhibits a strong cytotoxic activity in cultured cells and has an antiviral action against polyomavirus: Potential clinical use. *J. Exp. Clin. Cancer Res.* **2009**, *28*, 96. [[CrossRef](#)] [[PubMed](#)]
42. Kim, E.N.; Kim, M.Y.; Lim, J.H.; Kim, Y.; Shin, S.J.; Park, C.W.; Kim, Y.-S.; Chang, Y.S.; Yoon, H.E.; Choi, B.S. The protective effect of resveratrol on vascular aging by modulation of the renin-angiotensin system. *Atherosclerosis* **2018**, *270*, 123–131. [[CrossRef](#)]
43. Cheng, H.; Wang, Y.; Wang, G.-Q. Organ-protective effect of angiotensin-converting enzyme 2 and its effect on the prognosis of COVID-19. *J. Med. Virol.* **2020**, *92*, 726–730. [[CrossRef](#)]
44. Verdecchia, P.; Cavallini, C.; Spanevello, A.; Angeli, F. The pivotal link between ACE2 deficiency and SARS-CoV-2 infection. *Eur. J. Intern. Med.* **2020**, *10*, 1016.
45. Jang, I.-A.; Kim, E.N.; Lim, J.H.; Kim, M.Y.; Ban, T.H.; Yoon, H.E.; Park, C.W.; Chang, Y.S.; Choi, B.S. Effects of Resveratrol on the Renin-Angiotensin System in the Aging Kidney. *Nutrients* **2018**, *10*, 1741. [[CrossRef](#)]
46. Baer, A.; Kehin-Hall, K. Viral concentration determination through plaque assays: Using traditional and novel overlay systems. *J. Vis. Exp.* **2014**, *93*, e52065. [[CrossRef](#)]
47. Corman, V.M.; Landt, O.; Kaiser, M.; Molenkamp, R.; Meijer, A.; Chu, D.K.; Bleicker, T.; Brünink, S.; Schneider, J.; Schmidt, M.L.; et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Eurosurveillance* **2020**, *25*, 2000045. [[CrossRef](#)]
48. Choy, K.-T.; Wong, A.Y.-L.; Kaewpreedee, P.; Sia, S.F.; Chen, D.; Hui, K.P.Y.; Chu, D.K.W.; Chan, M.C.W.; Cheung, P.P.-H.; Huang, X.; et al. Remdesivir, lopinavir, emetine, and homoharringtonine inhibit SARS-CoV-2 replication in vitro. *Antivir. Res.* **2020**, *178*, 104786. [[CrossRef](#)]
49. Gordon, D.E.; Jang, G.M.; Bouhaddou, M.; Xu, J.; Obernier, K.; White, K.M.; O'Meara, M.J.; Rezelj, V.V.; Guo, J.Z.; Swaney, D.L.; et al. A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. *Nature* **2020**, *583*, 1–13. [[CrossRef](#)]
50. Lopez-Cortes, L.F.; Ruiz-Valderas, R.; Sánchez-Rivas, E.; Lluch, A.; Gutierrez-Valencia, A.; Torres-Cornejo, A.; BenMarzouk-Hidalgo, O.J.; Viciano, P. Lopinavir Plasma Concentrations and Virological Outcome with Lopinavir-Ritonavir Monotherapy in HIV-1-Infected Patients. *Antimicrob. Agents. Chemother.* **2013**, *57*, 3746–3751. [[CrossRef](#)]
51. Peng, F.; Tu, L.; Yang, Y.; Hu, P.; Wang, R.; Hu, Q.; Cao, F.; Jiang, T.; Sun, J.; Xu, G.; et al. Management and Treatment of COVID-19: The Chinese Experience. *Can. J. Cardiol.* **2020**, *36*, 915–930. [[CrossRef](#)]
52. Yang, M.; Wei, J.; Huang, T.; Lei, L.; Shen, C.; Lai, J.; Yang, M.; Liu, L.; Yang, Y.; Liu, G.; et al. Resveratrol inhibits the replication of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in cultured Vero cells. *Phytother. Res.* **2020**. [[CrossRef](#)] [[PubMed](#)]
53. Ter Ellen, B.M.; Kumar, N.D.; Bouma, E.M.; Troost, B.; van de Pol, D.P.; van der Ende-Metselaar, H.H.; Apperloo, L.; van Gosliga, D.; van den Berge, M.; Nawijn, M.C.; et al. Resveratrol And Pterostilbene Potently Inhibit SARS-CoV-2 Replication In Vitro. *bioRxiv* **2020**, 285940.
54. Wahedi, H.M.; Ahmad, S.; Abbasi, S.W. Stilbene-based natural compounds as promising drug candidates against COVID-19. *J. Biomol. Struct. Dyn.* **2020**, 1–10. [[CrossRef](#)] [[PubMed](#)]
55. Gambini, J.; Inglés, M.; Olaso, G.; Lopez-Grueso, R.; Bonet-Costa, V.; Gimeno-Mallench, L.; Mas-Bargues, C.; Abdelaziz, K.M.; Gomez-Cabrera, M.C.; Vina, J.; et al. Properties of Resveratrol: In Vitro and In Vivo Studies about Metabolism, Bioavailability, and Biological Effects in Animal Models and Humans. *Oxidative Med. Cell. Longev.* **2015**, *2015*, 837042. [[CrossRef](#)]
56. Amiot, M.J.; Romier, B.; Dao, T.-M.A.; Fanciullino, R.; Ciccolini, J.; Burelun, R.; Pechere, L.; Emond, C.; Savouret, J.-F.; Seree, E. Optimization of trans-Resveratrol bioavailability for human therapy. *Biochimie* **2013**, *95*, 1233–1238. [[CrossRef](#)]
57. Lv, C.; Zhang, Y.; Shen, L. Preliminary Clinical Effect Evaluation of Resveratrol in Adults with Allergic Rhinitis. *Int. Arch. Allergy Immunol.* **2018**, *175*, 231–236. [[CrossRef](#)] [[PubMed](#)]
58. Tomé-Carneiro, J.; Larrosa, M.; González-Sarriás, A.; Tomás-Barberán, F.A.; García-Conesa, M.T.; Espín, J.C. Resveratrol and Clinical Trials: The Crossroad from In Vitro Studies to Human Evidence. *Curr. Pharm. Des.* **2013**, *19*, 6064–6093. [[CrossRef](#)] [[PubMed](#)]
59. Sergides, C.; Chirilă, M.; Silvestro, L.; Pitta, D.; Pittas, A. Bioavailability and safety study of resveratrol 500 mg tablets in healthy male and female volunteers. *Exp. Ther. Med.* **2016**, *11*, 164–170. [[CrossRef](#)] [[PubMed](#)]
60. Montellier, A.; Voisin, A.; Furrer, P.; Allémann, E.; Cuendet, M. Intranasal administration of resveratrol successfully prevents lung cancer in A/J mice. *Sci. Rep.* **2018**, *8*, 14257. [[CrossRef](#)]

10. Extension de la recherche de traitements antiviraux ciblant les coronavirus à de nouvelles molécules.

10.1. Utilisation du modèle de screening HCoV-229E pour l'évaluation de traitements anti-SARS-CoV-2.

L'utilisation du HCoV-229E pour tester l'activité antivirale des traitements semble permettre d'éliminer les traitements ne permettant pas une réduction de la réplication des coronavirus. La réalisation d'un test préliminaire permettant d'évaluer la toxicité d'un traitement sur des cellules MRC5 ainsi que la mesure de son effet antiviral sur HCoV-229E constitue un pré-screening pouvant permettre d'augmenter le nombre de molécules testées et d'éliminer en amont celles ne présentant pas d'intérêt contre le SARS-CoV-2. Un intérêt supplémentaire de cette approche élargie est d'obtenir des traitements dont le spectre d'action comprend les coronavirus peu pathogènes, qui restent une menace pouvant entraîner des complications sévères chez les patients immunodéprimés [326]. Cela permet également d'obtenir des traitements dont l'efficacité serait moins affectée par les mutations des virus, et qui pourrait donc être utiles contre les différents variants.

10.2. Sélection et évaluation de l'effet cytotoxique de petites molécules.

Des composés phénoliques, des dérivés d'acide férulique et des dérivés d'acide caféique ont été sélectionnés pour leur homologie de structure avec des inhibiteurs de la protéase virale nsp5 du SARS-CoV-2. La toxicité des 10 molécules sélectionnées a été évaluée sur des cellules MRC5 traitées par doses croissantes. La mesure de cytotoxicité par test MTT a montré une forte toxicité de cinq molécules, à savoir MBA112, MBA33, MBA27-1, OS4-1 et MBA108-1. Les deux molécules MBA152 et 5c ont montré une toxicité modérée. Le niveau de toxicité observé pour ces molécules n'a pas permis l'évaluation de leur effet sur la réplication virale. Seules les molécules MBA140, LIJ2P40, et MBA28 ont montré une faible toxicité, permettant leur évaluation comme inhibiteurs des coronavirus.

10.3. Evaluation de l'effet anti-HCoV-229E.

La mesure de l'effet antiviral des trois molécules sélectionnées a été réalisée sur des MRC5 infectées par HCoV-229E. Les trois molécules ont montré une réduction de la réplication virale d'environ 60% pour les trois. MBA140 et MBA28 présentent les concentrations effectives les plus faibles. L'efficacité de ces trois molécules pour réduire la réplication du HCoV-229E associée à leur faible cytotoxicité a permis de valider leur évaluation contre le SARS-CoV-2.

10.4. Evaluation de l'effet sur le SARS-CoV-2

Les trois molécules ont été testées sur les cellules Vero E6 infectées avec le SARS-CoV-2. Parmi les trois molécules testées, MBA140 a montré un effet très limité sur la réplication du SARS-CoV-2. Les deux autres molécules ont montré un effet inhibiteur important sur la réplication du SARS-CoV-2. Cette inhibition atteint 95% avec MBA28 et 99% avec LIJ2P40. Ces résultats semblent indiquer que les composés MBA28 et LIJ2P40 pourraient être des candidats prometteurs dans le traitement de l'infection par SARS-CoV-2.

10.5. Discussion

Nous avons montré un effet antiviral de deux dérivés d'acide férulique sur le HCoV-229E et le SARS-CoV-2, nommément MBA28 et LIJ2P40. Le MBA28 s'est montré le plus efficace pour l'inhibition de la réplication du HCoV-229E. Le LIJ2P40 et le MBA140 ont montré un effet antiviral anti-HCoV-229E également, bien que plus limité. Les dérivés d'acide caféique et férulique ont montré précédemment une activité antivirale sur le VIH et le VHB [327]. Les récentes analyses de l'interaction avec nsp5, nsp15 et la sous-unité S2 de la protéine de spicule du SARS-CoV-2 ont montré que cette famille de composés possède une très forte affinité, et sont donc des bons candidats pour l'inhibition du SARS-CoV-2 [328].

Les FADs et CADs sont également des polyphénols. Cette famille de composés a été montrée comme ayant un effet antiviral par leur interaction avec nsp3 et nsp5, qui sont les protéases virales. Les polyphénols ont également un effet anti-inflammatoire, qui pourrait renforcer leur effet dans le cadre du traitement de la Covid-19 [329]. Les FADs et CADs présentent également des similitudes de structures avec de petites molécules précédemment rapportées comme pouvant cibler nsp5 [330]. La disruption de nsp5 entraîne une forte inhibition de la réplication [331].

Ces résultats préliminaires encourageants justifient de tester le MBA28 et le LIJ2P40, seuls ou combinés, sur des modèles plus proches des conditions cliniques, notamment des cultures d'épithélium nasal et des modèles animaux.

Cette étude a fait l'objet d'un article soumis au journal Scientific Reports en 2021, sous le titre *Ferulic acid derivatives block coronaviruses HCoV-229E and SARS-CoV-2 replication in vitro*.

10.6. Ferulic acid derivatives block coronaviruses HCoV-229E and SARS-CoV-2 replication in vitro.

Sébastien Pasquereau¹, Mathilde Galais²⁺, Maxime Bellefroid²⁺, Irene Pachón Angona³, Stéphanie Morot-Bizot⁴, Lhassane Ismaili³, Carine Van Lint² and Georges Herbein^{1,5*}

¹ Université de Franche-Comté, Université Bourgogne Franche-Comté (UBFC), Pathogens & Inflammation/EPILAB Laboratory, EA 4266, Besançon, France.

² Service of Molecular Virology, Department of Molecular Biology (DBM), Université Libre de Bruxelles (ULB), Gosselies, 6041, Belgium

³ Neurosciences Intégratives et Cliniques EA 481, Pôle de Chimie Organique et Thérapeutique, Univ. Bourgogne Franche-Comté, UFR Santé, Besançon, France;

⁴ Apex Biosolutions, Besançon, France

⁵ Department of Virology, CHU Besançon, Besançon, France.

+ contributed equally

* Correspondence: georges.herbein@univ-fcomte.fr

10.6.1. Abstract

A novel coronavirus, SARS-CoV-2, emerged in China at the end of 2019 causing a large global outbreak. As treatments are of the utmost importance, drugs with broad anti-coronavirus activity embody a rich and rapid drug discovery landscape, where candidate drug compounds could be identified and optimized. To this end, we tested ten small-molecules with chemical structures close to ferulic acid derivatives (FADs) (n=8), caffeic acid derivatives (CAFDs) (n=1) and phenolic compound (n=1) for their ability to reduce HCoV-229E replication, another member of the coronavirus family. Among these ten drugs tested five of them, namely MBA112, MBA33, MBA27-1, OS4-1 and MBA108-1 were highly cytotoxic and did not warrant further testing. In contrast, we observed a moderate cytotoxicity for two of them, MBA152 and 5c. Three drugs, namely MBA140, LIJ2P40, and MBA28 show low cytotoxicity with a reduction of the viral titer by 63% with MBA140 (EC₅₀=1.16μM), by 58% with LIJ2P40 (EC₅₀=12.3μM), and by 60% with MBA28 (EC₅₀=0.39μM). Similarly, among the three drugs with an anti-HCoV-229E activity and low cytotoxicity, two of them, FADs, LIJ2P40 and MBA28 showed a reduction of the viral titer on SARS-CoV-2 up to 99% with reduced cytotoxicity which opens the door to further evaluation to fight Covid-19.

Keywords: SARS-CoV-2; HCoV-229E; Ferulic acid derivatives; Caffeic acid derivatives; Coronavirus; Viral inhibition

10.6.2. Introduction

Human coronaviruses (HCoV) are positive-stranded RNA pathogens that are known to be rapidly evolving viruses. The coronaviridae family encompasses six different strains infecting humans divided into two genera: alphacoronavirus (alphaCoV) and betacoronavirus (betaCoV) [332]. The low pathogenic HCoV-229E and HCoV-NL63 strains are part of the alphaCoV. The betaCoV genera ramifies into the A lineage strains, including the more pathogenic HCoV-OC43 and HCoV-HKU1, and into the B and C lineage, respectively including the highly pathogenic severe acute respiratory syndrome coronavirus (SARS-CoV) and SARS-CoV-2, and the Middle East respiratory syndrome coronavirus (MERS-CoV) [333,334]. The lower and moderately pathogenic strains are endemic in the human populations, where they account for 15–30 % of mild, self-limiting respiratory infections. These strains, namely HCoV-229E, HCoV-NL63, HCoV-OC43, and HCoV-HKU1, still present a greater

incidence of lower respiratory tract infection in immunocompromised individuals [335]. The highly pathogenic strains include both the 2002 SARS-CoV and the 2012 MERS-CoV, which are linked to acute respiratory distress syndrome (ARDS). This syndrome potentially engenders a chronic reduction in lung function, arrhythmia, or death [336], with a mortality rate of 9.6% for SARS-CoV and 35.5% for MERS-CoV. SARS-CoV-2, the etiological agent behind Covid-19 disease, emerged in December 2019 in the city of Wuhan in China. After its pandemic outbreak, it became a major public health concern, inducing worldwide emergency measures [14]. While the average symptoms of infection are mild compared to SARS-CoV and MERS-CoV, SARS-CoV-2 can result in severe symptoms including hyperinflammation, cytokine storm and multiple organ complications. It can ultimately lead to death for some individuals [337].

Aside from fastly developed vaccines [15], no effective curative therapy is available against SARS-CoV-2. The development of new curative drugs is desperately needed [16]. It is well-known that it might take years for newly developed drugs to be clinically approved and reach the market [338], despite the tremendous efforts put into their development. Repurposing existing drugs could be more effective than de novo drug discovery, with a reduced cost and time. A rapid evaluation of *in vivo* and *in vitro* efficacy is possible for existing drugs [339]. Additionally, drugs that were shown to inhibit the replication of other coronaviruses, including HCoV-229E, could potentially inhibit the replication of SARS-CoV-2 and other betacoronaviruses[340].

Polyphenols have recently become an important group of compounds for the discovery of new drugs against human diseases[329], including against Covid-19, as some recent studies already reported[328]. Recently we reported that resveratrol, a well-known nutraceutical with a multitude of biological properties and therapeutic applications belonging to polyphenols' stilbenoids group [341], inhibits the replication of both HCoV-229E and SARS-CoV-2 [342]. Resveratrol is known for its anti-inflammatory activity [324], and for its anti-aging and antioxidant properties [343]. Resveratrol showed a potential as an anti-viral agent against some RNA and DNA viruses. Possible target RNA viruses include influenza virus [344], Zika virus [345], rhinovirus [346], rotavirus [347] and MERS-CoV [348], and DNA viruses such as poxvirus [349] and polyomavirus [350].

Therefore we decided to assess the anti-coronavirus activity of other phenolic compounds, namely ferulic acid derivatives (FADs), one caffeic acid derivative (CAFDs) and a carboxamid derivative. Phenolic compounds display antiviral activity against herpes simplex virus-1 and rabies virus [351]. Ferulic acid can be extracted from various fruits and cereals and is, with its derivatives, a phenolic compound. Ferulic acid derivatives (FADs) have been shown to inhibit H1N1 influenza virus replication *in vitro* [352]. Caffeic acids are found in coffee, fruits, and vegetables, and constitute one of the abundant plant-based phenols, which structures include 2 phenolic hydroxyl moieties. Caffeic acid and its derivatives previously showed an antiviral activity against herpes simplex virus [353], SFTS (severe fever with thrombocytopenia syndrome) virus [354], and influenza virus [355]. In addition, like resveratrol, phenolic compounds including FADS and CAFDs exhibit potent antioxidant and anti-inflammatory properties [356].

In the light of what was mentioned previously, we screened ten molecules with chemical structures close to already reported molecules with anti-SARS-CoV-2 protease activities [330] and belonging to the ferulic acid derivatives (FADs), caffeic acid derivatives (CAFDs), and phenolic acid compounds.

For any of these drugs that would show an inhibitory effect on HCoV-229E replication, *in vitro* testing for the inhibition of SARS-CoV-2 would be performed.

10.6.3. Materials and Methods

Viruses and reagents preparation

HCoV-229E was isolated from a man with mild upper respiratory illness (Human Coronavirus 229E ATCC VR-740) by using nasal and throat swabs. HCoV-229E stock virus (5 log PFU/mL) was propagated using MRC5 cells (RD Biotech, Besançon, France). MRC5 cells were cultured in DMEM media supplemented with 10% FCS. SARS-CoV-2 stock virus (7 log PFU/mL) was propagated in Vero E6 cells (ATCC) in DMEM media supplemented with 10% FCS. Viral titers were determined by plaque assays on Vero E6 cells and stored at -80°C until use. Compounds were sourced from the University of Franche-Comté (Besançon, France)[357–360].

Drug preparation

The ten small molecules tested were synthesized at the University of Franche-Comté. As described previously [357–360], the molecules were synthesized using the Ugi-4MCR method, starting from ferulic acid or caffeic acid. The resulting compounds were purified using flash column chromatography. After purification, drugs were stored in solid form at -20°C before preparation. Drugs were diluted in DMSO at a concentration of 10 mM, based on their molecular weight. The 10 mM stock was further diluted in cell culture media at final assay concentration at the time of use. DMSO concentration in the final assay was below 0.2%.

Viral replication inhibition assay

MRC5 cells were infected with HCoV-229E at MOI=1 and treated at time of infection with compounds diluted in culture during a 2-hour incubation period. After removing the inoculum the cells were overlaid with culture media containing diluted compounds. Supernatants were collected after a 48-hour incubation at 37 °C, to quantify viral loads by plaque forming unit (PFU) assay as previously described [342,361]. Briefly, supernatants were used to infect MRC5 cells for 2 hours. Cells were then overlaid with culture media containing agarose (1%). After a 48-hour incubation, plaque forming units were counted under a light microscope using MTT staining of the cells. The 50% effective concentrations (EC50), which are the compounds concentrations that inhibit viral replication by 50%, were determined using a four-parameter logistic regression (IC50 Toolkit IC50.org) to fit the dose-response curves.

Vero E6 cells were treated with compounds at time of infection by SARS-CoV-2 at MOI=0.01 in DMEM supplemented with 2% FCS. The viral inoculum was removed after a 2-hour incubation and the cells were overlaid with fresh DMEM media supplemented with 10% FCS containing the compounds. After a 48-hour incubation at 37 °C, supernatants were collected and frozen at -80°C prior to quantification and titration by endpoint dilution assay. Classic tissue culture infectious dose 50% (TCID50/mL) in Vero E6 cells were performed on viral stocks and on collected samples using the Reed & Muench statistical method.

Cytotoxicity assays

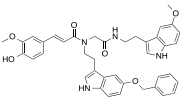
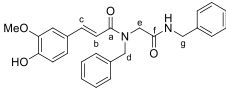
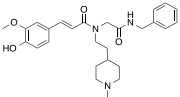
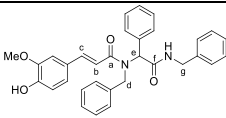
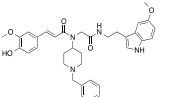
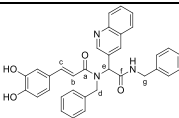
MRC5 cells were treated with compounds for 48 hours and MTT assay was performed as reported previously [342]. Cells were incubated with MTT reagent (1.2mM, Life Technologies, Eugene, OR, USA) for 2 hours. Acidic isopropanol was used to dissolve the formazan crystals and cell viability was measured by a spectrophotometer (Bio-Rad, Hercules, CA, USA). WST-1 assay was performed on Vero E6 cells treated for 48 hours with compounds, following the manufacturer's instructions (Takara Bio, Mountain View, CA, USA). The cytotoxic concentration 50 (CC50), which is the compounds concentrations that reduce cell viability by 50%, was determined for each compound and cell type by using a four-parameter logistic regression (IC50 Toolkit IC50.org).

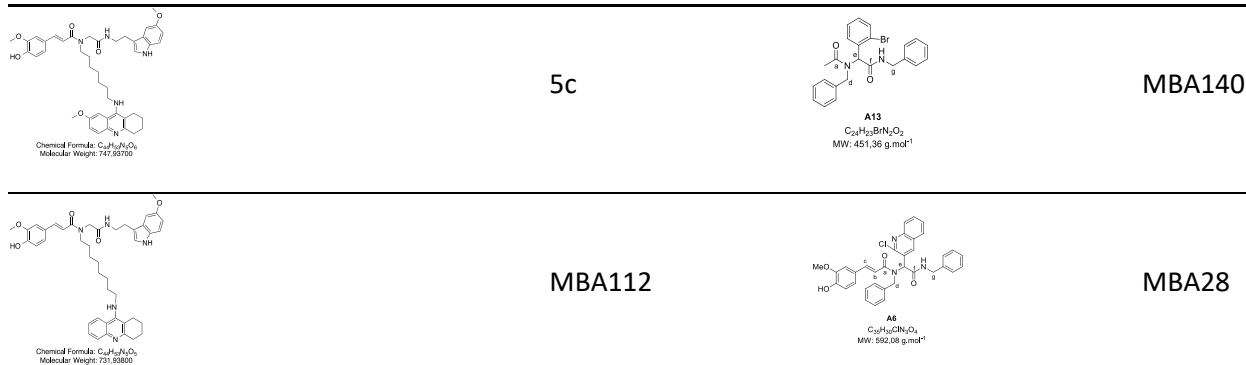
The Selectivity Index (SI) is indicative of the window between cytotoxicity and antiviral activity and was calculated as previously reported [342] by dividing the CC50 by the EC50 of each compound and virus tested. A high selectivity index reflects an important range of concentrations that show an effective antiviral effect with low toxicity.

10.6.4. Results

The ten small molecules tested were synthesized at the University of Franche-Comté (Table 1) based on their structure similarities with already reported inhibitors of nsp5 protease of SARS-CoV-2 [330]. Interestingly these ten small-molecule compounds tested have chemical structures close to ferulic acid derivatives (FADs), caffeic acid derivatives (CAFDs) and phenolic compound (Table 1) [357–360]. Among the ten compounds, eight were ferulic acid derivatives, namely LIJ2P40, OS4-1, MBA108-1, MBA33, MBA152, 5c, MBA112, and MBA28. MBA27-1 is a caffeic acid derivative. MBA140 is a phenolic derivative.

Table 1. The ten small-molecules tested belong to the FADs, the CAFDs, and the phenolic compounds.

Structure	Name	Structure	Name
 Chemical Formula: C ₂₈ H ₂₈ N ₂ O ₄ Molecular Weight: 472.76200	LIJ2P40	 A1 C ₂₈ H ₂₈ N ₂ O ₄ MW: 430.50 g.mol ⁻¹	OS4-1
 Chemical Formula: C ₂₇ H ₂₈ N ₂ O ₄ Molecular Weight: 541.69200	MBA 108-1	 A2 C ₂₈ H ₂₈ N ₂ O ₄ MW: 506.59 g.mol ⁻¹	MBA 33
 Chemical Formula: C ₂₇ H ₂₄ N ₂ O ₅ Molecular Weight: 506.72800	MBA 152	 A8 C ₂₈ H ₂₈ N ₂ O ₄ MW: 543.61 g.mol ⁻¹	MBA27-1



Among these ten small molecules tested, five, MBA112, MBA33, MBA27-1, OS4-1 and MBA108-1, show high cytotoxicity on MRC5 cells with a CC50 of 27.6 μ M, 0.51 μ M, 12.0 μ M, 0.17 μ M and 38.1 μ M respectively (Figure 1A, Table 2). In addition, two molecules MBA152 and 5c display moderate cytotoxicity with a CC50 of 97.1 μ M and 76.0 μ M respectively (Figure 1B, Table 2). By contrast, three molecules MBA140, LIJ2P40, and MBA28 show low cytotoxicity on MRC5 cells with a CC50 of 214.3 μ M, 125.9 μ M, and 1016.1 μ M respectively (Figure 1C, Table 3), and were further tested.

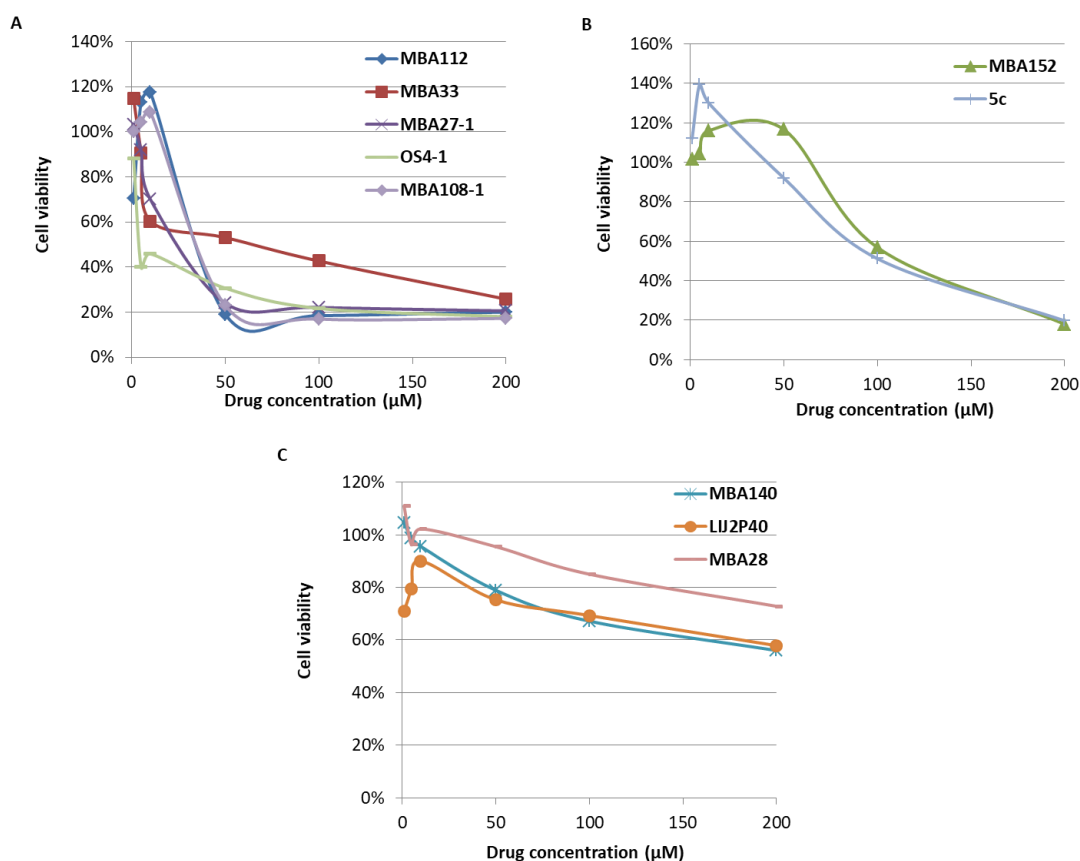


Figure 1. Viability of MRC5 cells under treatment with the ten drugs using MTT toxicity assay. (A) High cytotoxicity was observed for MBA112, MBA33, MBA27-1, OS4-1 and MBA108-1, (B) intermediate cytotoxicity for MBA152 and 5c and (C) low cytotoxicity for MBA140, LIJ2P40, and MBA28. MTT assays were performed after 48 hours. Untreated cells were used for normalization of MTT assay.

Table 2. CC50 of the seven drugs not selected for HCoV-229E replication inhibition in MRC5 cells due to high toxicity.

Drug tested	CC50 (μM)
MBA112	35.5
MBA33	39.4
MBA27-1	22.5
OS4-1	8.0
MBA108-1	34.4
MBA 152	108.3
5c	89.0

Table 3. EC50, CC50 and SI for the three drugs tested for HCoV-229E replication in MRC5 cells.

Drug tested	CC50 (μM)	EC50 (μM)	SI
MBA 140	>200	1.64	>120
LIJ2P40	>200	12.32	>16
MBA 28	>200	0.39	>500

Less cytopathic drugs (MBA28, MBA140 and LIJ2P40) were tested for their ability to inhibit viral replication of HCoV-229E in MRC5 cells. We measured an EC50 at 1.16 μM for MBA140, with a 63% reduction in replication even at higher concentrations (Figure 2, Table 3). For MBA28, we observed a similar inhibition (60%), with an EC50 below 1 μM (Figure 2, Table 3). The drug LIJ2P40 showed a higher EC50 at 12.32 μM , with a similar inhibition (58%) observed at higher concentrations (Figure 2, Table 3). Overall, the decrease in viral replication is up to 60% for these three molecules tested.

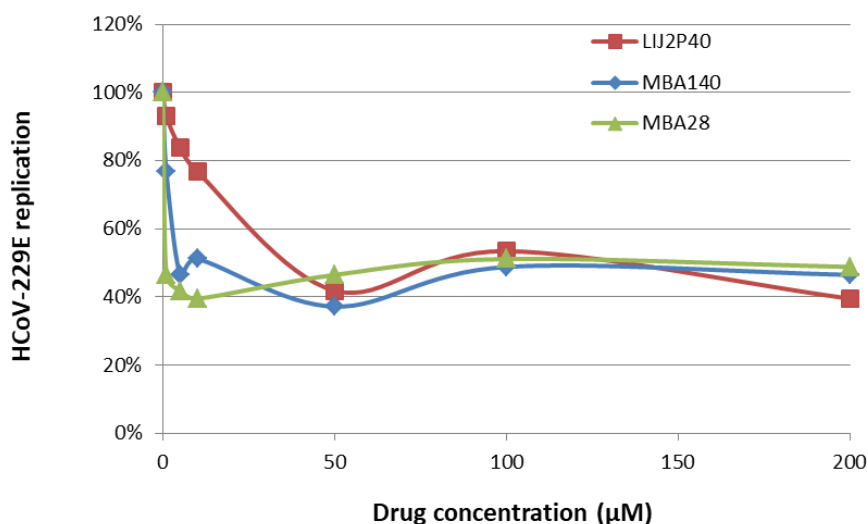


Figure 2. Inhibition of replication of high HCoV-229E viral load measured by PFU assay, for LIJ2P40, MBA140 and MBA28. Cells were treated by compounds at the time of infection with HCoV-229E (MOI=1). PFU assay was performed after 48 hours. Untreated cells infected with HCoV-229E were used for normalization of PFU assay.

We calculated the selectivity index for the inhibition of HCoV-229E in MRC5 cells for the three drugs tested. Both MBA140 and MBA28 present a high SI, at 185 and 2605, respectively (Table 3). The SI for LIJ2P40 is much lower (10.2), due to the higher EC₅₀ measured (Table 3).

Based on the results obtained for HCoV-229E, we performed testing of these three selected compounds on inhibition of replication of SARS-CoV-2 infection in VeroE6 cells. The cytotoxicity of LIJ2P40, MBA140 and MBA28 on Vero E6 cells was limited and occurred at concentrations higher than 100µM (Figure 3). Both MBA140 and LIJ2P40 showed a low toxicity at concentrations below 100µM, while MBA28 displayed the highest cytotoxic effect of the three drugs tested. Cell viability decreases below 70% even at low concentrations of MBA28. Only high concentrations (200µM) of MBA140 and LIJ2P40 induced a decrease of viability similar to MBA28 (Figure 3).

Among the three drugs tested, MBA140 showed a limited anti-SARS-CoV-2 activity (Figure 4). By contrast, the percentage of inhibition of SARS-CoV-2 ranges from 0 to 95% when increasing the concentration of MBA28 from 0 to 200µM with an EC₉₀ and EC₅₀ of 116 µM and 77.3 µM, respectively (Table 4). Similarly, the percentage of inhibition of SARS-CoV-2 ranges from 0 to 99% when increasing the concentration of LIJ2P40 from 0 to 200µM with an EC₅₀ of 9.98 µM (Figure 4, Table 4). The inhibition of SARS-CoV-2 replication observed in cells treated with MBA28 and to a lesser extent with LIJ2P40 did not follow the reduction in cell viability and was a genuine anti-SARS-CoV-2 effect of these two drugs *in vitro*.

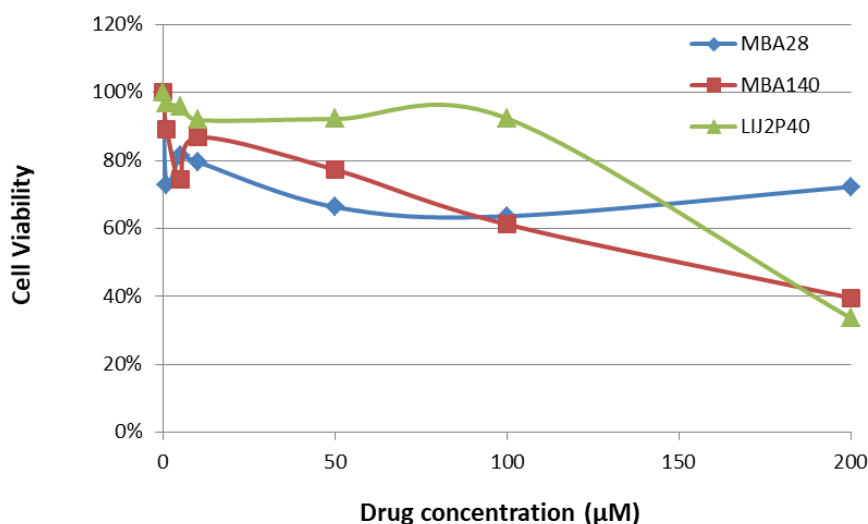


Figure 3. Viability of Vero E6 cells treated with MBA28, MBA140 or LIJ2P40 drugs measured by WST1 assay. WST1 assays were performed after 48 hours. Untreated cells were used for normalization of the WST1 assay.

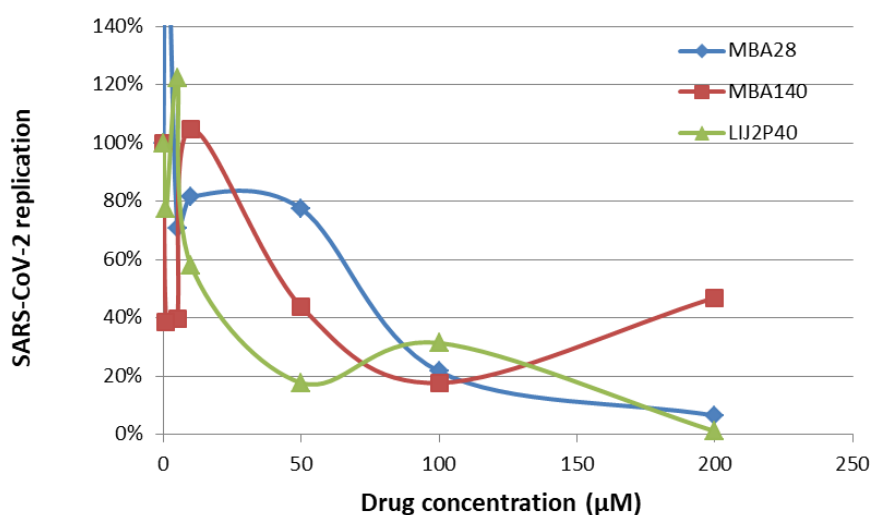


Figure 4. Inhibition of SARS-CoV-2 replication measured by endpoint dilution assay (TCID₅₀/mL), for MBA28, MBA140 or LIJ2P40. Cells were treated by compounds at the time of infection with SARS-CoV-2 (MOI=0.01). Endpoint dilution assay was performed 48 hours post-infection. Untreated cells infected with SARS-CoV2 were used for normalization of the assay.

Table 4. EC₅₀, CC₅₀ and SI for the 3 drugs tested for SARS-CoV-2 replication in VeroE6 cells.

Drug tested	CC ₅₀ (µM)	EC ₅₀ (µM)	SI
MBA 140	176.1	48.3	3.64
LIJ2P40	179.9	9.9	18.17
MBA 28	>200	70.4	>3

10.6.5. Discussion

We showed the antiviral effect of two ferulic acid derivatives (MBA28, LIJ2P40) on HCoV-229E and SARS-CoV-2 replication in vitro. To inhibit HCoV-229E replication, among ten molecules tested, the FAD MBA28 proved the most effective compound with an EC₅₀ below 1 μ M, a CC₅₀ of 1016 μ M, and a SI above 1000. While their SI are lower (above 120 and 16, respectively), the compound MBA140 and to a lesser extent the FAD LIJ2P40 also showed an inhibitory effect on HCoV-229E replication, with low toxicity (CC₅₀ above 200 μ M) and efficiency starting at low doses (EC₅₀=1.64 and 12.32 μ M, respectively). The overall efficiency of these three drugs against HCoV-229E is not enough (60% inhibition at best) to warrant their use as is, but they could be good candidates as base molecules to be modified for enhanced antiviral effect. We considered them having enough potential to be further tested on SARS-CoV-2.

We observed a limited cytotoxicity of MBA28 and LIJ2P40 on Vero E6 cells, with a CC₅₀ of 244.5 and 187 μ M, respectively. The testing of MBA140 however showed moderate cytotoxicity, with a CC₅₀ of 118.5 μ M. A small increase in viability at the highest concentration was globally observed for MBA28, compared to 100 μ M. This could be the results of the antioxidant properties of the molecule. Since the viability was measured using WST-1 assay, an antioxidant could impact the mitochondrial dehydrogenase activity, which is measured by such assay. This effect might be more pronounced at higher concentration.

The limited cytotoxicity measured in Vero E6 cells warranted the further testing of these three drugs for SARS-CoV-2 inhibition. In addition, we observed a significant decrease in SARS-CoV-2 replication under non-cytotoxic doses of MBA28 up to 200 μ M *in vitro* (EC₅₀= 77.3 μ M). Similarly we also found a significant inhibition of SARS-CoV-2 replication with LIJ2P40, although the latter was cytotoxic at the highest concentration tested of 200 μ M (EC₅₀= 9.98 μ M). We observed only very limited anti-SARS-CoV-2 activity of MBA140 (EC=48.3 μ M). Overall, two of the three tested drugs, namely MBA28 and to a lesser extent LIJ2P40, showed a promising inhibition of SARS-CoV-2 replication, with limited toxicity. To further confirm the anti-SARS-CoV-2 effect of these two drugs using an in vitro model closer to the in vivo pathophysiology, we will assess their effect on SARS-CoV-2 infections using organotypic nasal epithelial cultures which might better reflect the pathophysiology of SARS-CoV-2 infection than the renal epithelial Vero E6 cells.

Polyphenols have been previously showed to be efficient against viral infections, by acting on the modulation of immune system[362]. They were also shown to directly inhibit viral replication [363] or reduce viral entry[364]. CAFDs are phenolic compounds which possess prominent antiviral activity especially against hepatitis B virus (HBV) and HIV [327]. Recent docking analysis on CAFDs showed that they possessed high binding energies against the main protease Nsp5 (M^{Pro}), Nsp15 and the S2 spike subunit of SARS-CoV-2. They could be potent modulators of Covid-19, since their binding energy is even higher than nelfinavir [328].

FADs, as part of phenols, share properties with other polyphenols such as resveratrol. The observed antiviral effects could be linked to their interactions with both viral proteases (Nsp3 and Nsp5) but also with the spike protein. The phenol groups could allow the disruption of the enzymatic function of Nsp3 and Nsp5. The interaction with spike proteins could also prevent capsid formation, thus reducing the viral replication.

A similarity of FADs and CAFs chemical structure with previously reported small molecules [330] could explain their potential as antiviral drugs. These small molecules were predicted to target M^{Pro} of SARS- and MERS-CoV, by the presence of hydrophobic regions. The disruption of M^{Pro} protease activity results in an important inhibition of viral replication [331].

In conclusion, further testing is needed to confirm whether these two FADs, namely MBA28 and LIJ2P40, alone or in combination could be of use in a clinical setting. Finally, it is important to note that our results on SARS-CoV-2 inhibition are *in vitro* data and do not indicate that any of the drugs tested can be used to treat Covid-19 until double-blinded clinical assays have been successfully performed.

10.6.6. Conclusions

We confirm the anti-HCoV-229E activity of two FADs compounds, MBA28 and LIJ2P40, and the phenolic compound MBA140 *in vitro*. In addition, we show here the antiviral effect of two FADs, MBA28, and to a lesser extent LIJ2P40, both on HCoV-229E and SARS-CoV-2 replication *in vitro*. MBA28 proved to be the most effective compound to fight HCoV-229E infection with an EC₅₀ of 0.39 μM, a CC₅₀ of 1016.1 μM, and a SI of 2605. In addition, our testing for SARS-CoV-2 inhibition shows a strong anti-viral effect of MBA28 with limited cytotoxicity *in vitro* which will be assessed in future studies in organotypic nasal epithelial cultures before looking into their efficiency against Covid-19 in a clinical setting, either alone or in combination.

IV. Discussion, Conclusion et Perspectives

11. Discussion générale

11.1. Traitement du VIH

L'objectif de nos travaux était d'évaluer l'impact des différents types de traitements chez les patients infectés par le VIH. Cette évaluation nous a permis de quantifier les réservoirs de latence dans les deux réservoirs cellulaires principaux, les lymphocytes T CD4⁺ et les monocytes/macrophages. Nous avons également mesuré l'état d'activation des cellules infectées, par mesure de l'activation d'Akt, en fonction des traitements utilisés. Nous avons confirmé l'importance de Nef dans l'activation cellulaire et la réactivation virale.

11.1.1. Cibler les réservoirs de latence virale

Les réservoirs de latence représentent le principal obstacle à la guérison complète du VIH [202,365–367]. Bien que l'impact des traitements antirétroviraux sur la virémie résiduelle soit largement décrit, leur impact sur les réservoirs cellulaires du VIH, notamment dans les monocytes est moins bien documenté. Le nombre de monocytes infectés de manière productive est considéré 10 fois inférieur au nombre de cellules T CD4⁺ infectées [161].

Les inhibiteurs de protéases ont été montrés comme bloquant la propagation intercellulaire du VIH [368]. Plusieurs études ont montré qu'un traitement précoce lors de la primo-infection par le VIH permettait une diminution de la taille du réservoir viral [369].

Nous avons observé que les patients sous traitements cART incluant des PI présentent une diminution de la taille du réservoir viral par rapport aux patients sous traitement cART comprenant uniquement des NNRTI. Cette diminution est observée dans les lymphocytes T CD4⁺ quiescents et dans les monocytes/macrophages. Nous avons montré que les traitements cART étaient efficaces pour réduire la taille du réservoir du VIH dans les monocytes et les cellules T CD4⁺ quiescents par rapport aux patients naïfs de traitement.

Nous avons observé que les inhibiteurs de protéases pouvaient bloquer l'activation d'Akt et inhiber la réactivation virale à partir de cellules monocytoïdes U1 infectées de façon chronique. Nous avons observé que les lymphocytes T CD4⁺ quiescents de patients sous cART avec inhibiteurs de protéase montrent une limitation de la réactivation virale par rapport aux patients traités par cART sans inhibiteurs de protéase.

Globalement, les inhibiteurs de protéase semblent limiter la taille du réservoir de latence virale et sa réactivation. La limitation de la taille des réservoirs de latence permet d'envisager l'utilisation des IP de manière plus systématique. Cependant, les PI entraînent des effets secondaires adverses plus fréquents que d'autres classes de traitement, notamment les inhibiteurs de l'intégrase, et entraînent une apparition plus fréquente de résistances conduisant à des échecs thérapeutiques [316].

Pour les études réalisées en utilisant une quantification par mesure de la p24 post-réactivation et par une mesure directe de l'ADN, un biais dans la quantification peut entraîner une sous-estimation de la taille du réservoir en raison de la limitation de la réactivation observée lors du traitement par les PI, qui bloquent notamment Akt. Ainsi, une quantité similaire de provirus intégré pourrait donner

deux résultats différents en réactivation selon le traitement. Pour les études réalisées en utilisant une PCR en nids (Alu-Gag), la quantification mesure le nombre de copies du génome viral intégré au génome cellulaire. Cette méthode de mesure, qui est une mesure directe de la quantité de provirus, n'est pas impactée par les effets des traitements sur la réactivation virale. La mesure plus faible de la taille du réservoir viral sous PI par rapport aux NNRTI semble donc bien due à une réduction du nombre de cellules contenant un ADN proviral intégré.

L'utilisation d'inhibiteurs de l'intégrase permet de réduire de manière l'établissement des réservoirs de latence virale [369]. L'ajout de cette classe de molécules aux traitements cART a remplacé l'utilisation des inhibiteurs de protéases, en raison notamment des effets adverses relativement importants (troubles gastro-intestinaux, réactions cutanées, perturbations métaboliques notamment des fonctions rénales et du foie) observés chez les patients sous PI[370]. Ce type de traitement semble primordial dans le cadre d'une stratégie « shock and kill », pour limiter l'établissement de nouveaux réservoirs suite à la réactivation virale. L'utilisation de PI ne semble pas appropriée pour cette stratégie en raison de la diminution de la réactivation induite par les PI.

L'inhibition de la réactivation virale par les PI pourrait participer à la stratégie de « block and lock » et ainsi réduire le réservoir du VIH, en particulier dans les cellules myéloïdes. Les PI peuvent être associés à des traitements épigénétiques, notamment des inhibiteurs de Tat, permettant le silence transcriptionnel du provirus, en réduisant également l'activation cellulaire globale.

Ces stratégies ciblent principalement les lymphocytes T CD4+ et les cellules myéloïdes, qui sont les principaux réservoirs cellulaires de la latence virale. Cependant, les autres réservoirs, notamment dans le SNC, sont peu explorés lors de la recherche de nouveaux traitements. De plus, la persistance de cellules infectées au sein des différents organes solides (cerveau, foie, rein, poumons, rate et colon) a été montrée, mais les difficultés liées à l'étude de ce type d'échantillons (biopsies post-mortem principalement) ne permettent pas de connaître l'impact des différentes thérapies sur ces réservoirs [371].

11.1.2. Rôle de Nef et potentiel thérapeutique

Nous avons montré que les inhibiteurs de protéase réduisent l'activation d'Akt par Nef, ainsi que la réactivation virale à partir de cellules infectées de manière latente. Nous avons montré que l'interaction directe entre Nef et Akt entraîne l'activation des voies NFkB et du CD28RE. Cette activation est liée aux radeaux lipidiques et entraîne l'augmentation de la production d'IL-2. Nous avons montré que l'augmentation de l'expression d'Akt et son activation par phosphorylation par l'action de Nef peuvent être bloquées par les inhibiteurs de protéase.

Il a précédemment été démontré que la protéine Nef du VIH-1 active plusieurs molécules de signalisation clés, notamment Akt, MAPK et ERK [372,373]. À la suite d'un traitement par Nef, une multiplication par deux de l'activation de la LTR médiée par le NF-kB dans les cellules U937 transfectées par LTR-Luc et une multiplication par deux des niveaux de p24 dans les surnageants des cellules U1 infectées par le VIH de façon chronique ont été décrites précédemment [374–376]. Dans les cellules U1 traitées au TNF, on observe à la fois une activation du NF-kB et une réactivation du VIH-1 à partir de la latence [208,376].

NFκB est le facteur de transcription nécessaire à l'initiation de la transcription virale. L'activation par Nef, par le biais des différentes voies de signalisations activées et en particulier par l'activation d'Akt, de la production de NFκB par la cellule est un point important de la réactivation virale. Le ciblage de Nef dans le cadre de stratégies thérapeutiques pourrait augmenter l'efficacité des traitements. Dans le cadre d'une stratégie de réactivation (« shock and kill »), l'utilisation de Nef en complément des LRA pourrait permettre une augmentation de l'efficacité de la réactivation. Nef semble également entraîner une activation cellulaire globale, y compris dans les macrophages, ce qui permettrait également d'améliorer l'efficacité de la réactivation. Dans le cadre d'une stratégie de blocage, l'utilisation d'antagonistes de Nef serait complémentaire du ciblage du promoteur, afin de garantir l'absence d'activation. Des antagonistes de Nef, notamment des ligands artificiels de Nef comprenant un anticorps anti-Nef, ont été testés *in vitro* et ont montré une inhibition complète des fonctions de Nef [377].

11.1.3. Rôle central d'Akt

Nous avons montré l'effet de l'infection par le VIH sur l'activation cellulaire, par le biais de l'activation d'Akt. Cette protéine cellulaire joue un rôle central dans le métabolisme cellulaire, notamment dans la survie [318]. L'augmentation de l'activation d'Akt dans les cellules infectées par le VIH est réduite chez les patients traités par IP par rapport aux patients sous inhibiteurs de la transcriptase inverse. Nous avons montré que Nef active Akt et que cette activation est bloquée par les inhibiteurs de protéase.

L'activation d'Akt est nécessaire à la survie des cellules infectées. La voie de signalisation PI3K/Akt régule notamment l'apoptose et la prolifération cellulaire. Le ciblage de cette voie de signalisation fait l'objet d'un intérêt particulier dans le cadre du cancer [378]. L'activation d'Akt inhibe l'apoptose et favorise la prolifération cellulaire, ce qui dans le cadre de l'infection par le VIH, favorise la survie des cellules infectées et l'établissement des réservoirs de latence virale. L'inhibition de l'apoptose est nécessaire à la survie de la cellule lors de la phase de réplication active du virus. L'activation d'Akt semble également jouer un rôle important dans la réactivation virale, notamment par l'activation de NFκB [185].

Le rôle central d'Akt dans le métabolisme cellulaire et son rôle important dans la pathogénicité de l'infection par le VIH en fait une cible thérapeutique importante. L'utilisation d'inhibiteur d'Akt et de PI3K a montré une réduction de la réactivation du provirus latent. Les inhibiteurs d'Akt ont également montré un effet pro-apoptotique dans le traitement de cellules cancéreuses [315,379,380]. L'inhibition de l'activation d'Akt réduirait l'établissement des nouveaux réservoirs de latence virale. Cet effet pourrait être utilisé dans une stratégie « block and lock », en synergie avec des traitements inhibant la réactivation, en particulier des inhibiteurs de Tat. Cela permettrait également de réduire la survie des cellules conservant une réplication virale active, par la levée de l'inhibition de l'apoptose.

L'utilisation d'inhibiteurs d'Akt en combinaison avec des LRA pourrait permettre d'augmenter la clairance des cellules infectées dans le cadre d'une stratégie « shock and kill ». Cet effet pourrait cependant être limité par l'effet anti-réactivation de l'inhibition d'Akt. Les différents réservoirs viraux ne sont pas impactés de la même manière, avec une résistance des macrophages à l'apoptose plus élevée que les lymphocytes par exemple. La pénétration des traitements LRA et des inhibiteurs

d'Akt dans les réservoirs tissulaires, et notamment au niveau du SNC est vraisemblablement variable, et la réactivation pourrait conduire à l'expansion du réservoir.

11.1.4. Développement de traitements à effets longue durée

Les traitements cART classiques comprennent généralement trois composés d'au moins deux classes différentes. Cela inclut des inhibiteurs nucléosidiques de la transcriptase inverse, associés à un inhibiteur non-nucléosidique de la transcriptase inverse, un inhibiteur de protéase ou un inhibiteur de l'intégrase. Ces traitements réduisent la virémie et permettent un contrôle de l'infection. Des effets secondaires parfois importants, notamment avec les inhibiteurs de protéase, sont constatés chez les patients, de même que l'apparition de résistance au traitement, pouvant conduire à un échec thérapeutique[316]. Ces traitements doivent également faire l'objet d'une observance stricte [381,382]. Ces limitations ont conduit au développement de nouveaux traitements ayant comme spécificité un effet de longue durée, réduisant ainsi le nombre de prises de traitement [383]. Ces traitements incluent des antiviraux appartenant aux différentes catégories et des anticorps monoclonaux.

Parmi les traitements actuellement en essais cliniques de phase II et III, la rilpivirine est un inhibiteur non-nucléosidique de la transcriptase inverse et le cabotegravir est un inhibiteur de transfert de brin de l'intégrase [383]. La rilpivirine a été approuvée en 2011 par la FDA dans le cadre d'un traitement cART classique [384]. Ces deux traitements font l'objet de plusieurs essais cliniques de phase II et III destinés à montrer leur efficacité comme traitement à effet de longue durée. Les premiers résultats semblent indiquer que les traitements par rilpivirine et cabotegravir, espacés de plusieurs semaines, maintiennent la suppression de la virémie plasmatique de la même manière que l'utilisation d'un traitement cART classique [383].

Récemment, l'ibalizumab a été approuvé par la FDA pour utilisation comme traitement contre le VIH. Il s'agit du premier anticorps monoclonal approuvé dans ce cadre. Cet anticorps bloque l'entrée virale, par liaison non-compétitive au récepteur CD4 [385]. Les essais cliniques de phase 3 réalisés avant la mise sur le marché ont montré une réduction de la virémie pour 75% des patients en échec thérapeutique [385]. Les effets adhéses sévères du traitement ont concerné 30% des patients [385,386]. Le Leronlimab est un anticorps monoclonal dirigé contre les récepteurs CCR5. Sa liaison au récepteur entraîne l'inhibition de la phase finale de liaison du VIH avec la cellule [386]. Les essais cliniques en cours semblent montrer une suppression de la virémie chez 90% des patients traités par monothérapie de Leronlimab 700mg par semaine [386]. Ces traitements ne semblent cependant pas permettre une réduction de la taille des réservoirs de latence virale [387].

11.1.5. Traitements préventifs

La mise sur le marché de la prophylaxie pré-exposition a marqué un tournant dans les stratégies préventives contre le VIH. La stratégie de PrEP nécessite, comme pour les traitements cART, une observance stricte avec prise quotidienne de traitement. La mise en place des stratégies de PrEP, associés à des stratégies de dépistage importantes, semble réduire le nombre de contaminations[388]. Plusieurs études ont montré l'efficacité des traitements PrEP pour la réduction de la transmission de l'infection [196,389,390]. Ces études ont également montré l'absence d'effets adhéses sévères. Les cellules myéloïdes étant une cible principale des souches de VIH-1

macrophages-tropiques après une exposition au VIH, les études futures devront évaluer l'effet de la prophylaxie pré-exposition non seulement sur les cellules T CD4+ mais aussi sur le sous-ensemble de population monocytes/macrophages, ainsi que sur la taille des réservoirs chez les patients infectés malgré la PrEP.

Une stratégie émergente est l'utilisation de vaccins à ARNm ciblant le VIH. Plusieurs candidats vaccins ont été développés ces dernières années, avec un essai clinique de phase I démarrant en septembre 2021 (NCT05001373). Cet essai clinique porte sur deux ARNm, devant permettre l'induction de réponse cellulaire des lymphocytes B pour l'obtention d'anticorps neutralisants.

11.1.6. Perspectives

Malgré les progrès réalisés dans les traitements cART et les traitements préventifs, il n'existe toujours pas de traitement curatif efficace, c'est-à-dire permettant une guérison définitive. Plusieurs stratégies possibles pour l'obtention d'une guérison fonctionnelle sont débattues et testées, correspondants aux stratégies « shock and kill » et « block and lock ». Ces deux stratégies se heurtent à des difficultés, notamment dans l'élimination des cellules infectées. L'obtention d'une guérison fonctionnelle est conditionnée au ciblage de la latence virale, au niveau des mécanismes de restriction épigénétique, de séquestration des facteurs de transcription, de l'interférence transcriptionnelle et de la restriction de Tat [391]. Les stratégies visant ces mécanismes utilisent principalement des LRA, pour lesquels des essais cliniques les combinant avec des traitements permettant une clairance virale sont en cours. Jusqu'ici, il semble que les résultats obtenus montrent bien l'effet de réactivation virale, mais les traitements ne semblent pas permettre la déplétion des cellules infectées [392,393]. Les LRAs testés incluent entre autres la prostratine qui, associée au vorinostat ou au TSA, induit la réactivation [208]. Le vorinostat, associé à la bryostatine-1, induit également la réactivation [394]. La bryostatine-1, associée au panobinostat ou à la romidepsin semble induire la réactivation [395]. Le 5-azadC, associé au panobinostat ou à la romidepsin induisent également la réactivation [394].

11.2. Traitement des coronavirus

L'objectif de nos travaux était d'identifier de potentiels traitements antiviraux efficaces contre les coronavirus, en particulier le SARS-CoV-2. La réutilisation de traitements existants nous a permis d'identifier le resvératrol comme inhibiteur de la réplication du HCoV-229E et du SARS-CoV-2. La recherche de nouvelles molécules, plus adaptées et potentiellement plus efficace, nous a conduit à tester des petites molécules dérivées des acides féruliques et caféiques. Cela a permis l'identification de deux molécules ayant un effet inhibiteur de la réplication du HCoV-229E et du SARS-CoV-2.

11.2.1. Réutilisation de traitements existants

L'urgence de la pandémie mondiale de Covid-19 a entraîné un effort international de recherche de traitements. Le processus de développement de nouveaux médicaments pouvant durer plusieurs années, la réutilisation de traitements existants s'est imposée comme une stratégie intéressante. Ce processus présente des avantages de temps et de coût, grâce à la possibilité d'une évaluation rapide de l'efficacité des traitements testés.

Dans ce contexte, nous avons testé sept traitements, choisis pour leur potentiel effet antiviral précédemment décrit. Nous avons évalué la toxicité cellulaire de ces traitements, ce qui nous a conduit à ne conserver que trois d'entre eux. Ces trois traitements ont été testés pour leur capacité à inhiber la réplication du HCoV-229E. Nous avons montré un effet antiviral *in vitro*, en particulier pour le lopinavir/ritonavir et surtout pour le resvératrol.

Il a été mis en évidence précédemment que les agents thérapeutiques efficaces contre certains membres de la famille des coronavirus, comme le HCoV-229E, pourraient être des candidats pour l'inhibition des bêtacoronavirus [340]. Nous avons testé ces trois molécules pour leur capacité à inhiber la réplication du SARS-CoV-2. Nous avons montré que le resvératrol entraîne une inhibition de la réplication du SARS-CoV-2. Cet effet d'inhibition semble correspondre à l'inhibition de la réplication observée précédemment, à des doses similaires [323].

11.2.2. Recherche de nouvelles molécules

Bien que la réutilisation de traitements existants soit la stratégie la plus intéressante, le développement de traitements spécifiquement conçus pour cibler le SARS-CoV-2 peut permettre d'obtenir une meilleure efficacité. Une sélection des molécules à tester doit être faite, sur la base des similarités avec des inhibiteurs connus. Nous avons ainsi sélectionné dix molécules pour leur homologie structurelle avec des inhibiteurs connus de la protéase majeure MPro des bêtacoronavirus (nsp5).

Nous avons évalué la toxicité de ces molécules, pour ne retenir que les trois molécules les moins cytotoxiques. Nous avons alors évalué leur potentiel comme inhibiteur de la réplication de HCoV-229E. Les trois molécules testées, MBA140, MBA28 et LIJ2P40, ont montré une inhibition de HCoV-229E. Ces trois molécules ont été testées pour l'inhibition de la réplication du SARS-CoV-2. Nous avons montré que MBA28 et LIJ2P40 inhibent la réplication de SARS-CoV-2 *in vitro*. Ces deux molécules présentent également une faible cytotoxicité, ce qui en fait de bons candidats pour le traitement de l'infection par SARS-CoV-2.

11.2.3. Limitations et voies d'amélioration

Amélioration du pré-screening.

Une amélioration possible de notre méthode de test des molécules est une augmentation de la robustesse de notre présélection, basée sur l'inhibition du HCoV-229E. L'utilisation systématique d'une évaluation de la toxicité des molécules dans les différents modèles cellulaires (MRC5, Vero, Vero E6), associée à une mesure de l'inhibition de plusieurs coronavirus humains, permettrait de mieux tester l'efficacité des molécules candidates.

Ce pré-screening pourrait également inclure une évaluation *in silico* des interactions des molécules candidates avec les protéines virales ciblées. Cela permettrait l'identification plus rapide des molécules d'intérêt, et de potentiellement cibler des analogues ou dérivés de ces molécules.

Limitations liées aux modèles cellulaires utilisés.

Les modèles cellulaires utilisés souffrent des limitations habituelles des modèles *in vitro*, qui ne reproduisent pas fidèlement l'ensemble des processus interagissant *in vivo*. En particulier, ces modèles ne prennent pas en compte les relations entre les tissus infectés (représentés par les cultures cellulaires) et les cellules immunitaires. Étant donnée la nature inflammatoire systémique de la Covid-19, l'utilisation d'un modèle expérimental plus complexe, incluant des cellules immunitaires, produirait probablement des résultats plus facilement transposables. De plus, l'utilisation de reconstitutions d'épithéliums bronchiques, incluant l'ensemble des couches cellulaires et capable de production de mucus, permet également une restitution plus fidèle des interactions entre virus, cellules et traitement.

11.2.4. Remise en contexte

Globalement, nos résultats s'inscrivent dans la lignée des autres études réalisées sur le reconditionnement de traitements existants. Plusieurs études ont été menées par différents consortiums afin de tester massivement de potentiels traitements contre le SARS-CoV-2. Ces études ont montré l'absence d'efficacité *in vivo* de plusieurs molécules que nous avons également testées. Les résultats que nous avons obtenus, notamment avec la chloroquine, le lopinavir/ritonavir, la rapamycine et l'acide valproïque, sont similaires à ceux obtenus par d'autres équipes [321].

Depuis l'émergence du SARS-CoV-2, de nombreux traitements ont été testés pour leur potentiel effet antiviral. Les traitements actuellement utilisés en clinique sont principalement des traitements symptomatiques. Des inhibiteurs d'IL-6, notamment le Tocilizumab et le Sarilumab sont utilisés pour réduire la réponse hyper-inflammatoire observée dans les formes graves de la Covid-19 [279–281]. L'héparine est utilisée principalement pour son effet anticoagulant pour lutter contre la coagulopathie résultant des formes graves de la Covid-19, malgré les effets secondaires importants de ce type de traitement [396]. Parmi les traitements antiviraux utilisés, le Remdesivir est le traitement le plus répandu, actuellement approuvé pour utilisation chez les patients hospitalisés dans plus de 50 pays [397]. Ce traitement fait également l'objet d'essais cliniques de phase III, afin de confirmer son action antiviral sur le SARS-CoV-2. Un essai clinique, incluant trois autres traitements, a montré une absence d'efficacité significative [289]. D'autres traitements font également l'objet d'essais cliniques pour leur activité SARS-CoV-2, principalement des analogues nucléosidiques.

11.2.5. Développement de vaccins

Parallèlement à la recherche de traitements, le développement de vaccins contre le SARS-CoV-2 a été une priorité dans la lutte contre la pandémie de Covid-19. Plusieurs vaccins ont été mis au point, incluant notamment des vaccins utilisant les ARNm [293]. Ce nouveau type de vaccin, déjà expérimenté dans le traitement des cancers, a fait l'objet d'une attention médiatique particulière, s'agissant du premier déploiement à grande échelle de cette technologie vaccinale.

Des vaccins utilisant un vecteur viral Adénovirus exprimant la protéine S du SARS-CoV-2 ont été mis au point par Janssen Vaccines, par l'Université d'Oxford associée à AstraZeneca, et par l'Institut de

recherche Gamaleïa d'épidémiologie et de microbiologie. Des vaccins à ARNm, codants pour la protéine S du SARS-CoV-2, ont été développés par Moderna et par BioNTech.

Les vaccins à ARN ont l'avantage d'être plus immunogènes que les vaccins protéiques, en réduisant le risque de réaction adverse [398]. Cependant, des contraintes logistiques importantes, particulièrement les conditions de stockage à -80°C pour conserver la stabilité du vaccin, rend leur déploiement plus complexe, voire très difficile dans les pays en voie de développement.

11.2.6. Nouveaux traitements

Dans le même temps, la progression de la pandémie a entraîné l'apparition de nombreux variants du SARS-CoV-2. Ces variants, présentant principalement des mutations au niveau de la protéine S, possèdent une infectivité variable, incluant des variants hautement virulents [294]. Le déploiement de stratégies vaccinales à grande échelle devrait réduire l'urgence sanitaire mais la forte infectivité de certains variants maintient la nécessité de trouver des traitements curatifs

Etant donné la nature mutagène des protéines de l'enveloppe, en particulier la protéine S, le ciblage de protéines nécessaires à la réplication virale, comme les protéases nsp3 et nsp5, pourrait permettre d'obtenir des traitements plus efficaces et moins susceptibles aux différents variants. L'utilisation d'inhibiteurs de protéase s'est montrée efficace dans le traitement du VIH et du VHC. L'utilisation d'inhibiteurs de protéase efficace contre ces deux virus a été envisagée contre le SARS-CoV-2, mais cette réutilisation a montré une efficacité limitée [289,322]. Ces traitements incluent notamment le lopinavir/ritonavir.

Une possible stratégie dans le ciblage de ces protéines en particulier est l'utilisation de modélisations des interactions protéiques afin de définir quels types de molécules sont susceptibles de bloquer les protéases. Cette approche *in silico* pourrait également permettre de tester de nombreuses modifications chimiques des molécules d'intérêt pour obtenir la meilleure efficacité. Cette modélisation serait la première étape d'une stratégie de développement de nouveaux traitements. La deuxième étape consiste à tester les molécules sélectionnées *in vitro*, pour leur toxicité et leur effet antiviral. Les molécules sélectionnées après cette étape seront testées sur des épithéliums bronchiques ou nasaux humains, avant de passer par une étape critique de test dans un modèle animal pré-clinique. Ce modèle animal permet d'évaluer l'impact toxicologique des molécules testées, ainsi que leur effet antiviral *in vivo*, sur la réplication et sur la transmission entre animaux. Lorsque toutes ces étapes auront été réalisées et auront permis de démontrer l'efficacité et l'absence de toxicité d'un traitement, les phases d'essais cliniques pourront être réalisées, selon les protocoles appropriés.

12. Conclusions

L'augmentation de la fréquence de l'émergence de nouveaux virus, notamment des zoonoses, nous met face à la nécessité de développer de nouvelles stratégies thérapeutiques. Ces nouvelles approches nécessitent une connaissance approfondie des mécanismes moléculaires entourant la réplication virale, et leurs spécificités selon les familles et espèces virales.

Cette émergence de nouveaux virus s'accompagne également de la résurgence de virus anciens, bien connus ou non. Le dégel du permafrost pourrait entraîner la libération de pathogènes viraux préservés [399,400]. La hausse des températures dans la zone Arctique Russe semble corrélée à l'augmentation du nombre de cas d'infection par le virus de l'encéphalite à tique, ainsi qu'une résurgence des cas d'animaux porteurs du virus de la rage [401].

La compréhension des mécanismes moléculaires des virus non ou peu pathogènes pour l'homme peut permettre l'identification de cibles prioritaires pour les virus issus de la même famille, comme par exemple le ciblage des protéases des coronavirus.

Le ciblage par les molécules thérapeutiques de protéines spécifiques du virus permet d'augmenter l'efficacité du traitement et d'éviter les effets secondaires défavorables (effets off-target). Le choix de protéines nécessaires à la réplication virale, telles que les protéases virales, améliore grandement les chances de réussite. De plus, ce type de protéines essentielles présente peu de variabilité entre les souches d'un virus, et parfois même entre les espèces proches [402]. Cela permettrait de réduire l'échappement du virus aux thérapies.

Le ciblage des protéases virales semble constituer un choix thérapeutique intéressant. Nous avons montré que l'utilisation d'inhibiteurs de protéases entraîne la réduction de la taille du réservoir viral dans l'infection par le VIH, en plus de potentiellement réduire la capacité de réactivation du virus latent. Nous avons également montré que les molécules ciblant les protéases du SARS-CoV-2, notamment nsp5, entraînent une meilleure inhibition de la réplication que les traitements ciblant d'autres fonctions virales.

L'utilisation d'une approche systématique dans la recherche de traitements antiviraux permettrait d'obtenir plus rapidement des traitements lors de l'émergence d'un nouveau pathogène viral. Nous pourrions inclure dans cette approche une définition des cibles moléculaires d'intérêt associée à une validation *in silico* des interactions avec les traitements potentiels, permettant un pré-screening des classes de molécules à tester. Le cœur de cette approche systématique serait l'évaluation parallèle de la toxicité cellulaire des molécules et de leur efficacité *in vitro*. Cette évaluation de l'efficacité pourrait être réalisée dans un premier temps sur des virus de la même famille, si les cibles thérapeutiques sont globalement conservées. La comparaison des tests cellulaires avec les modélisations préalables pourrait permettre de définir d'éventuelles modifications des molécules pour garantir la meilleure efficacité.

L'accélération du rythme des pandémies virales au cours de ces 50 dernières années, en lien avec la baisse globale de la biodiversité et le changement climatique, nous a mis face à de nombreux virus émergents. Il est à prévoir que l'apparition de nouvelles maladies émergentes, notamment liées à

des pathogènes viraux, n'est pas terminée et que le développement de nouvelles approches thérapeutiques curatives et préventives n'en est qu'à son début.

13. Références

1. La Scola, B.; Desnues, C.; Pagnier, I.; Robert, C.; Barrassi, L.; Fournous, G.; Merchat, M.; Suzan-Monti, M.; Forterre, P.; Koonin, E.; et al. The Virophage as a Unique Parasite of the Giant Mimivirus. *Nature* **2008**, *455*, 100–104, doi:10.1038/nature07218.
2. Abergel, C.; Legendre, M.; Claverie, J.-M. The Rapidly Expanding Universe of Giant Viruses: Mimivirus, Pandoravirus, Pithovirus and Mollivirus. *FEMS Microbiology Reviews* **2015**, *39*, 779–796, doi:10.1093/femsre/fuv037.
3. Raoult, D.; Forterre, P. Redefining Viruses: Lessons from Mimivirus. *Nat Rev Microbiol* **2008**, *6*, 315–319, doi:10.1038/nrmicro1858.
4. Wolkowicz, R.; Schaechter, M. What Makes a Virus a Virus? *Nat Rev Microbiol* **2008**, *6*, 643–643, doi:10.1038/nrmicro1858-c1.
5. Baltimore, D. Expression of Animal Virus Genomes. *Bacteriol Rev* **1971**, *35*, 235–241.
6. Mahmoudabadi, G.; Phillips, R. A Comprehensive and Quantitative Exploration of Thousands of Viral Genomes. *eLife* **2015**, *7*, e31955, doi:10.7554/eLife.31955.
7. Warren, C.J.; Sawyer, S.L. How Host Genetics Dictates Successful Viral Zoonosis. *PLoS Biol* **2019**, *17*, e3000217, doi:10.1371/journal.pbio.3000217.
8. Vonesch, N.; Binazzi, A.; Bonafede, M.; Melis, P.; Ruggieri, A.; Iavicoli, S.; Tomao, P. Emerging Zoonotic Viral Infections of Occupational Health Importance. *Pathog Dis* **2019**, *77*, ftz018, doi:10.1093/femspd/ftz018.
9. Lloyd-Smith, J.O.; George, D.; Pepin, K.M.; Pitzer, V.E.; Pulliam, J.R.C.; Dobson, A.P.; Hudson, P.J.; Grenfell, B.T. Epidemic Dynamics at the Human-Animal Interface. *Science* **2009**, *326*, 1362–1367, doi:10.1126/science.1177345.
10. Gao, F.; Bailes, E.; Robertson, D.L.; Chen, Y.; Rodenburg, C.M.; Michael, S.F.; Cummins, L.B.; Arthur, L.O.; Peeters, M.; Shaw, G.M.; et al. Origin of HIV-1 in the Chimpanzee Pan Troglodytes Troglodytes. *Nature* **1999**, *397*, 436–441, doi:10.1038/17130.
11. Joint United Nations Programme on HIV/AIDS UNAIDS Data 2020.
12. Fantoni, M.; Del Borgo, C.; Autore, C. Evaluation and Management of Metabolic and Coagulative Disorders in HIV-Infected Patients Receiving Highly Active Antiretroviral Therapy. *AIDS* **2003**, *17 Suppl 1*, S162-169.
13. Colón-López, D.D.; Stefan, C.P.; Koehler, J.W. Emerging Viral Infections. *Genomic and Precision Medicine* **2019**, 141–154, doi:10.1016/B978-0-12-801496-7.00010-1.
14. Zheng, J. SARS-CoV-2: An Emerging Coronavirus That Causes a Global Threat. *Int J Biol Sci* **2020**, *16*, 1678–1685, doi:10.7150/ijbs.45053.
15. Krammer, F. SARS-CoV-2 Vaccines in Development. *Nature* **2020**, *586*, 516–527, doi:10.1038/s41586-020-2798-3.
16. Sanders, J.M.; Monogue, M.L.; Jodlowski, T.Z.; Cutrell, J.B. Pharmacologic Treatments for Coronavirus Disease 2019 (COVID-19): A Review. *JAMA* **2020**, doi:10.1001/jama.2020.6019.
17. Luciw, P.A.; Harada, S. Virology-S1 Virology: Overview. *AIDS-an International Monthly Journal* **1998**, *12*.
18. Barré-Sinoussi, F.; Chermann, J.C.; Rey, F.; Nugeyre, M.T.; Chamaret, S.; Gruest, J.; Dauguet, C.; Axler-Blin, C.; Vézinet-Brun, F.; Rouzioux, C.; et al. Isolation of a T-Lymphotropic Retrovirus from a Patient at Risk for Acquired Immune Deficiency Syndrome (AIDS). *Science* **1983**, *220*, 868–871, doi:10.1126/science.6189183.
19. Gallo, R.C.; Salahuddin, S.Z.; Popovic, M.; Shearer, G.M.; Kaplan, M.; Haynes, B.F.; Palker, T.J.; Redfield, R.; Oleske, J.; Safai, B. Frequent Detection and Isolation of Cytopathic Retroviruses (HTLV-III) from Patients with AIDS and at Risk for AIDS. *Science* **1984**, *224*, 500–503, doi:10.1126/science.6200936.

20. Korber, B.; Muldoon, M.; Theiler, J.; Gao, F.; Gupta, R.; Lapedes, A.; Hahn, B.H.; Wolinsky, S.; Bhattacharya, T. Timing the Ancestor of the HIV-1 Pandemic Strains. *Science* **2000**, *288*, 1789–1796, doi:10.1126/science.288.5472.1789.
21. Sharp, P.M.; Hahn, B.H. Origins of HIV and the AIDS Pandemic. *Cold Spring Harb Perspect Med* **2011**, *1*, a006841, doi:10.1101/cshperspect.a006841.
22. Faria, N.R.; Rambaut, A.; Suchard, M.A.; Baele, G.; Bedford, T.; Ward, M.J.; Tatem, A.J.; Sousa, J.D.; Arinaminpathy, N.; Pépin, J.; et al. HIV Epidemiology. The Early Spread and Epidemic Ignition of HIV-1 in Human Populations. *Science* **2014**, *346*, 56–61, doi:10.1126/science.1256739.
23. *Molecular Virology of Human Pathogenic Viruses*; Ryu, W.-S., Ed.; Academic Press: Boston, 2017; ISBN 978-0-12-800838-6.
24. Levy, J.A. HIV and the Pathogenesis of AIDS. *HIV and the pathogenesis of AIDS*. **2007**.
25. Sauter, D.; Unterwiesing, D.; Vogl, M.; Usmani, S.M.; Heigele, A.; Kluge, S.F.; Hermkes, E.; Moll, M.; Barker, E.; Peeters, M.; et al. Human Tetherin Exerts Strong Selection Pressure on the HIV-1 Group N Vpu Protein. *PLoS Pathog* **2012**, *8*, e1003093, doi:10.1371/journal.ppat.1003093.
26. Vicenzi, E.; Poli, G. Novel Factors Interfering with Human Immunodeficiency Virus-Type 1 Replication in Vivo and in Vitro. *Tissue Antigens* **2013**, *81*, 61–71, doi:10.1111/tan.12047.
27. Kuiken, C.; Foley, B.; Leitner, T.; Apetrei, C.; Hahn, B.; Mizrachi, I.; Mullins, J.; Rambaut, A.; Wolinsky, S.; Korber, B. *HIV Sequence Compendium 2010*; Los Alamos National Lab.(LANL), Los Alamos, NM (United States), 2010;
28. Simon, F.; Maucière, P.; Roques, P.; Loussert-Ajaka, I.; Müller-Trutwin, M.C.; Saragosti, S.; Georges-Courbot, M.C.; Barré-Sinoussi, F.; Brun-Vézinet, F. Identification of a New Human Immunodeficiency Virus Type 1 Distinct from Group M and Group O. *Nat Med* **1998**, *4*, 1032–1037, doi:10.1038/2017.
29. Plantier, J.-C.; Leoz, M.; Dickerson, J.E.; De Oliveira, F.; Cordonnier, F.; Lemée, V.; Damond, F.; Robertson, D.L.; Simon, F. A New Human Immunodeficiency Virus Derived from Gorillas. *Nat Med* **2009**, *15*, 871–872, doi:10.1038/nm.2016.
30. Vallari, A.; Holzmayer, V.; Harris, B.; Yamaguchi, J.; Ngansop, C.; Makamche, F.; Mbanya, D.; Kaptué, L.; Ndembu, N.; Gürtler, L.; et al. Confirmation of Putative HIV-1 Group P in Cameroon. *J Virol* **2011**, *85*, 1403–1407, doi:10.1128/JVI.02005-10.
31. Ward, M.J.; Lycett, S.J.; Kalish, M.L.; Rambaut, A.; Leigh Brown, A.J. Estimating the Rate of Intersubtype Recombination in Early HIV-1 Group M Strains. *J Virol* **2013**, *87*, 1967–1973, doi:10.1128/JVI.02478-12.
32. Chow, W.Z.; Al-Darraj, H.; Lee, Y.M.; Takebe, Y.; Kamarulzaman, A.; Tee, K.K. Genome Sequences of a Novel HIV-1 CRF53_01B Identified in Malaysia. *J Virol* **2012**, *86*, 11398–11399, doi:10.1128/JVI.01932-12.
33. Jia, L.; Li, L.; Li, H.; Liu, S.; Wang, X.; Bao, Z.; Li, T.; Zhuang, D.; Liu, Y.; Li, J. Recombination Pattern Reanalysis of Some HIV-1 Circulating Recombination Forms Suggest the Necessity and Difficulty of Revision. *PLoS One* **2014**, *9*, e107349, doi:10.1371/journal.pone.0107349.
34. Pessôa, R.; Watanabe, J.T.; Calabria, P.; Felix, A.C.; Loureiro, P.; Sabino, E.C.; Busch, M.P.; Sanabani, S.S.; International Component of the NHLBI Recipient Epidemiology and Donor Evaluation Study-III Deep Sequencing of HIV-1 near Full-Length Proviral Genomes Identifies High Rates of BF1 Recombinants Including Two Novel Circulating Recombinant Forms (CRF) 70_BF1 and a Disseminating 71_BF1 among Blood Donors in Pernambuco, Brazil. *PLoS One* **2014**, *9*, e112674, doi:10.1371/journal.pone.0112674.
35. Yu, F.; Wen, Y.; Wang, J.; Gong, Y.; Feng, K.; Ye, R.; Jiang, Y.; Zhao, Q.; Pan, P.; Wu, H.; et al. The Transmission and Evolution of HIV-1 Quasispecies within One Couple: A Follow-up Study Based on Next-Generation Sequencing. *Sci Rep* **2018**, *8*, 1404, doi:10.1038/s41598-018-19783-3.
36. Peeters, M.; Sharp, P.M. Genetic Diversity of HIV-1: The Moving Target. *AIDS* **2000**, *14 Suppl 3*, S129-140.

-
37. Gelderblom, H.R. Assembly and Morphology of HIV: Potential Effect of Structure on Viral Function. *AIDS* **1991**, *5*, 617–637.
 38. Parren, P.W.; Moore, J.P.; Burton, D.R.; Sattentau, Q.J. The Neutralizing Antibody Response to HIV-1: Viral Evasion and Escape from Humoral Immunity. *AIDS* **1999**, *13 Suppl A*, S137-162.
 39. Niedrig, M.; Gelderblom, H.R.; Pauli, G.; März, J.; Bickhard, H.; Wolf, H.; Modrow, S. Inhibition of Infectious Human Immunodeficiency Virus Type 1 Particle Formation by Gag Protein-Derived Peptides. *J Gen Virol* **1994**, *75 (Pt 6)*, 1469–1474, doi:10.1099/0022-1317-75-6-1469.
 40. Gelderblom, H.R.; Reupke, H.; Pauli, G. Loss of Envelope Antigens of HTLV-III/LAV, a Factor in AIDS Pathogenesis? *Lancet* **1985**, *2*, 1016–1017, doi:10.1016/s0140-6736(85)90570-7.
 41. Selhorst, P.; Gruppung, K.; Tong, T.; Crooks, E.T.; Martin, L.; Vanham, G.; Binley, J.M.; Ariën, K.K. M48U1 CD4 Mimetic Has a Sustained Inhibitory Effect on Cell-Associated HIV-1 by Attenuating Virion Infectivity through Gp120 Shedding. *Retrovirology* **2013**, *10*, 12, doi:10.1186/1742-4690-10-12.
 42. Hu, W.-S.; Hughes, S.H. HIV-1 Reverse Transcription. *Cold Spring Harb Perspect Med* **2012**, *2*, a006882, doi:10.1101/cshperspect.a006882.
 43. London, R.E. HIV-1 Reverse Transcriptase: A Metamorphic Protein with Three Stable States. *Structure* **2019**, *27*, 420–426, doi:10.1016/j.str.2018.11.011.
 44. Charneau, P.; Alizon, M.; Clavel, F. A Second Origin of DNA Plus-Strand Synthesis Is Required for Optimal Human Immunodeficiency Virus Replication. *J Virol* **1992**, *66*, 2814–2820, doi:10.1128/JVI.66.5.2814-2820.1992.
 45. Thomas, D.C.; Voronin, Y.A.; Nikolenko, G.N.; Chen, J.; Hu, W.-S.; Pathak, V.K. Determination of the Ex Vivo Rates of Human Immunodeficiency Virus Type 1 Reverse Transcription by Using Novel Strand-Specific Amplification Analysis. *J Virol* **2007**, *81*, 4798–4807, doi:10.1128/JVI.02471-06.
 46. Sarafianos, S.G.; Marchand, B.; Das, K.; Himmel, D.M.; Parniak, M.A.; Hughes, S.H.; Arnold, E. Structure and Function of HIV-1 Reverse Transcriptase: Molecular Mechanisms of Polymerization and Inhibition. *J Mol Biol* **2009**, *385*, 693–713, doi:10.1016/j.jmb.2008.10.071.
 47. Liao, C.; Marchand, C.; Burke, T.R.; Pommier, Y.; Nicklaus, M.C. Authentic HIV-1 Integrase Inhibitors. *Future Med Chem* **2010**, *2*, 1107–1122, doi:10.4155/fmc.10.199.
 48. Poeschla, E.M. Integrase, LEDGF/P75 and HIV Replication. *Cell Mol Life Sci* **2008**, *65*, 1403–1424, doi:10.1007/s00018-008-7540-5.
 49. Ingale, K.B.; Bhatia, M.S. HIV-1 Integrase Inhibitors: A Review of Their Chemical Development. *Antivir Chem Chemother* **2011**, *22*, 95–105, doi:10.3851/IMP1740.
 50. Dayer, M.R.; Dayer, M.S. Whiskers-Less HIV-Protease: A Possible Way for HIV-1 Deactivation. *J Biomed Sci* **2013**, *20*, 67, doi:10.1186/1423-0127-20-67.
 51. Potempa, M.; Lee, S.-K.; KurtYilmaz, N.; Nalivaika, E.A.; Rogers, A.; Spielvogel, E.; Carter, C.W.; Schiffer, C.A.; Swanstrom, R. HIV-1 Protease Uses Bi-Specific S2/S2' Subsites To Optimize Cleavage of Two Classes of Target Sites. *J Mol Biol* **2018**, *430*, 5182–5195, doi:10.1016/j.jmb.2018.10.022.
 52. Huang, L.; Li, L.; Tien, C.; LaBarbera, D.V.; Chen, C. Targeting HIV-1 Protease Autoprocessing for High-Throughput Drug Discovery and Drug Resistance Assessment. *Sci Rep* **2019**, *9*, 301, doi:10.1038/s41598-018-36730-4.
 53. Konvalinka, J.; Kräusslich, H.-G.; Müller, B. Retroviral Proteases and Their Roles in Virion Maturation. *Virology* **2015**, *479–480*, 403–417, doi:10.1016/j.virol.2015.03.021.
 54. Mbonye, U.; Karn, J. Transcriptional Control of HIV Latency: Cellular Signaling Pathways, Epigenetics, Happenstance and the Hope for a Cure. *Virology* **2014**, *454–455*, 328–339, doi:10.1016/j.virol.2014.02.008.
 55. Lange, U.C.; Verdikt, R.; Ait-Ammar, A.; Van Lint, C. Epigenetic Crosstalk in Chronic Infection with HIV-1. *Semin Immunopathol* **2020**, *42*, 187–200, doi:10.1007/s00281-020-00783-3.

56. Sobhian, B.; Laguette, N.; Yatim, A.; Nakamura, M.; Levy, Y.; Kiernan, R.; Benkirane, M. HIV-1 Tat Assembles a Multifunctional Transcription Elongation Complex and Stably Associates with the 7SK SnRNP. *Mol Cell* **2010**, *38*, 439–451, doi:10.1016/j.molcel.2010.04.012.
57. Ne, E.; Palstra, R.-J.; Mahmoudi, T. Transcription: Insights From the HIV-1 Promoter. *Int Rev Cell Mol Biol* **2018**, *335*, 191–243, doi:10.1016/bs.ircmb.2017.07.011.
58. Rafati, H.; Parra, M.; Hakre, S.; Moshkin, Y.; Verdin, E.; Mahmoudi, T. Repressive LTR Nucleosome Positioning by the BAF Complex Is Required for HIV Latency. *PLoS Biol* **2011**, *9*, e1001206, doi:10.1371/journal.pbio.1001206.
59. Fiume, G.; Vecchio, E.; De Laurentiis, A.; Trimboli, F.; Palmieri, C.; Pisano, A.; Falcone, C.; Pontoriero, M.; Rossi, A.; Scialdone, A.; et al. Human Immunodeficiency Virus-1 Tat Activates NF- κ B via Physical Interaction with I κ B- α and P65. *Nucleic Acids Res* **2012**, *40*, 3548–3562, doi:10.1093/nar/gkr1224.
60. Dutilleul, A.; Rodari, A.; Van Lint, C. Depicting HIV-1 Transcriptional Mechanisms: A Summary of What We Know. *Viruses* **2020**, *12*, 1385, doi:10.3390/v12121385.
61. Levy, J.A. Virus-Host Interactions in HIV Pathogenesis: Directions for Therapy. *Adv Dent Res* **2011**, *23*, 13–18, doi:10.1177/0022034511398874.
62. Tabarrini, O.; Desantis, J.; Massari, S. Recent Advances in the Identification of Tat-Mediated Transactivation Inhibitors: Progressing toward a Functional Cure of HIV. *Future Med Chem* **2016**, *8*, 421–442, doi:10.4155/fmc.16.3.
63. Truman, C.T.-S.; Järvelin, A.; Davis, I.; Castello, A. HIV Rev-Isited. *Open Biol* **2020**, *10*, 200320, doi:10.1098/rsob.200320.
64. Booth, D.S.; Cheng, Y.; Frankel, A.D. The Export Receptor Crm1 Forms a Dimer to Promote Nuclear Export of HIV RNA. *Elife* **2014**, *3*, e04121, doi:10.7554/eLife.04121.
65. Monette, A.; Panté, N.; Mouland, A.J. HIV-1 Remodels the Nuclear Pore Complex. *J Cell Biol* **2011**, *193*, 619–631, doi:10.1083/jcb.201008064.
66. Wang, Y.; Zhang, H.; Na, L.; Du, C.; Zhang, Z.; Zheng, Y.-H.; Wang, X. ANP32A and ANP32B Are Key Factors in the Rev-Dependent CRM1 Pathway for Nuclear Export of HIV-1 Unspliced MRNA. *J Biol Chem* **2019**, *294*, 15346–15357, doi:10.1074/jbc.RA119.008450.
67. Nguyen, K.-L.; Ilano, M.; Akari, H.; Miyagi, E.; Poeschla, E.M.; Strebler, K.; Bour, S. Codon Optimization of the HIV-1 Vpu and Vif Genes Stabilizes Their MRNA and Allows for Highly Efficient Rev-Independent Expression. *Virology* **2004**, *319*, 163–175, doi:10.1016/j.virol.2003.11.021.
68. Jacquenet, S.; Decimo, D.; Muriaux, D.; Darlix, J.-L. Dual Effect of the SR Proteins ASF/SF2, SC35 and 9G8 on HIV-1 RNA Splicing and Virion Production. *Retrovirology* **2005**, *2*, 33, doi:10.1186/1742-4690-2-33.
69. Cullen, B.R. Human Immunodeficiency Virus as a Prototypic Complex Retrovirus. *J Virol* **1991**, *65*, 1053–1056, doi:10.1128/JVI.65.3.1053-1056.1991.
70. Yu, G.; Felsted, R.L. Effect of Myristoylation on P27 Nef Subcellular Distribution and Suppression of HIV-LTR Transcription. *Virology* **1992**, *187*, 46–55, doi:10.1016/0042-6822(92)90293-x.
71. Staudt, R.P.; Alvarado, J.J.; Emert-Sedlak, L.A.; Shi, H.; Shu, S.T.; Wales, T.E.; Engen, J.R.; Smithgall, T.E. Structure, Function, and Inhibitor Targeting of HIV-1 Nef-Effector Kinase Complexes. *J Biol Chem* **2020**, *295*, 15158–15171, doi:10.1074/jbc.REV120.012317.
72. Aiken, C.; Konner, J.; Landau, N.R.; Lenburg, M.E.; Trono, D. Nef Induces CD4 Endocytosis: Requirement for a Critical Dileucine Motif in the Membrane-Proximal CD4 Cytoplasmic Domain. *Cell* **1994**, *76*, 853–864, doi:10.1016/0092-8674(94)90360-3.
73. Kwon, Y.; Kaake, R.M.; Echeverria, I.; Suarez, M.; Shamsabadi, M.K.; Stoneham, C.; Ramirez, P.W.; Kress, J.; Singh, R.; Sali, A.; et al. Structural Basis of CD4 Downregulation by HIV-1 Nef. *Nat Struct Mol Biol* **2020**, *27*, 822–828, doi:10.1038/s41594-020-0463-z.
74. Hung, C.-H.; Thomas, L.; Ruby, C.E.; Atkins, K.M.; Morris, N.P.; Knight, Z.A.; Scholz, I.; Barklis, E.; Weinberg, A.D.; Shokat, K.M.; et al. HIV-1 Nef Assembles a Src Family Kinase-

-
- ZAP-70/Syk-PI3K Cascade to Downregulate Cell-Surface MHC-I. *Cell Host Microbe* **2007**, *1*, 121–133, doi:10.1016/j.chom.2007.03.004.
75. Kasper, M.R.; Roeth, J.F.; Williams, M.; Filzen, T.M.; Fleis, R.I.; Collins, K.L. HIV-1 Nef Disrupts Antigen Presentation Early in the Secretory Pathway. *J Biol Chem* **2005**, *280*, 12840–12848, doi:10.1074/jbc.M413538200.
76. Rosa, A.; Chande, A.; Ziglio, S.; De Sanctis, V.; Bertorelli, R.; Goh, S.L.; McCauley, S.M.; Nowosielska, A.; Antonarakis, S.E.; Luban, J.; et al. HIV-1 Nef Promotes Infection by Excluding SERINC5 from Virion Incorporation. *Nature* **2015**, *526*, 212–217, doi:10.1038/nature15399.
77. Usami, Y.; Wu, Y.; Göttinger, H.G. SERINC3 and SERINC5 Restrict HIV-1 Infectivity and Are Counteracted by Nef. *Nature* **2015**, *526*, 218–223, doi:10.1038/nature15400.
78. Briggs, S.D.; Sharkey, M.; Stevenson, M.; Smithgall, T.E. SH3-Mediated Hck Tyrosine Kinase Activation and Fibroblast Transformation by the Nef Protein of HIV-1. *J Biol Chem* **1997**, *272*, 17899–17902, doi:10.1074/jbc.272.29.17899.
79. Tarafdar, S.; Poe, J.A.; Smithgall, T.E. The Accessory Factor Nef Links HIV-1 to Tec/Btk Kinases in an Src Homology 3 Domain-Dependent Manner. *J Biol Chem* **2014**, *289*, 15718–15728, doi:10.1074/jbc.M114.572099.
80. Basmaciogullari, S.; Pizzato, M. The Activity of Nef on HIV-1 Infectivity. *Front Microbiol* **2014**, *5*, 232, doi:10.3389/fmicb.2014.00232.
81. Rhodes, D.I.; Ashton, L.; Solomon, A.; Carr, A.; Cooper, D.; Kaldor, J.; Deacon, N. Characterization of Three Nef-Defective Human Immunodeficiency Virus Type 1 Strains Associated with Long-Term Nonprogression. Australian Long-Term Nonprogressor Study Group. *J Virol* **2000**, *74*, 10581–10588, doi:10.1128/jvi.74.22.10581-10588.2000.
82. Churchill, M.J.; Rhodes, D.I.; Learmont, J.C.; Sullivan, J.S.; Wesselingh, S.L.; Cooke, I.R.C.; Deacon, N.J.; Gorry, P.R. Longitudinal Analysis of Human Immunodeficiency Virus Type 1 Nef/Long Terminal Repeat Sequences in a Cohort of Long-Term Survivors Infected from a Single Source. *J Virol* **2006**, *80*, 1047–1052, doi:10.1128/JVI.80.2.1047-1052.2006.
83. Cohen, E.A.; Terwilliger, E.F.; Jalinoos, Y.; Proulx, J.; Sodroski, J.G.; Haseltine, W.A. Identification of HIV-1 Vpr Product and Function. *J Acquir Immune Defic Syndr (1988)* **1990**, *3*, 11–18.
84. Cohen, E.A.; Dehni, G.; Sodroski, J.G.; Haseltine, W.A. Human Immunodeficiency Virus Vpr Product Is a Virion-Associated Regulatory Protein. *J Virol* **1990**, *64*, 3097–3099, doi:10.1128/JVI.64.6.3097-3099.1990.
85. Zhao, L.J.; Wang, L.; Mukherjee, S.; Narayan, O. Biochemical Mechanism of HIV-1 Vpr Function. Oligomerization Mediated by the N-Terminal Domain. *J Biol Chem* **1994**, *269*, 32131–32137.
86. Wang, J.J.; Lu, Y.; Ratner, L. Particle Assembly and Vpr Expression in Human Immunodeficiency Virus Type 1-Infected Cells Demonstrated by Immunoelectron Microscopy. *J Gen Virol* **1994**, *75* (Pt 10), 2607–2614, doi:10.1099/0022-1317-75-10-2607.
87. Lubow, J.; Collins, K.L. Vpr Is a VIP: HIV Vpr and Infected Macrophages Promote Viral Pathogenesis. *Viruses* **2020**, *12*, 809, doi:10.3390/v12080809.
88. Eckstein, D.A.; Sherman, M.P.; Penn, M.L.; Chin, P.S.; De Noronha, C.M.C.; Greene, W.C.; Goldsmith, M.A. HIV-1 Vpr Enhances Viral Burden by Facilitating Infection of Tissue Macrophages but Not Nondividing CD4+ T Cells. *J Exp Med* **2001**, *194*, 1407–1419.
89. Lahouassa, H.; Blondot, M.-L.; Chauveau, L.; Chougui, G.; Morel, M.; Leduc, M.; Guillonnet, F.; Ramirez, B.C.; Schwartz, O.; Margottin-Goguet, F. HIV-1 Vpr Degrades the HLTF DNA Translocase in T Cells and Macrophages. *Proc Natl Acad Sci U S A* **2016**, *113*, 5311–5316, doi:10.1073/pnas.1600485113.
90. Mashiba, M.; Collins, D.R.; Terry, V.H.; Collins, K.L. Vpr Overcomes Macrophage-Specific Restriction of HIV-1 Env Expression and Virion Production. *Cell Host Microbe* **2014**, *16*, 722–735, doi:10.1016/j.chom.2014.10.014.

91. Wang, Q.; Su, L. Vpr Enhances HIV-1 Env Processing and Virion Infectivity in Macrophages by Modulating TET2-Dependent IFITM3 Expression. *mBio* **2019**, *10*, e01344-19, doi:10.1128/mBio.01344-19.
92. Popov, S.; Rexach, M.; Zybarth, G.; Reiling, N.; Lee, M.A.; Ratner, L.; Lane, C.M.; Moore, M.S.; Blobel, G.; Bukrinsky, M. Viral Protein R Regulates Nuclear Import of the HIV-1 Pre-Integration Complex. *EMBO J* **1998**, *17*, 909–917, doi:10.1093/emboj/17.4.909.
93. Okumura, A.; Alce, T.; Lubyova, B.; Ezelle, H.; Strebel, K.; Pitha, P.M. HIV-1 Accessory Proteins VPR and Vif Modulate Antiviral Response by Targeting IRF-3 for Degradation. *Virology* **2008**, *373*, 85–97, doi:10.1016/j.virol.2007.10.042.
94. Jowett, J.B.; Planelles, V.; Poon, B.; Shah, N.P.; Chen, M.L.; Chen, I.S. The Human Immunodeficiency Virus Type 1 Vpr Gene Arrests Infected T Cells in the G₂ + M Phase of the Cell Cycle. *J Virol* **1995**, *69*, 6304–6313, doi:10.1128/JVI.69.10.6304-6313.1995.
95. He, J.; Choe, S.; Walker, R.; Di Marzio, P.; Morgan, D.O.; Landau, N.R. Human Immunodeficiency Virus Type 1 Viral Protein R (Vpr) Arrests Cells in the G₂ Phase of the Cell Cycle by Inhibiting P34cdc2 Activity. *J Virol* **1995**, *69*, 6705–6711, doi:10.1128/JVI.69.11.6705-6711.1995.
96. Kamata, M.; Watanabe, N.; Nagaoka, Y.; Chen, I.S.Y. Human Immunodeficiency Virus Type 1 Vpr Binds to the N Lobe of the Wee1 Kinase Domain and Enhances Kinase Activity for CDC2. *J Virol* **2008**, *82*, 5672–5682, doi:10.1128/JVI.01330-07.
97. Huard, S.; Elder, R.T.; Liang, D.; Li, G.; Zhao, R.Y. Human Immunodeficiency Virus Type 1 Vpr Induces Cell Cycle G₂ Arrest through Srk1/MK2-Mediated Phosphorylation of Cdc25. *J Virol* **2008**, *82*, 2904–2917, doi:10.1128/JVI.01098-07.
98. Wu, Y.; Zhou, X.; Barnes, C.O.; DeLucia, M.; Cohen, A.E.; Gronenborn, A.M.; Ahn, J.; Calero, G. The DDB1-DCAF1-Vpr-UNG2 Crystal Structure Reveals How HIV-1 Vpr Steers Human UNG2 toward Destruction. *Nat Struct Mol Biol* **2016**, *23*, 933–940, doi:10.1038/nsmb.3284.
99. Nakagawa, T.; Lv, L.; Nakagawa, M.; Yu, Y.; Yu, C.; D'Alessio, A.C.; Nakayama, K.; Fan, H.-Y.; Chen, X.; Xiong, Y. CRL4(VprBP) E3 Ligase Promotes Monoubiquitylation and Chromatin Binding of TET Dioxygenases. *Mol Cell* **2015**, *57*, 247–260, doi:10.1016/j.molcel.2014.12.002.
100. Marno, K.M.; Ogunkolade, B.W.; Pade, C.; Oliveira, N.M.M.; O'Sullivan, E.; McKnight, Á. Novel Restriction Factor RNA-Associated Early-Stage Anti-Viral Factor (REAF) Inhibits Human and Simian Immunodeficiency Viruses. *Retrovirology* **2014**, *11*, 3, doi:10.1186/1742-4690-11-3.
101. Lubow, J.; Virgilio, M.C.; Merlino, M.; Collins, D.R.; Mashiba, M.; Peterson, B.G.; Lukic, Z.; Painter, M.M.; Gomez-Rivera, F.; Terry, V.; et al. Mannose Receptor Is an HIV Restriction Factor Counteracted by Vpr in Macrophages. *Elife* **2020**, *9*, e51035, doi:10.7554/eLife.51035.
102. Michaels, F.H.; Hattori, N.; Gallo, R.C.; Franchini, G. The Human Immunodeficiency Virus Type 1 (HIV-1) Vif Protein Is Located in the Cytoplasm of Infected Cells and Its Effect on Viral Replication Is Equivalent in HIV-2. *AIDS Res Hum Retroviruses* **1993**, *9*, 1025–1030, doi:10.1089/aid.1993.9.1025.
103. Borman, A.M.; Quillent, C.; Charneau, P.; Dauguet, C.; Clavel, F. Human Immunodeficiency Virus Type 1 Vif- Mutant Particles from Restrictive Cells: Role of Vif in Correct Particle Assembly and Infectivity. *J Virol* **1995**, *69*, 2058–2067, doi:10.1128/JVI.69.4.2058-2067.1995.
104. Sova, P.; Volsky, D.J. Efficiency of Viral DNA Synthesis during Infection of Permissive and Nonpermissive Cells with Vif-Negative Human Immunodeficiency Virus Type 1. *J Virol* **1993**, *67*, 6322–6326, doi:10.1128/JVI.67.10.6322-6326.1993.
105. Arias, J.F.; Koyama, T.; Kinomoto, M.; Tokunaga, K. Retroelements versus APOBEC3 Family Members: No Great Escape from the Magnificent Seven. *Front Microbiol* **2012**, *3*, 275, doi:10.3389/fmicb.2012.00275.
106. Ara, A.; Love, R.P.; Chelico, L. Different Mutagenic Potential of HIV-1 Restriction Factors APOBEC3G and APOBEC3F Is Determined by Distinct Single-Stranded DNA Scanning Mechanisms. *PLoS Pathog* **2014**, *10*, e1004024, doi:10.1371/journal.ppat.1004024.

-
107. Ebrahimi, D.; Alinejad-Rokny, H.; Davenport, M.P. Insights into the Motif Preference of APOBEC3 Enzymes. *PLoS One* **2014**, *9*, e87679, doi:10.1371/journal.pone.0087679.
 108. Sheehy, A.M.; Gaddis, N.C.; Malim, M.H. The Antiretroviral Enzyme APOBEC3G Is Degraded by the Proteasome in Response to HIV-1 Vif. *Nat Med* **2003**, *9*, 1404–1407, doi:10.1038/nm945.
 109. Zhao, K.; Du, J.; Rui, Y.; Zheng, W.; Kang, J.; Hou, J.; Wang, K.; Zhang, W.; Simon, V.A.; Yu, X.-F. Evolutionarily Conserved Pressure for the Existence of Distinct G2/M Cell Cycle Arrest and A3H Inactivation Functions in HIV-1 Vif. *Cell Cycle* **2015**, *14*, 838–847, doi:10.1080/15384101.2014.1000212.
 110. Greenwood, E.J.; Matheson, N.J.; Wals, K.; van den Boomen, D.J.; Antrobus, R.; Williamson, J.C.; Lehner, P.J. Temporal Proteomic Analysis of HIV Infection Reveals Remodelling of the Host Phosphoproteome by Lentiviral Vif Variants. *Elife* **2016**, *5*, e18296, doi:10.7554/eLife.18296.
 111. Salamango, D.J.; Ikeda, T.; Moghadasi, S.A.; Wang, J.; McCann, J.L.; Serebrenik, A.A.; Ebrahimi, D.; Jarvis, M.C.; Brown, W.L.; Harris, R.S. HIV-1 Vif Triggers Cell Cycle Arrest by Degrading Cellular PPP2R5 Phospho-Regulators. *Cell Rep* **2019**, *29*, 1057–1065.e4, doi:10.1016/j.celrep.2019.09.057.
 112. Nilsson, J. Protein Phosphatases in the Regulation of Mitosis. *J Cell Biol* **2019**, *218*, 395–409, doi:10.1083/jcb.201809138.
 113. Thompson, J.J.; Williams, C.S. Protein Phosphatase 2A in the Regulation of Wnt Signaling, Stem Cells, and Cancer. *Genes (Basel)* **2018**, *9*, E121, doi:10.3390/genes9030121.
 114. Vigan, R.; Neil, S.J.D. Separable Determinants of Subcellular Localization and Interaction Account for the Inability of Group O HIV-1 Vpu To Counteract Tetherin ν . *J Virol* **2011**, *85*, 9737–9748, doi:10.1128/JVI.00479-11.
 115. González, M.E. Vpu Protein: The Viroporin Encoded by HIV-1. *Viruses* **2015**, *7*, 4352–4368, doi:10.3390/v7082824.
 116. Xu, Z.; Lodge, R.; Power, C.; Cohen, E.A.; Hobman, T.C. The HIV-1 Accessory Protein Vpu Downregulates Peroxisome Biogenesis. *mBio* **2020**, *11*, e03395-19, doi:10.1128/mBio.03395-19.
 117. Hopfensperger, K.; Sauter, D. Decreased, Deformed, Defective—How HIV-1 Vpu Targets Peroxisomes. *mBio* **2020**, *11*, e00967-20, doi:10.1128/mBio.00967-20.
 118. Sauter, D.; Hotter, D.; Van Driessche, B.; Stürzel, C.M.; Kluge, S.F.; Wildum, S.; Yu, H.; Baumann, B.; Wirth, T.; Plantier, J.-C.; et al. Differential Regulation of NF-KB-Mediated Proviral and Antiviral Host Gene Expression by Primate Lentiviral Nef and Vpu Proteins. *Cell Rep* **2015**, *10*, 586–599, doi:10.1016/j.celrep.2014.12.047.
 119. Langer, S.; Hammer, C.; Hopfensperger, K.; Klein, L.; Hotter, D.; De Jesus, P.D.; Herbert, K.M.; Pache, L.; Smith, N.; van der Merwe, J.A.; et al. HIV-1 Vpu Is a Potent Transcriptional Suppressor of NF-KB-Elicited Antiviral Immune Responses. *eLife* **8**, e41930, doi:10.7554/eLife.41930.
 120. Klimkait, T.; Strebel, K.; Hoggan, M.D.; Martin, M.A.; Orenstein, J.M. The Human Immunodeficiency Virus Type 1-Specific Protein Vpu Is Required for Efficient Virus Maturation and Release. *J Virol* **1990**, *64*, 621–629, doi:10.1128/JVI.64.2.621-629.1990.
 121. Liu, Y.; Liu, H.; Kim, B.O.; Gattone, V.H.; Li, J.; Nath, A.; Blum, J.; He, J.J. CD4-Independent Infection of Astrocytes by Human Immunodeficiency Virus Type 1: Requirement for the Human Mannose Receptor. *J Virol* **2004**, *78*, 4120–4133, doi:10.1128/jvi.78.8.4120-4133.2004.
 122. Dean, M.; Carrington, M.; Winkler, C.; Huttley, G.A.; Smith, M.W.; Allikmets, R.; Goedert, J.J.; Buchbinder, S.P.; Vittinghoff, E.; Gomperts, E.; et al. Genetic Restriction of HIV-1 Infection and Progression to AIDS by a Deletion Allele of the CKR5 Structural Gene. Hemophilia Growth and Development Study, Multicenter AIDS Cohort Study, Multicenter Hemophilia Cohort Study, San Francisco City Cohort, ALIVE Study. *Science* **1996**, *273*, 1856–1862, doi:10.1126/science.273.5283.1856.

123. Feng, Y.; Broder, C.C.; Kennedy, P.E.; Berger, E.A. HIV-1 Entry Cofactor: Functional CDNA Cloning of a Seven-Transmembrane, G Protein-Coupled Receptor. *Science* **1996**, *272*, 872–877, doi:10.1126/science.272.5263.872.
124. Peden, K.W.C.; Farbert, J.M. Coreceptors for human immunodeficiency virus and simian immunodeficiency virus. In *Advances in Pharmacology; HIV-1: Molecular Biology and Pathogenesis Viral Mechanisms*; Academic Press, 2000; Vol. 48, pp. 409–478.
125. Zaitseva, M.; Peden, K.; Golding, H. HIV Coreceptors: Role of Structure, Posttranslational Modifications, and Internalization in Viral-Cell Fusion and as Targets for Entry Inhibitors. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **2003**, *1614*, 51–61, doi:10.1016/S0005-2736(03)00162-7.
126. Chen, B. Molecular Mechanism of HIV-1 Entry. *Trends Microbiol* **2019**, *27*, 878–891, doi:10.1016/j.tim.2019.06.002.
127. Archin, N.M.; Sung, J.M.; Garrido, C.; Soriano-Sarabia, N.; Margolis, D.M. Eradicating HIV-1 Infection: Seeking to Clear a Persistent Pathogen. *Nat Rev Microbiol* **2014**, *12*, 750–764, doi:10.1038/nrmicro3352.
128. Stein, B.S.; Gowda, S.D.; Lifson, J.D.; Penhallow, R.C.; Bensch, K.G.; Engleman, E.G. PH-Independent HIV Entry into CD4-Positive T Cells via Virus Envelope Fusion to the Plasma Membrane. *Cell* **1987**, *49*, 659–668, doi:10.1016/0092-8674(87)90542-3.
129. Sousa, R.; Chung, Y.J.; Rose, J.P.; Wang, B.C. Crystal Structure of Bacteriophage T7 RNA Polymerase at 3.3 Å Resolution. *Nature* **1993**, *364*, 593–599, doi:10.1038/364593a0.
130. Smith, A.J.; Srinivasakumar, N.; Hammarskjöld, M.L.; Rekosh, D. Requirements for Incorporation of Pr160gag-Pol from Human Immunodeficiency Virus Type 1 into Virus-like Particles. *J Virol* **1993**, *67*, 2266–2275, doi:10.1128/JVI.67.4.2266-2275.1993.
131. Dalgleish, A.G.; Beverley, P.C.; Clapham, P.R.; Crawford, D.H.; Greaves, M.F.; Weiss, R.A. The CD4 (T4) Antigen Is an Essential Component of the Receptor for the AIDS Retrovirus. *Nature* **1984**, *312*, 763–767, doi:10.1038/312763a0.
132. Klatzmann, D.; Champagne, E.; Chamaret, S.; Gruest, J.; Guetard, D.; Hercend, T.; Gluckman, J.C.; Montagnier, L. T-Lymphocyte T4 Molecule Behaves as the Receptor for Human Retrovirus LAV. *Nature* **1984**, *312*, 767–768, doi:10.1038/312767a0.
133. Gorry, P.R.; Churchill, M.; Crowe, S.M.; Cunningham, A.L.; Gabuzda, D. Pathogenesis of Macrophage Tropic HIV-1. *Curr HIV Res* **2005**, *3*, 53–60, doi:10.2174/1570162052772951.
134. Pope, M.; Betjes, M.G.; Romani, N.; Hirmand, H.; Cameron, P.U.; Hoffman, L.; Gezelter, S.; Schuler, G.; Steinman, R.M. Conjugates of Dendritic Cells and Memory T Lymphocytes from Skin Facilitate Productive Infection with HIV-1. *Cell* **1994**, *78*, 389–398, doi:10.1016/0092-8674(94)90418-9.
135. Evans, L.A.; McHugh, T.M.; Stites, D.P.; Levy, J.A. Differential Ability of Human Immunodeficiency Virus Isolates to Productively Infect Human Cells. *J Immunol* **1987**, *138*, 3415–3418.
136. Finzi, D.; Hermankova, M.; Pierson, T.; Carruth, L.M.; Buck, C.; Chaisson, R.E.; Quinn, T.C.; Chadwick, K.; Margolick, J.; Brookmeyer, R.; et al. Identification of a Reservoir for HIV-1 in Patients on Highly Active Antiretroviral Therapy. *Science* **1997**, *278*, 1295–1300.
137. Swiggard, W.J.; Baytop, C.; Yu, J.J.; Dai, J.; Li, C.; Schretzenmair, R.; Theodosopoulos, T.; O'Doherty, U. Human Immunodeficiency Virus Type 1 Can Establish Latent Infection in Resting CD4+ T Cells in the Absence of Activating Stimuli. *J. Virol.* **2005**, *79*, 14179–14188, doi:10.1128/JVI.79.22.14179-14188.2005.
138. Finzi, D.; Blankson, J.; Siliciano, J.D.; Margolick, J.B.; Chadwick, K.; Pierson, T.; Smith, K.; Lisziewicz, J.; Lori, F.; Flexner, C.; et al. Latent Infection of CD4+ T Cells Provides a Mechanism for Lifelong Persistence of HIV-1, Even in Patients on Effective Combination Therapy. *Nat Med* **1999**, *5*, 512–517, doi:10.1038/8394.

-
139. Colin, L.; Van Lint, C. Molecular Control of HIV-1 Postintegration Latency: Implications for the Development of New Therapeutic Strategies. *Retrovirology* **2009**, *6*, 111, doi:10.1186/1742-4690-6-111.
 140. Jordan, A.; Bisgrove, D.; Verdin, E. HIV Reproducibly Establishes a Latent Infection after Acute Infection of T Cells in Vitro. *EMBO J.* **2003**, *22*, 1868–1877, doi:10.1093/emboj/cdg188.
 141. de Medeiros, R.M.; Valverde-Villegas, J.M.; Junqueira, D.M.; Gräf, T.; Lindenau, J.D.; de Mello, M.G.; Vianna, P.; Almeida, S.E.M.; Chies, J.A.B. Rapid and Slow Progressors Show Increased IL-6 and IL-10 Levels in the Pre-AIDS Stage of HIV Infection. *PLoS One* **2016**, *11*, e0156163, doi:10.1371/journal.pone.0156163.
 142. Wendel, B.S.; Fu, Y.; He, C.; Hernandez, S.M.; Qu, M.; Zhang, Z.; Jiang, Y.; Han, X.; Xu, J.; Ding, H.; et al. Rapid HIV Progression Is Associated with Extensive Ongoing Somatic Hypermutation. *J Immunol* **2020**, *205*, 587–594, doi:10.4049/jimmunol.1901161.
 143. Gonzalo-Gil, E.; Ikediobi, U.; Sutton, R.E. Mechanisms of Virologic Control and Clinical Characteristics of HIV+ Elite/Viremic Controllers. *Yale J Biol Med* **2017**, *90*, 245–259.
 144. Zerbato, J.M.; McMahon, D.K.; Sobolewski, M.D.; Mellors, J.W.; Sluis-Cremer, N. Naive CD4+ T Cells Harbor a Large Inducible Reservoir of Latent, Replication-Competent Human Immunodeficiency Virus Type 1. *Clin Infect Dis* **2019**, *69*, 1919–1925, doi:10.1093/cid/ciz108.
 145. Hiener, B.; Horsburgh, B.A.; Eden, J.-S.; Barton, K.; Schlub, T.E.; Lee, E.; von Stockenstrom, S.; Odevall, L.; Milush, J.M.; Liegler, T.; et al. Identification of Genetically Intact HIV-1 Proviruses in Specific CD4+ T Cells from Effectively Treated Participants. *Cell Rep* **2017**, *21*, 813–822, doi:10.1016/j.celrep.2017.09.081.
 146. Lee, G.Q.; Orlova-Fink, N.; Einkauf, K.; Chowdhury, F.Z.; Sun, X.; Harrington, S.; Kuo, H.-H.; Hua, S.; Chen, H.-R.; Ouyang, Z.; et al. Clonal Expansion of Genome-Intact HIV-1 in Functionally Polarized Th1 CD4+ T Cells. *J Clin Invest* **2017**, *127*, 2689–2696, doi:10.1172/JCI93289.
 147. Gosselin, A.; Wiche Salinas, T.R.; Planas, D.; Wacleche, V.S.; Zhang, Y.; Fromentin, R.; Chomont, N.; Cohen, É.A.; Shacklett, B.; Mehraj, V.; et al. HIV Persists in CCR6+CD4+ T Cells from Colon and Blood during Antiretroviral Therapy. *AIDS* **2017**, *31*, 35–48, doi:10.1097/QAD.0000000000001309.
 148. Muranski, P.; Restifo, N.P. Essentials of Th17 Cell Commitment and Plasticity. *Blood* **2013**, *121*, 2402–2414, doi:10.1182/blood-2012-09-378653.
 149. Darcis, G.; Berkhout, B.; Pasternak, A.O. The Quest for Cellular Markers of HIV Reservoirs: Any Color You Like. *Front Immunol* **2019**, *10*, 2251, doi:10.3389/fimmu.2019.02251.
 150. Iglesias-Ussel, M.; Vandergeeten, C.; Marchionni, L.; Chomont, N.; Romerio, F. High Levels of CD2 Expression Identify HIV-1 Latently Infected Resting Memory CD4+ T Cells in Virally Suppressed Subjects. *J Virol* **2013**, *87*, 9148–9158, doi:10.1128/JVI.01297-13.
 151. Romagnani, S.; Annunziato, F.; Manetti, R.; Almerigogna, F.; Biagiotti, R.; Giudizi, M.G.; Ravina, A.; Giannò, V.; Tomasévic, L.; Maggi, E. Role for CD30 in HIV Expression. *Immunol Lett* **1996**, *51*, 83–88, doi:10.1016/0165-2478(96)02559-x.
 152. Hogan, L.E.; Vasquez, J.; Hobbs, K.S.; Hanhauser, E.; Aguilar-Rodriguez, B.; Hussien, R.; Thanh, C.; Gibson, E.A.; Carvidi, A.B.; Smith, L.C.B.; et al. Increased HIV-1 Transcriptional Activity and Infectious Burden in Peripheral Blood and Gut-Associated CD4+ T Cells Expressing CD30. *PLoS Pathog* **2018**, *14*, e1006856, doi:10.1371/journal.ppat.1006856.
 153. Darcis, G.; Kootstra, N.A.; Hooibrink, B.; van Montfort, T.; Maurer, I.; Groen, K.; Jurriaans, S.; Bakker, M.; van Lint, C.; Berkhout, B.; et al. CD32+CD4+ T Cells Are Highly Enriched for HIV DNA and Can Support Transcriptional Latency. *Cell Rep* **2020**, *30*, 2284–2296.e3, doi:10.1016/j.celrep.2020.01.071.
 154. Descours, B.; Petitjean, G.; López-Zaragoza, J.-L.; Bruel, T.; Raffel, R.; Psomas, C.; Reynes, J.; Lacabaratz, C.; Levy, Y.; Schwartz, O.; et al. CD32a Is a Marker of a CD4 T-Cell HIV Reservoir Harbouring Replication-Competent Proviruses. *Nature* **2017**, *advance online publication*, doi:10.1038/nature21710.

155. Badia, R.; Ballana, E.; Castellví, M.; García-Vidal, E.; Pujantell, M.; Clotet, B.; Prado, J.G.; Puig, J.; Martínez, M.A.; Riveira-Muñoz, E.; et al. CD32 Expression Is Associated to T-Cell Activation and Is Not a Marker of the HIV-1 Reservoir. *Nat Commun* **2018**, *9*, 2739, doi:10.1038/s41467-018-05157-w.
156. Bertagnolli, L.N.; White, J.A.; Simonetti, F.R.; Beg, S.A.; Lai, J.; Tomescu, C.; Murray, A.J.; Antar, A.A.R.; Zhang, H.; Margolick, J.B.; et al. The Role of CD32 during HIV-1 Infection. *Nature* **2018**, *561*, E17–E19, doi:10.1038/s41586-018-0494-3.
157. Kumar, A.; Abbas, W.; Herbein, G. HIV-1 Latency in Monocytes/Macrophages. *Viruses* **2014**, *6*, 1837–1860, doi:10.3390/v6041837.
158. Triques, K.; Stevenson, M. Characterization of Restrictions to Human Immunodeficiency Virus Type 1 Infection of Monocytes. *J Virol* **2004**, *78*, 5523–5527, doi:10.1128/jvi.78.10.5523-5527.2004.
159. Orenstein, J.M.; Fox, C.; Wahl, S.M. Macrophages as a Source of HIV during Opportunistic Infections. *Science* **1997**, *276*, 1857–1861, doi:10.1126/science.276.5320.1857.
160. Siliciano, R.F.; Greene, W.C. HIV Latency. *Cold Spring Harb Perspect Med* **2011**, *1*, a007096, doi:10.1101/cshperspect.a007096.
161. Coleman, C.M.; Wu, L. HIV Interactions with Monocytes and Dendritic Cells: Viral Latency and Reservoirs. *Retrovirology* **2009**, *6*, 51, doi:10.1186/1742-4690-6-51.
162. Dong, C.; Kwas, C.; Wu, L. Transcriptional Restriction of Human Immunodeficiency Virus Type 1 Gene Expression in Undifferentiated Primary Monocytes. *J. Virol.* **2009**, *83*, 3518–3527, doi:10.1128/JVI.02665-08.
163. Gibellini, D.; Borderi, M.; De Crignis, E.; Cicola, R.; Cimatti, L.; Vitone, F.; Chiodo, F.; Re, M.C. HIV-1 DNA Load Analysis in Peripheral Blood Lymphocytes and Monocytes from Naïve and HAART-Treated Individuals. *J Infect* **2008**, *56*, 219–225, doi:10.1016/j.jinf.2008.01.001.
164. Josefsson, L.; von Stockenström, S.; Faria, N.R.; Sinclair, E.; Bacchetti, P.; Killian, M.; Epling, L.; Tan, A.; Ho, T.; Lemey, P.; et al. The HIV-1 Reservoir in Eight Patients on Long-Term Suppressive Antiretroviral Therapy Is Stable with Few Genetic Changes over Time. *Proc Natl Acad Sci U S A* **2013**, *110*, E4987–E4996, doi:10.1073/pnas.1308313110.
165. Clapham, P.R.; Blanc, D.; Weiss, R.A. Specific Cell Surface Requirements for the Infection of CD4-Positive Cells by Human Immunodeficiency Virus Types 1 and 2 and by Simian Immunodeficiency Virus. *Virology* **1991**, *181*, 703–715, doi:10.1016/0042-6822(91)90904-p.
166. Veenstra, M.; León-Rivera, R.; Li, M.; Gama, L.; Clements, J.E.; Berman, J.W. Mechanisms of CNS Viral Seeding by HIV+ CD14+ CD16+ Monocytes: Establishment and Reseeding of Viral Reservoirs Contributing to HIV-Associated Neurocognitive Disorders. *mBio* **2017**, *8*, e01280-17, doi:10.1128/mBio.01280-17.
167. Mlcochova, P.; Sutherland, K.A.; Watters, S.A.; Bertoli, C.; de Bruin, R.A.; Rehwinkel, J.; Neil, S.J.; Lenzi, G.M.; Kim, B.; Khwaja, A.; et al. A G1-like State Allows HIV-1 to Bypass SAMHD1 Restriction in Macrophages. *EMBO J* **2017**, *36*, 604–616, doi:10.15252/embj.201696025.
168. Ko, A.; Kang, G.; Hattler, J.B.; Galadima, H.I.; Zhang, J.; Li, Q.; Kim, W.-K. Macrophages but Not Astrocytes Harbor HIV DNA in the Brains of HIV-1-Infected Aviremic Individuals on Suppressive Antiretroviral Therapy. *J Neuroimmune Pharmacol* **2019**, *14*, 110–119, doi:10.1007/s11481-018-9809-2.
169. Réu, P.; Khosravi, A.; Bernard, S.; Mold, J.E.; Salehpour, M.; Alkass, K.; Perl, S.; Tisdale, J.; Possnert, G.; Druid, H.; et al. The Lifespan and Turnover of Microglia in the Human Brain. *Cell Rep* **2017**, *20*, 779–784, doi:10.1016/j.celrep.2017.07.004.
170. Sutherland, E.J.; Brew, B.J. Human Immunodeficiency Virus and the Nervous System. *Neurol Clin* **2018**, *36*, 751–765, doi:10.1016/j.ncl.2018.07.002.
171. Wallet, C.; De Rovere, M.; Van Assche, J.; Daouad, F.; De Wit, S.; Gautier, V.; Mallon, P.W.G.; Marcello, A.; Van Lint, C.; Rohr, O.; et al. Microglial Cells: The Main HIV-1 Reservoir in the Brain. *Front Cell Infect Microbiol* **2019**, *9*, 362, doi:10.3389/fcimb.2019.00362.
172. Rohr, O.; Marban, C.; Aunis, D.; Schaeffer, E. Regulation of HIV-1 Gene Transcription: From Lymphocytes to Microglial Cells. *J Leukoc Biol* **2003**, *74*, 736–749, doi:10.1189/jlb.0403180.

-
173. Marban, C.; Suzanne, S.; Dequiedt, F.; de Walque, S.; Redel, L.; Van Lint, C.; Aunis, D.; Rohr, O. Recruitment of Chromatin-Modifying Enzymes by CTIP2 Promotes HIV-1 Transcriptional Silencing. *EMBO J* **2007**, *26*, 412–423, doi:10.1038/sj.emboj.7601516.
174. Williams, S.A.; Chen, L.-F.; Kwon, H.; Ruiz-Jarabo, C.M.; Verdin, E.; Greene, W.C. NF-KappaB P50 Promotes HIV Latency through HDAC Recruitment and Repression of Transcriptional Initiation. *EMBO J* **2006**, *25*, 139–149, doi:10.1038/sj.emboj.7600900.
175. He, G.; Margolis, D.M. Counterregulation of Chromatin Deacetylation and Histone Deacetylase Occupancy at the Integrated Promoter of Human Immunodeficiency Virus Type 1 (HIV-1) by the HIV-1 Repressor YY1 and HIV-1 Activator Tat. *Mol Cell Biol* **2002**, *22*, 2965–2973, doi:10.1128/MCB.22.9.2965-2973.2002.
176. Friedman, J.; Cho, W.-K.; Chu, C.K.; Keedy, K.S.; Archin, N.M.; Margolis, D.M.; Karn, J. Epigenetic Silencing of HIV-1 by the Histone H3 Lysine 27 Methyltransferase Enhancer of Zeste 2. *J. Virol.* **2011**, *85*, 9078–9089, doi:10.1128/JVI.00836-11.
177. Kauder, S.E.; Bosque, A.; Lindqvist, A.; Planelles, V.; Verdin, E. Epigenetic Regulation of HIV-1 Latency by Cytosine Methylation. *PLoS Pathog* **2009**, *5*, e1000495, doi:10.1371/journal.ppat.1000495.
178. Schröder, A.R.W.; Shinn, P.; Chen, H.; Berry, C.; Ecker, J.R.; Bushman, F. HIV-1 Integration in the Human Genome Favors Active Genes and Local Hotspots. *Cell* **2002**, *110*, 521–529, doi:10.1016/s0092-8674(02)00864-4.
179. Ciuffi, A.; Llano, M.; Poeschla, E.; Hoffmann, C.; Leipzig, J.; Shinn, P.; Ecker, J.R.; Bushman, F. A Role for LEDGF/P75 in Targeting HIV DNA Integration. *Nat Med* **2005**, *11*, 1287–1289, doi:10.1038/nm1329.
180. Churchill, M.J.; Cowley, D.J.; Wesselingh, S.L.; Gorry, P.R.; Gray, L.R. HIV-1 Transcriptional Regulation in the Central Nervous System and Implications for HIV Cure Research. *J Neurovirol* **2015**, *21*, 290–300, doi:10.1007/s13365-014-0271-5.
181. Vorster, P.J.; Guo, J.; Yoder, A.; Wang, W.; Zheng, Y.; Xu, X.; Yu, D.; Spear, M.; Wu, Y. LIM Kinase 1 Modulates Cortical Actin and CXCR4 Cycling and Is Activated by HIV-1 to Initiate Viral Infection. *J Biol Chem* **2011**, *286*, 12554–12564, doi:10.1074/jbc.M110.182238.
182. Ahr, B.; Robert-Hebmann, V.; Devaux, C.; Biard-Piechaczyk, M. Apoptosis of Uninfected Cells Induced by HIV Envelope Glycoproteins. *Retrovirology* **2004**, *1*, 12, doi:10.1186/1742-4690-1-12.
183. Diehl, N.; Schaal, H. Make Yourself at Home: Viral Hijacking of the PI3K/Akt Signaling Pathway. *Viruses* **2013**, *5*, 3192–3212, doi:10.3390/v5123192.
184. Contreras, X.; Barboric, M.; Lenasi, T.; Peterlin, B.M. HMBA Releases P-TEFb from HEXIM1 and 7SK SnRNA via PI3K/Akt and Activates HIV Transcription. *PLoS Pathog* **2007**, *3*, 1459–1469, doi:10.1371/journal.ppat.0030146.
185. Zhang, H.-S.; Sang, W.-W.; Ruan, Z.; Wang, Y.-O. Akt/Nox2/NF-KB Signaling Pathway Is Involved in Tat-Induced HIV-1 Long Terminal Repeat (LTR) Transactivation. *Arch Biochem Biophys* **2011**, *505*, 266–272, doi:10.1016/j.abb.2010.10.018.
186. Zhang, H.-S.; Liu, Y.; Wu, T.-C.; Du, G.-Y.; Zhang, F.-J. EZH2 Phosphorylation Regulates Tat-Induced HIV-1 Transactivation via ROS/Akt Signaling Pathway. *FEBS Lett* **2015**, *589*, 4106–4111, doi:10.1016/j.febslet.2015.11.033.
187. Margolis, D.M.; Koup, R.A.; Ferrari, G. HIV Antibodies for Treatment of HIV Infection. *Immunol Rev* **2017**, *275*, 313–323, doi:10.1111/imr.12506.
188. Geskus, R.B.; Prins, M.; Hubert, J.-B.; Miedema, F.; Berkhout, B.; Rouzioux, C.; Delfraissy, J.-F.; Meyer, L. The HIV RNA Setpoint Theory Revisited. *Retrovirology* **2007**, *4*, 65, doi:10.1186/1742-4690-4-65.
189. Sainz, B.; LaMarca, H.L.; Garry, R.F.; Morris, C.A. Synergistic Inhibition of Human Cytomegalovirus Replication by Interferon-Alpha/Beta and Interferon-Gamma. *Virol. J.* **2005**, *2*, 14, doi:10.1186/1743-422X-2-14.

190. Panos, G.; Samonis, G.; Alexiou, V.G.; Kavarnou, G.A.; Charatsis, G.; Falagas, M.E. Mortality and Morbidity of HIV Infected Patients Receiving HAART: A Cohort Study. *Curr. HIV Res.* **2008**, *6*, 257–260.
191. Lifson, A.R.; Grund, B.; Gardner, E.M.; Kaplan, R.; Denning, E.; Engen, N.; Carey, C.L.; Chen, F.; Dao, S.; Florence, E.; et al. Improved Quality of Life with Immediate versus Deferred Initiation of Antiretroviral Therapy in Early Asymptomatic HIV Infection. *AIDS* **2017**, *31*, 953–963, doi:10.1097/QAD.0000000000001417.
192. Chen, W.-T.; Shiu, C.-S.; Yang, J.P.; Simoni, J.M.; Fredriksen-Goldsen, K.I.; Lee, T.S.-H.; Zhao, H. Antiretroviral Therapy (ART) Side Effect Impacted on Quality of Life, and Depressive Symptomatology: A Mixed-Method Study. *J AIDS Clin Res* **2013**, *4*, 218, doi:10.4172/2155-6113.1000218.
193. Montessori, V.; Press, N.; Harris, M.; Akagi, L.; Montaner, J.S.G. Adverse Effects of Antiretroviral Therapy for HIV Infection. *CMAJ* **2004**, *170*, 229–238.
194. d'Arminio Monforte, A.; Lepri, A.C.; Rezza, G.; Pezzotti, P.; Antinori, A.; Phillips, A.N.; Angarano, G.; Colangeli, V.; De Luca, A.; Ippolito, G.; et al. Insights into the Reasons for Discontinuation of the First Highly Active Antiretroviral Therapy (HAART) Regimen in a Cohort of Antiretroviral Naïve Patients. I.CO.N.A. Study Group. Italian Cohort of Antiretroviral-Naïve Patients. *AIDS* **2000**, *14*, 499–507.
195. Eakle, R.; Venter, F.; Rees, H. Pre-Exposure Prophylaxis (PrEP) in an Era of Stalled HIV Prevention: Can It Change the Game? *Retrovirology* **2018**, *15*, 29, doi:10.1186/s12977-018-0408-3.
196. Fonner, V.A.; Dalglish, S.L.; Kennedy, C.E.; Baggaley, R.; O'Reilly, K.R.; Koechlin, F.M.; Rodolph, M.; Hodges-Mameletzis, I.; Grant, R.M. Effectiveness and Safety of Oral HIV Preexposure Prophylaxis for All Populations. *AIDS* **2016**, *30*, 1973–1983, doi:10.1097/QAD.0000000000001145.
197. Cottrell, M.L.; Yang, K.H.; Prince, H.M.A.; Sykes, C.; White, N.; Malone, S.; Dellon, E.S.; Madanick, R.D.; Shaheen, N.J.; Hudgens, M.G.; et al. A Translational Pharmacology Approach to Predicting Outcomes of Preexposure Prophylaxis Against HIV in Men and Women Using Tenofovir Disoproxil Fumarate With or Without Emtricitabine. *J Infect Dis* **2016**, *214*, 55–64, doi:10.1093/infdis/jiw077.
198. Hamlyn, E.; Ewings, F.M.; Porter, K.; Cooper, D.A.; Tambussi, G.; Schechter, M.; Pedersen, C.; Okulicz, J.F.; McClure, M.; Babiker, A.; et al. Plasma HIV Viral Rebound Following Protocol-Indicated Cessation of ART Commenced in Primary and Chronic HIV Infection. *PLoS ONE* **2012**, *7*, e43754, doi:10.1371/journal.pone.0043754.
199. Le Douce, V.; Herbein, G.; Rohr, O.; Schwartz, C. Molecular Mechanisms of HIV-1 Persistence in the Monocyte-Macrophage Lineage. *Retrovirology* **2010**, *7*, 32, doi:10.1186/1742-4690-7-32.
200. Abbas, W.; Tariq, M.; Iqbal, M.; Kumar, A.; Herbein, G. Eradication of HIV-1 from the Macrophage Reservoir: An Uncertain Goal? *Viruses* **2015**, *7*, 1578–1598, doi:10.3390/v7041578.
201. Churchill, M.J.; Deeks, S.G.; Margolis, D.M.; Siliciano, R.F.; Swanstrom, R. HIV Reservoirs: What, Where and How to Target Them. *Nat. Rev. Microbiol.* **2016**, *14*, 55–60, doi:10.1038/nrmicro.2015.5.
202. Darcis, G.; Van Driessche, B.; Van Lint, C. HIV Latency: Should We Shock or Lock? *Trends Immunol.* **2017**, *38*, 217–228, doi:10.1016/j.it.2016.12.003.
203. Kumar, A.; Darcis, G.; Van Lint, C.; Herbein, G. Epigenetic Control of HIV-1 Post Integration Latency: Implications for Therapy. *Clinical Epigenetics* **2015**, *7*, 103, doi:10.1186/s13148-015-0137-6.
204. Khan, S.; Iqbal, M.; Tariq, M.; Baig, S.M.; Abbas, W. Epigenetic Regulation of HIV-1 Latency: Focus on Polycomb Group (PcG) Proteins. *Clin Epigenetics* **2018**, *10*, 14, doi:10.1186/s13148-018-0441-z.
205. Nehme, Z.; Pasquereau, S.; Herbein, G. Control of Viral Infections by Epigenetic-Targeted Therapy. *Clin Epigenetics* **2019**, *11*, doi:10.1186/s13148-019-0654-9.
206. Deeks, S.G. HIV: Shock and Kill. *Nature* **2012**, *487*, 439–440, doi:10.1038/487439a.

-
207. Archin, N.M.; Liberty, A.L.; Kashuba, A.D.; Choudhary, S.K.; Kuruc, J.D.; Crooks, A.M.; Parker, D.C.; Anderson, E.M.; Kearney, M.F.; Strain, M.C.; et al. Administration of Vorinostat Disrupts HIV-1 Latency in Patients on Antiretroviral Therapy. *Nature* **2012**, *487*, 482–485, doi:10.1038/nature11286.
 208. Reuse, S.; Calao, M.; Kabeya, K.; Guiguen, A.; Gatot, J.-S.; Quivy, V.; Vanhulle, C.; Lamine, A.; Vaira, D.; Demonte, D.; et al. Synergistic Activation of HIV-1 Expression by Deacetylase Inhibitors and Prostratin: Implications for Treatment of Latent Infection. *PLoS ONE* **2009**, *4*, e6093, doi:10.1371/journal.pone.0006093.
 209. Thorlund, K.; Horwitz, M.S.; Fife, B.T.; Lester, R.; Cameron, D.W. Landscape Review of Current HIV ‘Kick and Kill’ Cure Research - Some Kicking, Not Enough Killing. *BMC Infectious Diseases* **2017**, *17*, 595, doi:10.1186/s12879-017-2683-3.
 210. Park, S.Y.; Kim, K.-C.; Hong, K.-J.; Kim, S.S.; Choi, B.-S. Histone Deacetylase Inhibitor SAHA Induces a Synergistic HIV-1 Reactivation by 12-O-Tetradecanoylphorbol-13-Acetate in Latently Infected Cells. *Intervirology* **2013**, *56*, 242–248, doi:10.1159/000350563.
 211. Ait-Ammar, A.; Kula, A.; Darcis, G.; Verdikt, R.; De Wit, S.; Gautier, V.; Mallon, P.W.G.; Marcello, A.; Rohr, O.; Van Lint, C. Current Status of Latency Reversing Agents Facing the Heterogeneity of HIV-1 Cellular and Tissue Reservoirs. *Front. Microbiol.* **2020**, *10*, doi:10.3389/fmicb.2019.03060.
 212. Das, A.T.; Harwig, A.; Berkhout, B. The HIV-1 Tat Protein Has a Versatile Role in Activating Viral Transcription. *J Virol* **2011**, *85*, 9506–9516, doi:10.1128/JVI.00650-11.
 213. Mousseau, G.; Clementz, M.A.; Bakeman, W.N.; Nagarsheth, N.; Cameron, M.; Shi, J.; Baran, P.; Fromentin, R.; Chomont, N.; Valente, S.T. An Analog of the Natural Steroidal Alkaloid Cortistatin A Potently Suppresses Tat-Dependent HIV Transcription. *Cell Host Microbe* **2012**, *12*, 97–108, doi:10.1016/j.chom.2012.05.016.
 214. Chugh, P.; Bradel-Tretheway, B.; Monteiro-Filho, C.M.R.; Planelles, V.; Maggirwar, S.B.; Dewhurst, S.; Kim, B. Akt Inhibitors as an HIV-1 Infected Macrophage-Specific Anti-Viral Therapy. *Retrovirology* **2008**, *5*, 11, doi:10.1186/1742-4690-5-11.
 215. Chun, T.-W.; Nickle, D.C.; Justement, J.S.; Large, D.; Semerjian, A.; Curlin, M.E.; O’Shea, M.A.; Hallahan, C.W.; Daucher, M.; Ward, D.J.; et al. HIV-Infected Individuals Receiving Effective Antiviral Therapy for Extended Periods of Time Continually Replenish Their Viral Reservoir. *J Clin Invest* **2005**, *115*, 3250–3255, doi:10.1172/JCI26197.
 216. Coronaviridae Study Group of the International Committee on Taxonomy of Viruses The Species Severe Acute Respiratory Syndrome-Related Coronavirus: Classifying 2019-NCoV and Naming It SARS-CoV-2. *Nat Microbiol* **2020**, *5*, 536–544, doi:10.1038/s41564-020-0695-z.
 217. Corman, V.M.; Muth, D.; Niemeyer, D.; Drosten, C. Hosts and Sources of Endemic Human Coronaviruses. *Adv Virus Res* **2018**, *100*, 163–188, doi:10.1016/bs.aivir.2018.01.001.
 218. Su, S.; Wong, G.; Shi, W.; Liu, J.; Lai, A.C.K.; Zhou, J.; Liu, W.; Bi, Y.; Gao, G.F. Epidemiology, Genetic Recombination, and Pathogenesis of Coronaviruses. *Trends Microbiol* **2016**, *24*, 490–502, doi:10.1016/j.tim.2016.03.003.
 219. Forni, D.; Cagliani, R.; Clerici, M.; Sironi, M. Molecular Evolution of Human Coronavirus Genomes. *Trends Microbiol* **2017**, *25*, 35–48, doi:10.1016/j.tim.2016.09.001.
 220. Gorbalenya, A.E.; Enjuanes, L.; Ziebuhr, J.; Snijder, E.J. Nidovirales: Evolving the Largest RNA Virus Genome. *Virus Res* **2006**, *117*, 17–37, doi:10.1016/j.virusres.2006.01.017.
 221. Lang, Y.; Li, W.; Li, Z.; Koerhuis, D.; van den Burg, A.C.S.; Rozemuller, E.; Bosch, B.-J.; van Kuppeveld, F.J.M.; Boons, G.-J.; Huizinga, E.G.; et al. Coronavirus Hemagglutinin-Esterase and Spike Proteins Coevolve for Functional Balance and Optimal Virion Avidity. *Proc Natl Acad Sci U S A* **2020**, *117*, 25759–25770, doi:10.1073/pnas.2006299117.
 222. Perlman, S.; Netland, J. Coronaviruses Post-SARS: Update on Replication and Pathogenesis. *Nat Rev Microbiol* **2009**, *7*, 439–450, doi:10.1038/nrmicro2147.
 223. Masters, P.S. The Molecular Biology of Coronaviruses. *Adv Virus Res* **2006**, *66*, 193–292, doi:10.1016/S0065-3527(06)66005-3.

224. Liu, D.X.; Fung, T.S.; Chong, K.K.-L.; Shukla, A.; Hilgenfeld, R. Accessory Proteins of SARS-CoV and Other Coronaviruses. *Antiviral Res* **2014**, *109*, 97–109, doi:10.1016/j.antiviral.2014.06.013.
225. McIntosh, K. Coronaviruses: A Comparative Review. In Proceedings of the Current Topics in Microbiology and Immunology / Ergebnisse der Mikrobiologie und Immunitätsforschung; Arber, W., Haas, R., Henle, W., Hofschneider, P.H., Jerne, N.K., Koldovský, P., Koprowski, H., Maaløe, O., Rott, R., Schweiger, H.G., Sela, M., Syruček, L., Vogt, P.K., Wecker, E., Eds.; Springer: Berlin, Heidelberg, 1974; pp. 85–129.
226. Becker, W.B.; McIntosh, K.; Dees, J.H.; Chanock, R.M. Morphogenesis of Avian Infectious Bronchitis Virus and a Related Human Virus (Strain 229E). *J Virol* **1967**, *1*, 1019–1027.
227. Bosch, B.J.; van der Zee, R.; de Haan, C.A.M.; Rottier, P.J.M. The Coronavirus Spike Protein Is a Class I Virus Fusion Protein: Structural and Functional Characterization of the Fusion Core Complex. *J Virol* **2003**, *77*, 8801–8811, doi:10.1128/jvi.77.16.8801-8811.2003.
228. Millet, J.K.; Whittaker, G.R. Host Cell Proteases: Critical Determinants of Coronavirus Tropism and Pathogenesis. *Virus Res* **2015**, *202*, 120–134, doi:10.1016/j.virusres.2014.11.021.
229. Cheng, Y.-W.; Chao, T.-L.; Li, C.-L.; Chiu, M.-F.; Kao, H.-C.; Wang, S.-H.; Pang, Y.-H.; Lin, C.-H.; Tsai, Y.-M.; Lee, W.-H.; et al. Furin Inhibitors Block SARS-CoV-2 Spike Protein Cleavage to Suppress Virus Production and Cytopathic Effects. *Cell Rep* **2020**, *33*, 108254, doi:10.1016/j.celrep.2020.108254.
230. Seidah, N.G.; Prat, A. The Biology and Therapeutic Targeting of the Proprotein Convertases. *Nat Rev Drug Discov* **2012**, *11*, 367–383, doi:10.1038/nrd3699.
231. Belouzard, S.; Millet, J.K.; Licitra, B.N.; Whittaker, G.R. Mechanisms of Coronavirus Cell Entry Mediated by the Viral Spike Protein. *Viruses* **2012**, *4*, 1011–1033, doi:10.3390/v4061011.
232. Zeng, Q.; Langereis, M.A.; van Vliet, A.L.W.; Huizinga, E.G.; de Groot, R.J. Structure of Coronavirus Hemagglutinin-Esterase Offers Insight into Corona and Influenza Virus Evolution. *Proc Natl Acad Sci U S A* **2008**, *105*, 9065–9069, doi:10.1073/pnas.0800502105.
233. Schoeman, D.; Fielding, B.C. Coronavirus Envelope Protein: Current Knowledge. *Virology Journal* **2019**, *16*, 69, doi:10.1186/s12985-019-1182-0.
234. Wang, Y.; Liu, L. The Membrane Protein of Severe Acute Respiratory Syndrome Coronavirus Functions as a Novel Cytosolic Pathogen-Associated Molecular Pattern To Promote Beta Interferon Induction via a Toll-Like-Receptor-Related TRAF3-Independent Mechanism. *mBio* **2016**, *7*, e01872-01815, doi:10.1128/mBio.01872-15.
235. Venkatagopalan, P.; Daskalova, S.M.; Lopez, L.A.; Dolezal, K.A.; Hogue, B.G. Coronavirus Envelope (E) Protein Remains at the Site of Assembly. *Virology* **2015**, *478*, 75–85, doi:10.1016/j.virol.2015.02.005.
236. DeDiego, M.L.; Alvarez, E.; Almazán, F.; Rejas, M.T.; Lamirande, E.; Roberts, A.; Shieh, W.-J.; Zaki, S.R.; Subbarao, K.; Enjuanes, L. A Severe Acute Respiratory Syndrome Coronavirus That Lacks the E Gene Is Attenuated in Vitro and in Vivo. *J Virol* **2007**, *81*, 1701–1713, doi:10.1128/JVI.01467-06.
237. Nieto-Torres, J.L.; Dediego, M.L.; Alvarez, E.; Jiménez-Guardeño, J.M.; Regla-Nava, J.A.; Llorente, M.; Kremer, L.; Shuo, S.; Enjuanes, L. Subcellular Location and Topology of Severe Acute Respiratory Syndrome Coronavirus Envelope Protein. *Virology* **2011**, *415*, 69–82, doi:10.1016/j.virol.2011.03.029.
238. Chang, C.; Lo, S.-C.; Wang, Y.-S.; Hou, M.-H. Recent Insights into the Development of Therapeutics against Coronavirus Diseases by Targeting N Protein. *Drug Discov Today* **2016**, *21*, 562–572, doi:10.1016/j.drudis.2015.11.015.
239. de Haan, C.A.M.; Rottier, P.J.M. Molecular Interactions in the Assembly of Coronaviruses. *Adv Virus Res* **2005**, *64*, 165–230, doi:10.1016/S0065-3527(05)64006-7.
240. McBride, R.; van Zyl, M.; Fielding, B.C. The Coronavirus Nucleocapsid Is a Multifunctional Protein. *Viruses* **2014**, *6*, 2991–3018, doi:10.3390/v6082991.

-
241. Siu, Y.L.; Teoh, K.T.; Lo, J.; Chan, C.M.; Kien, F.; Escriou, N.; Tsao, S.W.; Nicholls, J.M.; Altmeyer, R.; Peiris, J.S.M.; et al. The M, E, and N Structural Proteins of the Severe Acute Respiratory Syndrome Coronavirus Are Required for Efficient Assembly, Trafficking, and Release of Virus-like Particles. *J Virol* **2008**, *82*, 11318–11330, doi:10.1128/JVI.01052-08.
242. Ruch, T.R.; Machamer, C.E. The Coronavirus E Protein: Assembly and Beyond. *Viruses* **2012**, *4*, 363–382, doi:10.3390/v4030363.
243. Rohaim, M.A.; El Naggar, R.F.; Clayton, E.; Munir, M. Structural and Functional Insights into Non-Structural Proteins of Coronaviruses. *Microb Pathog* **2021**, *150*, 104641, doi:10.1016/j.micpath.2020.104641.
244. Smith, E.C.; Blanc, H.; Surdel, M.C.; Vignuzzi, M.; Denison, M.R. Coronaviruses Lacking Exoribonuclease Activity Are Susceptible to Lethal Mutagenesis: Evidence for Proofreading and Potential Therapeutics. *PLoS Pathog* **2013**, *9*, e1003565, doi:10.1371/journal.ppat.1003565.
245. Matthews, K.L.; Coleman, C.M.; van der Meer, Y.; Snijder, E.J.; Frieman, M.B. The ORF4b-Encoded Accessory Proteins of Middle East Respiratory Syndrome Coronavirus and Two Related Bat Coronaviruses Localize to the Nucleus and Inhibit Innate Immune Signalling. *J Gen Virol* **2014**, *95*, 874–882, doi:10.1099/vir.0.062059-0.
246. Yeager, C.L.; Ashmun, R.A.; Williams, R.K.; Cardellichio, C.B.; Shapiro, L.H.; Look, A.T.; Holmes, K.V. Human Aminopeptidase N Is a Receptor for Human Coronavirus 229E. *Nature* **1992**, *357*, 420–422, doi:10.1038/357420a0.
247. Li, W.; Moore, M.J.; Vasilieva, N.; Sui, J.; Wong, S.K.; Berne, M.A.; Somasundaran, M.; Sullivan, J.L.; Luzuriaga, K.; Greenough, T.C.; et al. Angiotensin-Converting Enzyme 2 Is a Functional Receptor for the SARS Coronavirus. *Nature* **2003**, *426*, 450–454, doi:10.1038/nature02145.
248. Hofmann, H.; Pyrc, K.; van der Hoek, L.; Geier, M.; Berkhout, B.; Pöhlmann, S. Human Coronavirus NL63 Employs the Severe Acute Respiratory Syndrome Coronavirus Receptor for Cellular Entry. *Proc Natl Acad Sci U S A* **2005**, *102*, 7988–7993, doi:10.1073/pnas.0409465102.
249. Raj, V.S.; Mou, H.; Smits, S.L.; Dekkers, D.H.W.; Müller, M.A.; Dijkman, R.; Muth, D.; Demmers, J.A.A.; Zaki, A.; Fouchier, R.A.M.; et al. Dipeptidyl Peptidase 4 Is a Functional Receptor for the Emerging Human Coronavirus-EMC. *Nature* **2013**, *495*, 251–254, doi:10.1038/nature12005.
250. Jin, Y.; Yang, H.; Ji, W.; Wu, W.; Chen, S.; Zhang, W.; Duan, G. Virology, Epidemiology, Pathogenesis, and Control of COVID-19. *Viruses* **2020**, *12*, 372, doi:10.3390/v12040372.
251. Kamitani, W.; Narayanan, K.; Huang, C.; Lokugamage, K.; Ikegami, T.; Ito, N.; Kubo, H.; Makino, S. Severe Acute Respiratory Syndrome Coronavirus Nsp1 Protein Suppresses Host Gene Expression by Promoting Host mRNA Degradation. *Proc Natl Acad Sci U S A* **2006**, *103*, 12885–12890, doi:10.1073/pnas.0603144103.
252. Snijder, E.J.; Decroly, E.; Ziebuhr, J. The Nonstructural Proteins Directing Coronavirus RNA Synthesis and Processing. *Adv Virus Res* **2016**, *96*, 59–126, doi:10.1016/bs.aivir.2016.08.008.
253. Tvarogová, J.; Madhugiri, R.; Bylapudi, G.; Ferguson, L.J.; Karl, N.; Ziebuhr, J. Identification and Characterization of a Human Coronavirus 229E Nonstructural Protein 8-Associated RNA 3'-Terminal Adenylyltransferase Activity. *J Virol* **2019**, *93*, e00291-19, doi:10.1128/JVI.00291-19.
254. Eckerle, L.D.; Lu, X.; Sperry, S.M.; Choi, L.; Denison, M.R. High Fidelity of Murine Hepatitis Virus Replication Is Decreased in Nsp14 Exoribonuclease Mutants. *J Virol* **2007**, *81*, 12135–12144, doi:10.1128/JVI.01296-07.
255. Ivanov, K.A.; Ziebuhr, J. Human Coronavirus 229E Nonstructural Protein 13: Characterization of Duplex-Unwinding, Nucleoside Triphosphatase, and RNA 5'-Triphosphatase Activities. *J Virol* **2004**, *78*, 7833–7838, doi:10.1128/JVI.78.14.7833-7838.2004.
256. Chen, Y.; Su, C.; Ke, M.; Jin, X.; Xu, L.; Zhang, Z.; Wu, A.; Sun, Y.; Yang, Z.; Tien, P.; et al. Biochemical and Structural Insights into the Mechanisms of SARS Coronavirus RNA Ribose 2'-O-Methylation by Nsp16/Nsp10 Protein Complex. *PLoS Pathog* **2011**, *7*, e1002294, doi:10.1371/journal.ppat.1002294.

257. Chen, Y.; Cai, H.; Pan, J.; Xiang, N.; Tien, P.; Ahola, T.; Guo, D. Functional Screen Reveals SARS Coronavirus Nonstructural Protein Nsp14 as a Novel Cap N7 Methyltransferase. *Proc Natl Acad Sci U S A* **2009**, *106*, 3484–3489, doi:10.1073/pnas.0808790106.
258. Knoops, K.; Swett-Tapia, C.; van den Worm, S.H.E.; Te Velhuis, A.J.W.; Koster, A.J.; Mommaas, A.M.; Snijder, E.J.; Kikkert, M. Integrity of the Early Secretory Pathway Promotes, but Is Not Required for, Severe Acute Respiratory Syndrome Coronavirus RNA Synthesis and Virus-Induced Remodeling of Endoplasmic Reticulum Membranes. *J Virol* **2010**, *84*, 833–846, doi:10.1128/JVI.01826-09.
259. Reggiori, F.; Monastyrska, I.; Verheije, M.H.; Cali, T.; Ulasli, M.; Bianchi, S.; Bernasconi, R.; de Haan, C.A.M.; Molinari, M. Coronaviruses Hijack the LC3-I-Positive EDEMosomes, ER-Derived Vesicles Exporting Short-Lived ERAD Regulators, for Replication. *Cell Host Microbe* **2010**, *7*, 500–508, doi:10.1016/j.chom.2010.05.013.
260. Wong, H.H.; Kumar, P.; Tay, F.P.L.; Moreau, D.; Liu, D.X.; Bard, F. Genome-Wide Screen Reveals Valosin-Containing Protein Requirement for Coronavirus Exit from Endosomes. *J Virol* **2015**, *89*, 11116–11128, doi:10.1128/JVI.01360-15.
261. Li, J.; Liu, Y.; Zhang, X. Murine Coronavirus Induces Type I Interferon in Oligodendrocytes through Recognition by RIG-I and MDA5. *J Virol* **2010**, *84*, 6472–6482, doi:10.1128/JVI.00016-10.
262. Cervantes-Barragan, L.; Züst, R.; Weber, F.; Spiegel, M.; Lang, K.S.; Akira, S.; Thiel, V.; Ludewig, B. Control of Coronavirus Infection through Plasmacytoid Dendritic-Cell-Derived Type I Interferon. *Blood* **2007**, *109*, 1131–1137, doi:10.1182/blood-2006-05-023770.
263. Zhang, W.; Bailey-Elkin, B.A.; Knaap, R.C.M.; Khare, B.; Dalebout, T.J.; Johnson, G.G.; van Kasteren, P.B.; McLeish, N.J.; Gu, J.; He, W.; et al. Potent and Selective Inhibition of Pathogenic Viruses by Engineered Ubiquitin Variants. *PLoS Pathog* **2017**, *13*, e1006372, doi:10.1371/journal.ppat.1006372.
264. Clementz, M.A.; Chen, Z.; Banach, B.S.; Wang, Y.; Sun, L.; Ratia, K.; Baez-Santos, Y.M.; Wang, J.; Takayama, J.; Ghosh, A.K.; et al. Deubiquitinating and Interferon Antagonism Activities of Coronavirus Papain-like Proteases. *J Virol* **2010**, *84*, 4619–4629, doi:10.1128/JVI.02406-09.
265. Grunewald, M.E.; Chen, Y.; Kuny, C.; Maejima, T.; Lease, R.; Ferraris, D.; Aikawa, M.; Sullivan, C.S.; Perlman, S.; Fehr, A.R. The Coronavirus Macrodome Is Required to Prevent PARP-Mediated Inhibition of Virus Replication and Enhancement of IFN Expression. *PLoS Pathog* **2019**, *15*, e1007756, doi:10.1371/journal.ppat.1007756.
266. Daffis, S.; Szretter, K.J.; Schriewer, J.; Li, J.; Youn, S.; Errett, J.; Lin, T.-Y.; Schneller, S.; Züst, R.; Dong, H.; et al. 2'-O Methylation of the Viral MRNA Cap Evades Host Restriction by IFIT Family Members. *Nature* **2010**, *468*, 452–456, doi:10.1038/nature09489.
267. Kindler, E.; Gil-Cruz, C.; Spanier, J.; Li, Y.; Wilhelm, J.; Rabouw, H.H.; Züst, R.; Hwang, M.; V'kovski, P.; Stalder, H.; et al. Early Endonuclease-Mediated Evasion of RNA Sensing Ensures Efficient Coronavirus Replication. *PLoS Pathog* **2017**, *13*, e1006195, doi:10.1371/journal.ppat.1006195.
268. Deng, X.; Hackbart, M.; Mettelman, R.C.; O'Brien, A.; Mielech, A.M.; Yi, G.; Kao, C.C.; Baker, S.C. Coronavirus Nonstructural Protein 15 Mediates Evasion of DsRNA Sensors and Limits Apoptosis in Macrophages. *Proc Natl Acad Sci U S A* **2017**, *114*, E4251–E4260, doi:10.1073/pnas.1618310114.
269. Tay, M.Z.; Poh, C.M.; Rénia, L.; MacAry, P.A.; Ng, L.F.P. The Trinity of COVID-19: Immunity, Inflammation and Intervention. *Nat Rev Immunol* **2020**, *20*, 363–374, doi:10.1038/s41577-020-0311-8.
270. Channappanavar, R.; Fehr, A.R.; Vijay, R.; Mack, M.; Zhao, J.; Meyerholz, D.K.; Perlman, S. Dysregulated Type I Interferon and Inflammatory Monocyte-Macrophage Responses Cause Lethal Pneumonia in SARS-CoV-Infected Mice. *Cell Host Microbe* **2016**, *19*, 181–193, doi:10.1016/j.chom.2016.01.007.
271. Giamarellos-Bourboulis, E.J.; Netea, M.G.; Rovina, N.; Akinosoglou, K.; Antoniadou, A.; Antonakos, N.; Damoraki, G.; Gkavogianni, T.; Adami, M.-E.; Katsaounou, P.; et al. Complex

-
- Immune Dysregulation in COVID-19 Patients with Severe Respiratory Failure. *Cell Host Microbe* **2020**, *27*, 992-1000.e3, doi:10.1016/j.chom.2020.04.009.
272. Liao, M.; Liu, Y.; Yuan, J.; Wen, Y.; Xu, G.; Zhao, J.; Cheng, L.; Li, J.; Wang, X.; Wang, F.; et al. Single-Cell Landscape of Bronchoalveolar Immune Cells in Patients with COVID-19. *Nat Med* **2020**, *26*, 842-844, doi:10.1038/s41591-020-0901-9.
273. Vabret, N.; Britton, G.J.; Gruber, C.; Hegde, S.; Kim, J.; Kuksin, M.; Levantovsky, R.; Malle, L.; Moreira, A.; Park, M.D.; et al. Immunology of COVID-19: Current State of the Science. *Immunity* **2020**, *52*, 910-941, doi:10.1016/j.immuni.2020.05.002.
274. Zhong, J.; Tang, J.; Ye, C.; Dong, L. The Immunology of COVID-19: Is Immune Modulation an Option for Treatment? *Lancet Rheumatol* **2020**, *2*, e428-e436, doi:10.1016/S2665-9913(20)30120-X.
275. Li, H.; Chen, C.; Hu, F.; Wang, J.; Zhao, Q.; Gale, R.P.; Liang, Y. Impact of Corticosteroid Therapy on Outcomes of Persons with SARS-CoV-2, SARS-CoV, or MERS-CoV Infection: A Systematic Review and Meta-Analysis. *Leukemia* **2020**, *34*, 1503-1511, doi:10.1038/s41375-020-0848-3.
276. Villar, J.; Ferrando, C.; Martínez, D.; Ambrós, A.; Muñoz, T.; Soler, J.A.; Aguilar, G.; Alba, F.; González-Higueras, E.; Conesa, L.A.; et al. Dexamethasone Treatment for the Acute Respiratory Distress Syndrome: A Multicentre, Randomised Controlled Trial. *Lancet Respir Med* **2020**, *8*, 267-276, doi:10.1016/S2213-2600(19)30417-5.
277. Dexamethasone in Hospitalized Patients with Covid-19. *New England Journal of Medicine* **2021**, *384*, 693-704, doi:10.1056/NEJMoa2021436.
278. Hung, I.F.-N.; Lung, K.-C.; Tso, E.Y.-K.; Liu, R.; Chung, T.W.-H.; Chu, M.-Y.; Ng, Y.-Y.; Lo, J.; Chan, J.; Tam, A.R.; et al. Triple Combination of Interferon Beta-1b, Lopinavir-Ritonavir, and Ribavirin in the Treatment of Patients Admitted to Hospital with COVID-19: An Open-Label, Randomised, Phase 2 Trial. *Lancet* **2020**, *395*, 1695-1704, doi:10.1016/S0140-6736(20)31042-4.
279. Gremese, E.; Cingolani, A.; Bosello, S.L.; Alivernini, S.; Tolusso, B.; Perniola, S.; Landi, F.; Pompili, M.; Murri, R.; Santoliquido, A.; et al. Sarilumab Use in Severe SARS-CoV-2 Pneumonia. *EclinicalMedicine* **2020**, *27*, 100553, doi:10.1016/j.eclinm.2020.100553.
280. Lescure, F.-X.; Honda, H.; Fowler, R.A.; Lazar, J.S.; Shi, G.; Wung, P.; Patel, N.; Hagino, O. Sarilumab in Patients Admitted to Hospital with Severe or Critical COVID-19: A Randomised, Double-Blind, Placebo-Controlled, Phase 3 Trial. *Lancet Respir Med* **2021**, *9*, 522-532, doi:10.1016/S2213-2600(21)00099-0.
281. Pelaia, C.; Calabrese, C.; Garofalo, E.; Bruni, A.; Vatrella, A.; Pelaia, G. Therapeutic Role of Tocilizumab in SARS-CoV-2-Induced Cytokine Storm: Rationale and Current Evidence. *Int J Mol Sci* **2021**, *22*, 3059, doi:10.3390/ijms22063059.
282. Zhang, Y.; Xiao, M.; Zhang, S.; Xia, P.; Cao, W.; Jiang, W.; Chen, H.; Ding, X.; Zhao, H.; Zhang, H.; et al. Coagulopathy and Antiphospholipid Antibodies in Patients with Covid-19. *N Engl J Med* **2020**, e38, doi:10.1056/NEJMc2007575.
283. Ozolina, A.; Sarkele, M.; Sabelnikovs, O.; Skesters, A.; Jaunalksne, I.; Serova, J.; Ievins, T.; Bjertnaes, L.J.; Vanags, I. Activation of Coagulation and Fibrinolysis in Acute Respiratory Distress Syndrome: A Prospective Pilot Study. *Front Med (Lausanne)* **2016**, *3*, 64, doi:10.3389/fmed.2016.00064.
284. Tang, N.; Bai, H.; Chen, X.; Gong, J.; Li, D.; Sun, Z. Anticoagulant Treatment Is Associated with Decreased Mortality in Severe Coronavirus Disease 2019 Patients with Coagulopathy. *J Thromb Haemost* **2020**, *18*, 1094-1099, doi:10.1111/jth.14817.
285. Cortegiani, A.; Ippolito, M.; Ingoglia, G.; Iozzo, P.; Giarratano, A.; Einav, S. Update I. A Systematic Review on the Efficacy and Safety of Chloroquine/Hydroxychloroquine for COVID-19. *J Crit Care* **2020**, *59*, 176-190, doi:10.1016/j.jccrc.2020.06.019.
286. Cao, B.; Wang, Y.; Wen, D.; Liu, W.; Wang, J.; Fan, G.; Ruan, L.; Song, B.; Cai, Y.; Wei, M.; et al. A Trial of Lopinavir-Ritonavir in Adults Hospitalized with Severe Covid-19. *New England Journal of Medicine* **2020**, *382*, 1787-1799, doi:10.1056/NEJMoa2001282.

287. Choy, K.-T.; Wong, A.Y.-L.; Kaewpreedee, P.; Sia, S.F.; Chen, D.; Hui, K.P.Y.; Chu, D.K.W.; Chan, M.C.W.; Cheung, P.P.-H.; Huang, X.; et al. Remdesivir, Lopinavir, Emetine, and Homoharringtonine Inhibit SARS-CoV-2 Replication in Vitro. *Antiviral Research* **2020**, *178*, 104786, doi:10.1016/j.antiviral.2020.104786.
288. Goldman, J.D.; Lye, D.C.B.; Hui, D.S.; Marks, K.M.; Bruno, R.; Montejano, R.; Spinner, C.D.; Galli, M.; Ahn, M.-Y.; Nahass, R.G.; et al. Remdesivir for 5 or 10 Days in Patients with Severe Covid-19. *N Engl J Med* **2020**, NEJMoa2015301, doi:10.1056/NEJMoa2015301.
289. WHO Solidarity Trial Consortium; Pan, H.; Peto, R.; Henao-Restrepo, A.-M.; Preziosi, M.-P.; Sathiyamoorthy, V.; Abdool Karim, Q.; Alejandria, M.M.; Hernández García, C.; Kieny, M.-P.; et al. Repurposed Antiviral Drugs for Covid-19 - Interim WHO Solidarity Trial Results. *N Engl J Med* **2021**, *384*, 497–511, doi:10.1056/NEJMoa2023184.
290. Wang, J.J.; Edin, M.L.; Zeldin, D.C.; Li, C.; Wang, D.W.; Chen, C. Good or Bad: Application of RAAS Inhibitors in COVID-19 Patients with Cardiovascular Comorbidities. *Pharmacol Ther* **2020**, *215*, 107628, doi:10.1016/j.pharmthera.2020.107628.
291. Valle, C.; Martin, B.; Touret, F.; Shannon, A.; Canard, B.; Guillemot, J.; Coutard, B.; Decroly, E. Drugs against SARS-CoV-2: What Do We Know about Their Mode of Action? *Rev Med Virol* **2020**, e2143, doi:10.1002/rmv.2143.
292. Knoll, M.D.; Wonodi, C. Oxford–AstraZeneca COVID-19 Vaccine Efficacy. *Lancet* **2021**, *397*, 72–74, doi:10.1016/S0140-6736(20)32623-4.
293. Baden, L.R.; El Sahly, H.M.; Essink, B.; Kotloff, K.; Frey, S.; Novak, R.; Diemert, D.; Spector, S.A.; Rouphael, N.; Creech, C.B.; et al. Efficacy and Safety of the MRNA-1273 SARS-CoV-2 Vaccine. *N Engl J Med* **2021**, *384*, 403–416, doi:10.1056/NEJMoa2035389.
294. Luring, A.S.; Hodcroft, E.B. Genetic Variants of SARS-CoV-2-What Do They Mean? *JAMA* **2021**, *325*, 529–531, doi:10.1001/jama.2020.27124.
295. Aggarwal, B.B.; Gupta, S.C.; Kim, J.H. Historical Perspectives on Tumor Necrosis Factor and Its Superfamily: 25 Years Later, a Golden Journey. *Blood* **2012**, *119*, 651–665, doi:10.1182/blood-2011-04-325225.
296. Herbein, G.; O'Brien, W.A. Tumor Necrosis Factor (TNF)-Alpha and TNF Receptors in Viral Pathogenesis. *Proc Soc Exp Biol Med* **2000**, *223*, 241–257, doi:10.1046/j.1525-1373.2000.22335.x.
297. Di Marzio, P.; Tse, J.; Landau, N.R. Chemokine Receptor Regulation and HIV Type 1 Tropism in Monocyte-Macrophages. *AIDS Res Hum Retroviruses* **1998**, *14*, 129–138, doi:10.1089/aid.1998.14.129.
298. Herbein, G.; Doyle, A.G.; Montaner, L.J.; Gordon, S. Lipopolysaccharide (LPS) down-Regulates CD4 Expression in Primary Human Macrophages through Induction of Endogenous Tumour Necrosis Factor (TNF) and IL-1 Beta. *Clin Exp Immunol* **1995**, *102*, 430–437, doi:10.1111/j.1365-2249.1995.tb03801.x.
299. Coffey, M.J.; Woffendin, C.; Phare, S.M.; Strieter, R.M.; Markovitz, D.M. RANTES Inhibits HIV-1 Replication in Human Peripheral Blood Monocytes and Alveolar Macrophages. *Am J Physiol* **1997**, *272*, L1025–1029, doi:10.1152/ajplung.1997.272.5.L1025.
300. Kitai, R.; Zhao, M.L.; Zhang, N.; Hua, L.L.; Lee, S.C. Role of MIP-1beta and RANTES in HIV-1 Infection of Microglia: Inhibition of Infection and Induction by IFNbeta. *J Neuroimmunol* **2000**, *110*, 230–239, doi:10.1016/s0165-5728(00)00315-5.
301. Lane, B.R.; Markovitz, D.M.; Woodford, N.L.; Rochford, R.; Strieter, R.M.; Coffey, M.J. TNF-Alpha Inhibits HIV-1 Replication in Peripheral Blood Monocytes and Alveolar Macrophages by Inducing the Production of RANTES and Decreasing C-C Chemokine Receptor 5 (CCR5) Expression. *J Immunol* **1999**, *163*, 3653–3661.
302. Herbein, G.; Khan, K.A. Is HIV Infection a TNF Receptor Signalling-Driven Disease? *Trends Immunol* **2008**, *29*, 61–67, doi:10.1016/j.it.2007.10.008.
303. Hoshino, S.; Konishi, M.; Mori, M.; Shimura, M.; Nishitani, C.; Kuroki, Y.; Koyanagi, Y.; Kano, S.; Itabe, H.; Ishizaka, Y. HIV-1 Vpr Induces TLR4/MyD88-Mediated IL-6 Production and

-
- Reactivates Viral Production from Latency. *J Leukoc Biol* **2010**, *87*, 1133–1143, doi:10.1189/jlb.0809547.
304. Roesch, F.; Richard, L.; Rua, R.; Porrot, F.; Casartelli, N.; Schwartz, O. Vpr Enhances Tumor Necrosis Factor Production by HIV-1-Infected T Cells. *J. Virol.* **2015**, *89*, 12118–12130, doi:10.1128/JVI.02098-15.
305. Olivetta, E.; Percario, Z.; Fiorucci, G.; Mattia, G.; Schiavoni, I.; Dennis, C.; Jäger, J.; Harris, M.; Romeo, G.; Affabris, E.; et al. HIV-1 Nef Induces the Release of Inflammatory Factors from Human Monocyte/Macrophages: Involvement of Nef Endocytotic Signals and NF-Kappa B Activation. *J Immunol* **2003**, *170*, 1716–1727, doi:10.4049/jimmunol.170.4.1716.
306. Ostalecki, C.; Wittki, S.; Lee, J.-H.; Geist, M.M.; Tibroni, N.; Harrer, T.; Schuler, G.; Fackler, O.T.; Baur, A.S. HIV Nef- and Notch1-Dependent Endocytosis of ADAM17 Induces Vesicular TNF Secretion in Chronic HIV Infection. *EBioMedicine* **2016**, *13*, 294–304, doi:10.1016/j.ebiom.2016.10.027.
307. Kumar, A.; Manna, S.K.; Dhawan, S.; Aggarwal, B.B. HIV-Tat Protein Activates c-Jun N-Terminal Kinase and Activator Protein-1. *J Immunol* **1998**, *161*, 776–781.
308. Planès, R.; Ben Haij, N.; Leghmari, K.; Serrero, M.; BenMohamed, L.; Bahraoui, E. HIV-1 Tat Protein Activates Both the MyD88 and TRIF Pathways To Induce Tumor Necrosis Factor Alpha and Interleukin-10 in Human Monocytes. *J. Virol.* **2016**, *90*, 5886–5898, doi:10.1128/JVI.00262-16.
309. Ting, P.T.; Koo, J.Y. Use of Etanercept in Human Immunodeficiency Virus (HIV) and Acquired Immunodeficiency Syndrome (AIDS) Patients. *Int J Dermatol* **2006**, *45*, 689–692, doi:10.1111/j.1365-4632.2005.02642.x.
310. Calabrese, L.H.; Zein, N.; Vassilopoulos, D. Safety of Antitumour Necrosis Factor (Anti-TNF) Therapy in Patients with Chronic Viral Infections: Hepatitis C, Hepatitis B, and HIV Infection. *Ann Rheum Dis* **2004**, *63 Suppl 2*, ii18–ii24, doi:10.1136/ard.2004.028209.
311. Sha, B.E.; Valdez, H.; Gelman, R.S.; Landay, A.L.; Agosti, J.; Mitsuyasu, R.; Pollard, R.B.; Mildvan, D.; Namkung, A.; Ogata-Arakaki, D.M.; et al. Effect of Etanercept (Enbrel) on Interleukin 6, Tumor Necrosis Factor Alpha, and Markers of Immune Activation in HIV-Infected Subjects Receiving Interleukin 2. *AIDS Res Hum Retroviruses* **2002**, *18*, 661–665, doi:10.1089/088922202760019365.
312. Gallitano, S.M.; McDermott, L.; Brar, K.; Lowenstein, E. Use of Tumor Necrosis Factor (TNF) Inhibitors in Patients with HIV/AIDS. *J. Am. Acad. Dermatol.* **2016**, *74*, 974–980, doi:10.1016/j.jaad.2015.11.043.
313. Pasquereau, S.; Kumar, A.; Abbas, W.; Herbein, G. Counteracting Akt Activation by HIV Protease Inhibitors in Monocytes/Macrophages. *Viruses* **2018**, *10*, 190, doi:10.3390/v10040190.
314. Gupta, A.K.; Li, B.; Cerniglia, G.J.; Ahmed, M.S.; Hahn, S.M.; Maity, A. The HIV Protease Inhibitor Nelfinavir Downregulates Akt Phosphorylation by Inhibiting Proteasomal Activity and Inducing the Unfolded Protein Response. *Neoplasia* **2007**, *9*, 271–278.
315. Yang, J.; Pi, C.; Wang, G. Inhibition of PI3K/Akt/MTOR Pathway by Apigenin Induces Apoptosis and Autophagy in Hepatocellular Carcinoma Cells. *Biomed Pharmacother* **2018**, *103*, 699–707, doi:10.1016/j.biopha.2018.04.072.
316. Zaccarelli, M.; Tozzi, V.; Lorenzini, P.; Trotta, M.P.; Forbici, F.; Visco-Comandini, U.; Gori, C.; Narciso, P.; Perno, C.F.; Antinori, A.; et al. Multiple Drug Class-Wide Resistance Associated with Poorer Survival after Treatment Failure in a Cohort of HIV-Infected Patients. *AIDS* **2005**, *19*, 1081–1089, doi:10.1097/01.aids.0000174455.01369.ad.
317. Avalos, C.R.; Price, S.L.; Forsyth, E.R.; Pin, J.N.; Shirk, E.N.; Bullock, B.T.; Queen, S.E.; Li, M.; Gellerup, D.; O'Connor, S.L.; et al. Quantitation of Productively Infected Monocytes and Macrophages of Simian Immunodeficiency Virus-Infected Macaques. *J. Virol.* **2016**, *90*, 5643–5656, doi:10.1128/JVI.00290-16.

318. Lucas, A.; Kim, Y.; Rivera-Pabon, O.; Chae, S.; Kim, D.-H.; Kim, B. Targeting the PI3K/Akt Cell Survival Pathway to Induce Cell Death of HIV-1 Infected Macrophages with Alkylphospholipid Compounds. *PLoS ONE* **2010**, *5*, doi:10.1371/journal.pone.0013121.
319. Aquaro, S.; Svicher, V.; Schols, D.; Pollicita, M.; Antinori, A.; Balzarini, J.; Perno, C.F. Mechanisms Underlying Activity of Antiretroviral Drugs in HIV-1-Infected Macrophages: New Therapeutic Strategies. *J. Leukoc. Biol.* **2006**, *80*, 1103–1110, doi:10.1189/jlb.0606376.
320. Li, G.; De Clercq, E. Therapeutic Options for the 2019 Novel Coronavirus (2019-nCoV). *Nature Reviews Drug Discovery* **2020**, *19*, 149–150, doi:10.1038/d41573-020-00016-0.
321. Gordon, D.E.; Jang, G.M.; Bouhaddou, M.; Xu, J.; Obernier, K.; White, K.M.; O’Meara, M.J.; Rezelj, V.V.; Guo, J.Z.; Swaney, D.L.; et al. A SARS-CoV-2 Protein Interaction Map Reveals Targets for Drug Repurposing. *Nature* **2020**, 1–13, doi:10.1038/s41586-020-2286-9.
322. RECOVERY Collaborative Group Lopinavir-Ritonavir in Patients Admitted to Hospital with COVID-19 (RECOVERY): A Randomised, Controlled, Open-Label, Platform Trial. *Lancet* **2020**, *396*, 1345–1352, doi:10.1016/S0140-6736(20)32013-4.
323. ter Ellen, B.M.; Dinesh Kumar, N.; Bouma, E.M.; Troost, B.; van de Pol, D.P.I.; van der Ende-Metselaar, H.H.; Apperloo, L.; van Gosliga, D.; van den Berge, M.; Nawijn, M.C.; et al. Resveratrol and Pterostilbene Inhibit SARS-CoV-2 Replication in Air–Liquid Interface Cultured Human Primary Bronchial Epithelial Cells. *Viruses* **2021**, *13*, 1335, doi:10.3390/v13071335.
324. Zhou, Z.-X.; Mou, S.-F.; Chen, X.-Q.; Gong, L.-L.; Ge, W.-S. Anti-Inflammatory Activity of Resveratrol Prevents Inflammation by Inhibiting NF- κ B in Animal Models of Acute Pharyngitis. *Mol Med Rep* **2018**, *17*, 1269–1274, doi:10.3892/mmr.2017.7933.
325. Gambini, J.; Inglés, M.; Olaso, G.; Lopez-Grueso, R.; Bonet-Costa, V.; Gimeno-Mallench, L.; Mas-Bargues, C.; Abdelaziz, K.M.; Gomez-Cabrera, M.C.; Vina, J.; et al. Properties of Resveratrol: In Vitro and In Vivo Studies about Metabolism, Bioavailability, and Biological Effects in Animal Models and Humans. *Oxid Med Cell Longev* **2015**, *2015*, doi:10.1155/2015/837042.
326. Makoti, P.; Fielding, B.C. HIV and Human Coronavirus Coinfections: A Historical Perspective. *Viruses* **2020**, *12*, E937, doi:10.3390/v12090937.
327. Pluymers, W.; Pais, G.; Van Maele, B.; Pannecouque, C.; Fikkert, V.; Burke, Jr., T.R.; De Clercq, E.; Witvrouw, M.; Neamati, N.; Debyser, Z. Inhibition of Human Immunodeficiency Virus Type 1 Integration by Diketo Derivatives. *Antimicrob Agents Chemother* **2002**, *46*, 3292–3297, doi:10.1128/AAC.46.10.3292-3297.2002.
328. Adem, Ş.; Eyupoglu, V.; Sarfraz, I.; Rasul, A.; Zahoor, A.F.; Ali, M.; Abdalla, M.; Ibrahim, I.M.; Elfiky, A.A. Caffeic Acid Derivatives (CAFDs) as Inhibitors of SARS-CoV-2: CAFDs-Based Functional Foods as a Potential Alternative Approach to Combat COVID-19. *Phytomedicine* **2021**, *85*, 153310, doi:10.1016/j.phymed.2020.153310.
329. Dos Santos, C.N.; Menezes, R.; Stewart, D. Polyphenols as New Leads in Drug Discovery: Biological Activity and Mechanisms. *Curr Pharm Des* **2018**, *24*, 2041–2042, doi:10.2174/138161282419180924094610.
330. Morse, J.S.; Lalonde, T.; Xu, S.; Liu, W.R. Learning from the Past: Possible Urgent Prevention and Treatment Options for Severe Acute Respiratory Infections Caused by 2019-nCoV. *Chembiochem* **2020**, *21*, 730–738, doi:10.1002/cbic.202000047.
331. Qiao, J.; Li, Y.-S.; Zeng, R.; Liu, F.-L.; Luo, R.-H.; Huang, C.; Wang, Y.-F.; Zhang, J.; Quan, B.; Shen, C.; et al. SARS-CoV-2 Mpro Inhibitors with Antiviral Activity in a Transgenic Mouse Model. *Science* **2021**, *371*, 1374–1378, doi:10.1126/science.abf1611.
332. Phan, M.V.T.; Ngo Tri, T.; Hong Anh, P.; Baker, S.; Kellam, P.; Cotten, M. Identification and Characterization of Coronaviridae Genomes from Vietnamese Bats and Rats Based on Conserved Protein Domains. *Virus Evolution* **2018**, *4*, doi:10.1093/ve/vey035.
333. Lim, Y.X.; Ng, Y.L.; Tam, J.P.; Liu, D.X. Human Coronaviruses: A Review of Virus–Host Interactions. *Diseases* **2016**, *4*, doi:10.3390/diseases4030026.

-
334. Chan, J.F.-W.; Kok, K.-H.; Zhu, Z.; Chu, H.; To, K.K.-W.; Yuan, S.; Yuen, K.-Y. Genomic Characterization of the 2019 Novel Human-Pathogenic Coronavirus Isolated from a Patient with Atypical Pneumonia after Visiting Wuhan. *Emerg Microbes Infect* **2020**, *9*, 221–236, doi:10.1080/22221751.2020.1719902.
335. van der Hoek, L. Human Coronaviruses: What Do They Cause? *Antivir. Ther. (Lond.)* **2007**, *12*, 651–658.
336. Fehr, A.R.; Perlman, S. Coronaviruses: An Overview of Their Replication and Pathogenesis. *Methods Mol. Biol.* **2015**, *1282*, 1–23, doi:10.1007/978-1-4939-2438-7_1.
337. Geng, Y.-J.; Wei, Z.-Y.; Qian, H.-Y.; Huang, J.; Lodato, R.; Castriotta, R.J. Pathophysiological Characteristics and Therapeutic Approaches for Pulmonary Injury and Cardiovascular Complications of Coronavirus Disease 2019. *Cardiovasc Pathol* **2020**, doi:10.1016/j.carpath.2020.107228.
338. Chen, W.-H.; Strych, U.; Hotez, P.J.; Bottazzi, M.E. The SARS-CoV-2 Vaccine Pipeline: An Overview. *Curr Trop Med Rep* **2020**, 1–4, doi:10.1007/s40475-020-00201-6.
339. Zhou, Y.; Hou, Y.; Shen, J.; Huang, Y.; Martin, W.; Cheng, F. Network-Based Drug Repurposing for Novel Coronavirus 2019-NCoV/SARS-CoV-2. *Cell Discovery* **2020**, *6*, 1–18, doi:10.1038/s41421-020-0153-3.
340. Pyrc, K.; Berkhout, B.; van der Hoek, L. Antiviral Strategies against Human Coronaviruses. *Infect Disord Drug Targets* **2007**, *7*, 59–66, doi:10.2174/187152607780090757.
341. Salehi, B.; Mishra, A.P.; Nigam, M.; Sener, B.; Kilic, M.; Sharifi-Rad, M.; Fokou, P.V.T.; Martins, N.; Sharifi-Rad, J. Resveratrol: A Double-Edged Sword in Health Benefits. *Biomedicines* **2018**, *6*, doi:10.3390/biomedicines6030091.
342. Pasquereau, S.; Nehme, Z.; Haidar Ahmad, S.; Daouad, F.; Van Assche, J.; Wallet, C.; Schwartz, C.; Rohr, O.; Morot-Bizot, S.; Herbein, G. Resveratrol Inhibits HCoV-229E and SARS-CoV-2 Coronavirus Replication In Vitro. *Viruses* **2021**, *13*, 354, doi:10.3390/v13020354.
343. Camins, A.; Junyent, F.; Verdaguer, E.; Beas-Zarate, C.; Rojas-Mayorquín, A.E.; Ortuño-Sahagún, D.; Pallàs, M. Resveratrol: An Antiaging Drug with Potential Therapeutic Applications in Treating Diseases. *Pharmaceuticals (Basel)* **2009**, *2*, 194–205, doi:10.3390/ph2030194.
344. Palamara, A.T.; Nencioni, L.; Aquilano, K.; De Chiara, G.; Hernandez, L.; Cozzolino, F.; Ciriolo, M.R.; Garaci, E. Inhibition of Influenza A Virus Replication by Resveratrol. *J. Infect. Dis.* **2005**, *191*, 1719–1729, doi:10.1086/429694.
345. Mohd, A.; Zainal, N.; Tan, K.-K.; AbuBakar, S. Resveratrol Affects Zika Virus Replication in Vitro. *Scientific Reports* **2019**, *9*, 14336, doi:10.1038/s41598-019-50674-3.
346. Mastromarino, P.; Capobianco, D.; Cannata, F.; Nardis, C.; Mattia, E.; De Leo, A.; Restignoli, R.; Francioso, A.; Mosca, L. Resveratrol Inhibits Rhinovirus Replication and Expression of Inflammatory Mediators in Nasal Epithelia. *Antiviral Research* **2015**, *123*, 15–21, doi:10.1016/j.antiviral.2015.08.010.
347. Huang, H.; Liao, D.; Zhou, G.; Zhu, Z.; Cui, Y.; Pu, R. Antiviral Activities of Resveratrol against Rotavirus in Vitro and in Vivo. *Phytomedicine* **2020**, 153230, doi:10.1016/j.phymed.2020.153230.
348. Lin, S.-C.; Ho, C.-T.; Chuo, W.-H.; Li, S.; Wang, T.T.; Lin, C.-C. Effective Inhibition of MERS-CoV Infection by Resveratrol. *BMC Infectious Diseases* **2017**, *17*, 144, doi:10.1186/s12879-017-2253-8.
349. Cao, S.; Realegeno, S.; Pant, A.; Satheshkumar, P.S.; Yang, Z. Suppression of Poxvirus Replication by Resveratrol. *Front Microbiol* **2017**, *8*, doi:10.3389/fmicb.2017.02196.
350. Berardi, V.; Ricci, F.; Castelli, M.; Galati, G.; Risuleo, G. Resveratrol Exhibits a Strong Cytotoxic Activity in Cultured Cells and Has an Antiviral Action against Polyomavirus: Potential Clinical Use. *Journal of Experimental & Clinical Cancer Research* **2009**, *28*, 96, doi:10.1186/1756-9966-28-96.

351. Chávez, J.H.; Leal, P.C.; Yunes, R.A.; Nunes, R.J.; Barardi, C.R.M.; Pinto, A.R.; Simões, C.M.O.; Zanetti, C.R. Evaluation of Antiviral Activity of Phenolic Compounds and Derivatives against Rabies Virus. *Vet Microbiol* **2006**, *116*, 53–59, doi:10.1016/j.vetmic.2006.03.019.
352. Hariono, M.; Abdullah, N.; Damodaran, K.V.; Kamarulzaman, E.E.; Mohamed, N.; Hassan, S.S.; Shamsuddin, S.; Wahab, H.A. Potential New H1N1 Neuraminidase Inhibitors from Ferulic Acid and Vanillin: Molecular Modelling, Synthesis and in Vitro Assay. *Sci Rep* **2016**, *6*, 38692, doi:10.1038/srep38692.
353. Langland, J.; Jacobs, B.; Wagner, C.E.; Ruiz, G.; Cahill, T.M. Antiviral Activity of Metal Chelates of Caffeic Acid and Similar Compounds towards Herpes Simplex, VSV-Ebola Pseudotyped and Vaccinia Viruses. *Antiviral Res* **2018**, *160*, 143–150, doi:10.1016/j.antiviral.2018.10.021.
354. Ogawa, M.; Shirasago, Y.; Ando, S.; Shimojima, M.; Saijo, M.; Fukasawa, M. Caffeic Acid, a Coffee-Related Organic Acid, Inhibits Infection by Severe Fever with Thrombocytopenia Syndrome Virus in Vitro. *J Infect Chemother* **2018**, *24*, 597–601, doi:10.1016/j.jiac.2018.03.005.
355. Utsunomiya, H.; Ichinose, M.; Ikeda, K.; Uozaki, M.; Morishita, J.; Kuwahara, T.; Koyama, A.H.; Yamasaki, H. Inhibition by Caffeic Acid of the Influenza A Virus Multiplication in Vitro. *Int J Mol Med* **2014**, *34*, 1020–1024, doi:10.3892/ijmm.2014.1859.
356. Alam, M.A. Anti-Hypertensive Effect of Cereal Antioxidant Ferulic Acid and Its Mechanism of Action. *Front. Nutr.* **2019**, *6*, doi:10.3389/fnut.2019.00121.
357. Pachón-Angona, I.; Martín, H.; Chhor, S.; Oset-Gasque, M.-J.; Refouvelet, B.; Marco-Contelles, J.; Ismaili, L. Synthesis of New Ferulic/Lipoic/Comenic Acid-Melatonin Hybrids as Antioxidants and Nrf2 Activators via Ugi Reaction. *Future Med Chem* **2019**, *11*, 3097–3108, doi:10.4155/fmc-2019-0191.
358. Benchekroun, M.; Romero, A.; Egea, J.; León, R.; Michalska, P.; Buendía, I.; Jimeno, M.L.; Jun, D.; Janockova, J.; Sepsova, V.; et al. The Antioxidant Additive Approach for Alzheimer's Disease Therapy: New Ferulic (Lipoic) Acid Plus Melatonin Modified Tacrines as Cholinesterases Inhibitors, Direct Antioxidants, and Nuclear Factor (Erythroid-Derived 2)-Like 2 Activators. *J Med Chem* **2016**, *59*, 9967–9973, doi:10.1021/acs.jmedchem.6b01178.
359. Benchekroun, M.; Ismaili, L.; Pudlo, M.; Luzet, V.; Gharbi, T.; Refouvelet, B.; Marco-Contelles, J. Donepezil-Ferulic Acid Hybrids as Anti-Alzheimer Drugs. *Future Med Chem* **2015**, *7*, 15–21, doi:10.4155/fmc.14.148.
360. Benchekroun, M.; Pachón-Angona, I.; Luzet, V.; Martín, H.; Oset-Gasque, M.-J.; Marco-Contelles, J.; Ismaili, L. Synthesis, Antioxidant and A β Anti-Aggregation Properties of New Ferulic, Caffeic and Lipoic Acid Derivatives Obtained by the Ugi Four-Component Reaction. *Bioorg Chem* **2019**, *85*, 221–228, doi:10.1016/j.bioorg.2018.12.029.
361. Baer, A.; Kehn-Hall, K. Viral Concentration Determination through Plaque Assays: Using Traditional and Novel Overlay Systems. *J Vis Exp* **2014**, e52065, doi:10.3791/52065.
362. Jasso-Miranda, C.; Herrera-Camacho, I.; Flores-Mendoza, L.K.; Dominguez, F.; Vallejo-Ruiz, V.; Sanchez-Burgos, G.G.; Pando-Robles, V.; Santos-Lopez, G.; Reyes-Leyva, J. Antiviral and Immunomodulatory Effects of Polyphenols on Macrophages Infected with Dengue Virus Serotypes 2 and 3 Enhanced or Not with Antibodies. *Infect Drug Resist* **2019**, *12*, 1833–1852, doi:10.2147/IDR.S210890.
363. Lalani, S.; Poh, C.L. Flavonoids as Antiviral Agents for Enterovirus A71 (EV-A71). *Viruses* **2020**, *12*, 184, doi:10.3390/v12020184.
364. Vázquez-Calvo, Á.; Jiménez de Oya, N.; Martín-Acebes, M.A.; Garcia-Moruno, E.; Saiz, J.-C. Antiviral Properties of the Natural Polyphenols Delphinidin and Epigallocatechin Gallate against the Flaviviruses West Nile Virus, Zika Virus, and Dengue Virus. *Front Microbiol* **2017**, *8*, 1314, doi:10.3389/fmicb.2017.01314.
365. Tyagi, M.; Bukrinsky, M. Human Immunodeficiency Virus (HIV) Latency: The Major Hurdle in HIV Eradication. *Mol. Med.* **2012**, *18*, 1096–1108, doi:10.2119/molmed.2012.00194.

-
366. Matsuda, Y.; Kobayashi-Ishihara, M.; Fujikawa, D.; Ishida, T.; Watanabe, T.; Yamagishi, M. Epigenetic Heterogeneity in HIV-1 Latency Establishment. *Sci Rep* **2015**, *5*, 7701, doi:10.1038/srep07701.
367. Kim, Y.; Anderson, J.L.; Lewin, S.R. Getting the “Kill” into “Shock and Kill”: Strategies to Eliminate Latent HIV. *Cell Host Microbe* **2018**, *23*, 14–26, doi:10.1016/j.chom.2017.12.004.
368. Muratori, C.; Ruggiero, E.; Sistigu, A.; Bona, R.; Federico, M. Human Immunodeficiency Virus Type 1 (HIV-1) Protease Inhibitors Block Cell-to-Cell HIV-1 Endocytosis in Dendritic Cells. *J Gen Virol* **2009**, *90*, 2777–2787, doi:10.1099/vir.0.012609-0.
369. Bachmann, N.; von Siebenthal, C.; Vongrad, V.; Turk, T.; Neumann, K.; Beerenwinkel, N.; Bogojeska, J.; Fellay, J.; Roth, V.; Kok, Y.L.; et al. Determinants of HIV-1 Reservoir Size and Long-Term Dynamics during Suppressive ART. *Nat Commun* **2019**, *10*, 3193, doi:10.1038/s41467-019-10884-9.
370. Manchanda, T.; Schiedel, D.; Fischer, D.; Dekaban, G.A.; Rieder, M.J. Adverse Drug Reactions to Protease Inhibitors. *Can J Clin Pharmacol* **2002**, *9*, 137–146.
371. Lamers, S.L.; Rose, R.; Maidji, E.; Agsalda-Garcia, M.; Nolan, D.J.; Fogel, G.B.; Salemi, M.; Garcia, D.L.; Bracci, P.; Yong, W.; et al. HIV DNA Is Frequently Present within Pathologic Tissues Evaluated at Autopsy from Combined Antiretroviral Therapy-Treated Patients with Undetectable Viral Loads. *J Virol* **2016**, *90*, 8968–8983, doi:10.1128/JVI.00674-16.
372. Schragar, J.A.; Der Minassian, V.; Marsh, J.W. HIV Nef Increases T Cell ERK MAP Kinase Activity. *J. Biol. Chem.* **2002**, *277*, 6137–6142, doi:10.1074/jbc.M107322200.
373. Xue, M.; Yao, S.; Hu, M.; Li, W.; Hao, T.; Zhou, F.; Zhu, X.; Lu, H.; Qin, D.; Yan, Q.; et al. HIV-1 Nef and KSHV Oncogene K1 Synergistically Promote Angiogenesis by Inducing Cellular MiR-718 to Regulate the PTEN/AKT/MTOR Signaling Pathway. *Nucleic Acids Res* **2014**, *42*, 9862–9879, doi:10.1093/nar/gku583.
374. Varin, A.; Manna, S.K.; Quivy, V.; Decrion, A.-Z.; Van Lint, C.; Herbein, G.; Aggarwal, B.B. Exogenous Nef Protein Activates NF-Kappa B, AP-1, and c-Jun N-Terminal Kinase and Stimulates HIV Transcription in Promonocytic Cells. Role in AIDS Pathogenesis. *J. Biol. Chem.* **2003**, *278*, 2219–2227, doi:10.1074/jbc.M209622200.
375. Nidai Ozes, O.; Mayo, L.D.; Gustin, J.A.; Pfeffer, S.R.; Pfeffer, L.M.; Donner, D.B. NF-KB Activation by Tumour Necrosis Factor Requires the Akt Serine–Threonine Kinase. *Nature* **1999**, *401*, 82–85, doi:10.1038/43466.
376. Herbein, G.; Varin, A.; Larbi, A.; Fortin, C.; Mahlknecht, U.; Fulop, T.; Aggarwal, B. Nef and TNF α Are Coplayers That Favor HIV-1 Replication in Monocytic Cells and Primary Macrophages. *Current HIV Research* **2008**, *6*, 117–129, doi:10.2174/157016208783884985.
377. Bouchet, J.; Hérate, C.; Guenzel, C.A.; Vérollet, C.; Järviluoma, A.; Mazzolini, J.; Rafie, S.; Chames, P.; Baty, D.; Saksela, K.; et al. Single-Domain Antibody-SH3 Fusions for Efficient Neutralization of HIV-1 Nef Functions. *J. Virol.* **2012**, *86*, 4856–4867, doi:10.1128/JVI.06329-11.
378. Shariati, M.; Meric-Bernstam, F. Targeting AKT for Cancer Therapy. *Expert Opin Investig Drugs* **2019**, *28*, 977–988, doi:10.1080/13543784.2019.1676726.
379. Jia, T.; Cao, Q.-Q.; Chen, X.-M.; Xu, F.-L. Effects of the Akt Inhibitor Src-Homology 5 on Proliferation and Apoptosis of the Laryngeal Squamous Cell Carcinoma. *J Cancer Res Ther* **2018**, *14*, 208–212, doi:10.4103/0973-1482.199454.
380. Lin, J.; Sampath, D.; Nannini, M.A.; Lee, B.B.; Degtyarev, M.; Oeh, J.; Savage, H.; Guan, Z.; Hong, R.; Kassees, R.; et al. Targeting Activated Akt with GDC-0068, a Novel Selective Akt Inhibitor That Is Efficacious in Multiple Tumor Models. *Clin Cancer Res* **2013**, *19*, 1760–1772, doi:10.1158/1078-0432.CCR-12-3072.
381. Kim, J.; Lee, E.; Park, B.-J.; Bang, J.H.; Lee, J.Y. Adherence to Antiretroviral Therapy and Factors Affecting Low Medication Adherence among Incident HIV-Infected Individuals during 2009–2016: A Nationwide Study. *Sci Rep* **2018**, *8*, 3133, doi:10.1038/s41598-018-21081-x.
382. Castillo-Mancilla, J.R.; Brown, T.T.; Erlandson, K.M.; Palella, F.J.; Gardner, E.M.; Macatangay, B.J.C.; Breen, E.C.; Jacobson, L.P.; Anderson, P.L.; Wada, N.I. Suboptimal Adherence to

- Combination Antiretroviral Therapy Is Associated With Higher Levels of Inflammation Despite HIV Suppression. *Clin. Infect. Dis.* **2016**, *63*, 1661–1667, doi:10.1093/cid/ciw650.
383. RANA, A.I.; CASTILLO-MANCILLA, J.R.; TASHIMA, K.T.; LANDOVITZ, R.L. Advances in Long-Acting Agents for the Treatment of HIV Infection. *Drugs* **2020**, *80*, 535–545, doi:10.1007/s40265-020-01284-1.
384. Molina, J.-M.; Cahn, P.; Grinsztejn, B.; Lazzarin, A.; Mills, A.; Saag, M.; Supparatpinyo, K.; Walmsley, S.; Crauwels, H.; Rimsky, L.T.; et al. Rilpivirine versus Efavirenz with Tenofovir and Emtricitabine in Treatment-Naive Adults Infected with HIV-1 (ECHO): A Phase 3 Randomised Double-Blind Active-Controlled Trial. *Lancet* **2011**, *378*, 238–246, doi:10.1016/S0140-6736(11)60936-7.
385. Blair, H.A. Ibalizumab: A Review in Multidrug-Resistant HIV-1 Infection. *Drugs* **2020**, *80*, 189–196, doi:10.1007/s40265-020-01258-3.
386. Kufel, W.D. Antibody-Based Strategies in HIV Therapy. *Int J Antimicrob Agents* **2020**, *56*, 106186, doi:10.1016/j.ijantimicag.2020.106186.
387. Lynch, R.M.; Boritz, E.; Coates, E.E.; DeZure, A.; Madden, P.; Costner, P.; Enama, M.E.; Plummer, S.; Holman, L.; Hendel, C.S.; et al. Virologic Effects of Broadly Neutralizing Antibody VRC01 Administration during Chronic HIV-1 Infection. *Sci Transl Med* **2015**, *7*, 319ra206, doi:10.1126/scitranslmed.aad5752.
388. Brown, A.E.; Mohammed, H.; Ogaz, D.; Kirwan, P.D.; Yung, M.; Nash, S.G.; Furegato, M.; Hughes, G.; Connor, N.; Delpech, V.C.; et al. Fall in New HIV Diagnoses among Men Who Have Sex with Men (MSM) at Selected London Sexual Health Clinics since Early 2015: Testing or Treatment or Pre-Exposure Prophylaxis (PrEP)? *Euro Surveill* **2017**, *22*, 30553, doi:10.2807/1560-7917.ES.2017.22.25.30553.
389. Grohskopf, L.A.; Chillag, K.L.; Gvetadze, R.; Liu, A.Y.; Thompson, M.; Mayer, K.H.; Collins, B.M.; Pathak, S.R.; O'hara, B.; Ackers, M.L.; et al. Randomized Trial of Clinical Safety of Daily Oral Tenofovir Disoproxil Fumarate among HIV-Uninfected Men Who Have Sex with Men in the United States. *J Acquir Immune Defic Syndr* **2013**, *64*, 79–86, doi:10.1097/QAI.0b013e31828ece33.
390. Grant, R.M.; Lama, J.R.; Anderson, P.L.; McMahan, V.; Liu, A.Y.; Vargas, L.; Goicochea, P.; Casapía, M.; Guanira-Carranza, J.V.; Ramirez-Cardich, M.E.; et al. Preexposure Chemoprophylaxis for HIV Prevention in Men Who Have Sex with Men. *N Engl J Med* **2010**, *363*, 2587–2599, doi:10.1056/NEJMoa1011205.
391. Margolis, D.M.; Archin, N.M.; Cohen, M.S.; Eron, J.J.; Ferrari, G.; Garcia, J.V.; Gay, C.L.; Goonetilleke, N.; Joseph, S.B.; Swanstrom, R.; et al. Curing HIV: Seeking to Target and Clear Persistent Infection. *Cell* **2020**, *181*, 189–206, doi:10.1016/j.cell.2020.03.005.
392. Fidler, S.; Stöhr, W.; Pace, M.; Dorrell, L.; Lever, A.; Pett, S.; Kinloch-de Loes, S.; Fox, J.; Clarke, A.; Nelson, M.; et al. Antiretroviral Therapy Alone versus Antiretroviral Therapy with a Kick and Kill Approach, on Measures of the HIV Reservoir in Participants with Recent HIV Infection (the RIVER Trial): A Phase 2, Randomised Trial. *Lancet* **2020**, *395*, 888–898, doi:10.1016/S0140-6736(19)32990-3.
393. Gay, C.L.; Kuruc, J.D.; Falcinelli, S.D.; Warren, J.A.; Reifeis, S.A.; Kirchherr, J.L.; James, K.S.; Dewey, M.G.; Helms, A.; Allard, B.; et al. Assessing the Impact of AGS-004, a Dendritic Cell-Based Immunotherapy, and Vorinostat on Persistent HIV-1 Infection. *Sci Rep* **2020**, *10*, 5134, doi:10.1038/s41598-020-61878-3.
394. Bouchat, S.; Delacourt, N.; Kula, A.; Darcis, G.; Van Driessche, B.; Corazza, F.; Gatot, J.; Melard, A.; Vanhulle, C.; Kabeya, K.; et al. Sequential Treatment with 5-aza-2'-deoxycytidine and Deacetylase Inhibitors Reactivates HIV-1. *EMBO Mol Med* **2016**, *8*, 117–138, doi:10.15252/emmm.201505557.
395. Laird, G.M.; Bullen, C.K.; Rosenbloom, D.I.S.; Martin, A.R.; Hill, A.L.; Durand, C.M.; Siliciano, J.D.; Siliciano, R.F. Ex Vivo Analysis Identifies Effective HIV-1 Latency-Reversing Drug Combinations. *J Clin Invest* **2015**, *125*, 1901–1912, doi:10.1172/JCI80142.

-
396. Shi, C.; Tingting, W.; Li, J.-P.; Sullivan, M.A.; Wang, C.; Wang, H.; Deng, B.; Zhang, Y. Comprehensive Landscape of Heparin Therapy for COVID-19. *Carbohydr Polym* **2021**, *254*, 117232, doi:10.1016/j.carbpol.2020.117232.
397. Hall, M.D.; Anderson, J.M.; Anderson, A.; Baker, D.; Bradner, J.; Brimacombe, K.R.; Campbell, E.A.; Corbett, K.S.; Carter, K.; Cherry, S.; et al. Report of the National Institutes of Health SARS-CoV-2 Antiviral Therapeutics Summit. *J Infect Dis* **2021**, *224*, S1–S21, doi:10.1093/infdis/jiab305.
398. Pardi, N.; Hogan, M.J.; Porter, F.W.; Weissman, D. mRNA Vaccines — a New Era in Vaccinology. *Nat Rev Drug Discov* **2018**, *17*, 261–279, doi:10.1038/nrd.2017.243.
399. Smith, A.W.; Skilling, D.E.; Castello, J.D.; Rogers, S.O. Ice as a Reservoir for Pathogenic Human Viruses: Specifically, Caliciviruses, Influenza Viruses, and Enteroviruses. *Med Hypotheses* **2004**, *63*, 560–566, doi:10.1016/j.mehy.2004.05.011.
400. Hofmeister, A.M.; Seckler, J.M.; Criss, G.M. Possible Roles of Permafrost Melting, Atmospheric Transport, and Solar Irradiance in the Development of Major Coronavirus and Influenza Pandemics. *Int J Environ Res Public Health* **2021**, *18*, 3055, doi:10.3390/ijerph18063055.
401. Revich, B.; Tokarevich, N.; Parkinson, A.J. Climate Change and Zoonotic Infections in the Russian Arctic. *Int J Circumpolar Health* **2012**, *71*, 10.3402/ijch.v71i0.18792, doi:10.3402/ijch.v71i0.18792.
402. Ullrich, S.; Nitsche, C. The SARS-CoV-2 Main Protease as Drug Target. *Bioorg Med Chem Lett* **2020**, *30*, 127377, doi:10.1016/j.bmcl.2020.127377.

V. Annexes

Liste des publications

1. Pasquereau, S.; Totoson, P.; Nehme, Z.; Abbas, W.; Kumar, A.; Verhoeven, F.; Prati, C.; Wendling, D.; Demougeot, C.; Herbein, G. Impact of Glucocorticoids on Systemic Sirtuin 1 Expression and Activity in Rats with Adjuvant-Induced Arthritis. *Epigenetics* **2021**, *16*, 132–143, doi:10.1080/15592294.2020.1790789.
2. Pasquereau, S.; Nehme, Z.; Haidar Ahmad, S.; Daouad, F.; Van Assche, J.; Wallet, C.; Schwartz, C.; Rohr, O.; Morot-Bizot, S.; Herbein, G. Resveratrol Inhibits HCoV-229E and SARS-CoV-2 Coronavirus Replication In Vitro. *Viruses* **2021**, *13*, 354, doi:10.3390/v13020354.
3. Nehme, Z.; Pasquereau, S.; Haidar Ahmad, S.; Coaquette, A.; Molimard, C.; Monnien, F.; Algros, M.-P.; Adotevi, O.; Diab Assaf, M.; Feugeas, J.-P.; et al. Polyploid Giant Cancer Cells, Stemness and Epithelial-Mesenchymal Plasticity Elicited by Human Cytomegalovirus. *Oncogene* **2021**, 1–17, doi:10.1038/s41388-021-01715-7.
4. Haidar Ahmad, S.; Al Moussawi, F.; El Baba, R.; Nehme, Z.; Pasquereau, S.; Kumar, A.; Molimard, C.; Monnien, F.; Algros, M.-P.; Karaky, R.; et al. Identification of UL69 Gene and Protein in Cytomegalovirus-Transformed Human Mammary Epithelial Cells. *Front Oncol* **2021**, *11*, 627866, doi:10.3389/fonc.2021.627866.
5. Nehme, Z.; Pasquereau, S.; Herbein, G. Control of Viral Infections by Epigenetic-Targeted Therapy. *Clin Epigenetics* **2019**, *11*, 55, doi:10.1186/s13148-019-0654-9.
6. Pasquereau, S.; Kumar, A.; Abbas, W.; Herbein, G. Counteracting Akt Activation by HIV Protease Inhibitors in Monocytes/Macrophages. *Viruses* **2018**, *10*, 190, doi:10.3390/v10040190.
7. Kumar, A.; Tripathy, M.K.; Pasquereau, S.; Al Moussawi, F.; Abbas, W.; Coquard, L.; Khan, K.A.; Russo, L.; Algros, M.-P.; Valmary-Degano, S.; et al. The Human Cytomegalovirus Strain DB Activates Oncogenic Pathways in Mammary Epithelial Cells. *EBioMedicine* **2018**, *30*, 167–183, doi:10.1016/j.ebiom.2018.03.015.
8. Al Moussawi, F.; Kumar, A.; Pasquereau, S.; Tripathy, M.K.; Karam, W.; Diab-Assaf, M.; Herbein, G. The Transcriptome of Human Mammary Epithelial Cells Infected with the HCMV-DB Strain Displays Oncogenic Traits. *Sci Rep* **2018**, *8*, doi:10.1038/s41598-018-30109-1.
9. Pasquereau, S.; Kumar, A.; Herbein, G. Targeting TNF and TNF Receptor Pathway in HIV-1 Infection: From Immune Activation to Viral Reservoirs. *Viruses* **2017**, *9*, doi:10.3390/v9040064.
10. Pasquereau, S.; Al Moussawi, F.; Karam, W.; Diab Assaf, M.; Kumar, A.; Herbein, G. Cytomegalovirus, Macrophages and Breast Cancer. *The Open Virology Journal* **2017**, *11*, doi:10.2174/1874357901711010015.
11. Kumar, A.; Coquard, L.; Pasquereau, S.; Russo, L.; Valmary-Degano, S.; Borg, C.; Pothier, P.; Herbein, G. Tumor Control by Human Cytomegalovirus in a Murine Model of Hepatocellular Carcinoma. *Mol Ther Oncolytics* **2016**, *3*, 16012, doi:10.1038/mto.2016.12.
12. Kumar, A.; Abbas, W.; Colin, L.; Khan, K.A.; Bouchat, S.; Varin, A.; Larbi, A.; Gatot, J.-S.; Kabeya, K.; Vanhulle, C.; et al. Tuning of AKT-Pathway by Nef and Its Blockade by Protease Inhibitors Results in Limited Recovery in Latently HIV Infected T-Cell Line. *Sci Rep* **2016**, *6*, 24090, doi:10.1038/srep24090.
13. Kumar, A.; Abbas, W.; Bouchat, S.; Gatot, J.-S.; Pasquereau, S.; Kabeya, K.; Clumeck, N.; De Wit, S.; Van Lint, C.; Herbein, G. Limited HIV-1 Reactivation in Resting CD4(+) T Cells from Aviremic Patients under Protease Inhibitors. *Sci Rep* **2016**, *6*, 38313, doi:10.1038/srep38313.

Titre : Latence du VIH-1 et réplication des Coronavirus : nouvelles approches thérapeutiques.

Mots clés : VIH, Latence, Réservoir, Coronavirus

Résumé : Les virus sont des pathogènes cellulaires, responsables de maladies chez différentes espèces animales, incluant l'Homme. La transmission d'un virus pathogène d'un animal à l'Homme entraîne l'émergence de zoonoses, maladies infectieuses régulièrement responsables de pandémies. Parmi les virus responsables de pandémies, le VIH continue, par l'absence de traitement curatif, de constituer une menace globale pour la santé humaine. Plus récemment, l'émergence de pandémies liées à différents coronavirus a montré que cette famille de virus présente un risque pour l'homme. La recherche de nouveaux traitements antiviraux est nécessaire pour faire face à l'émergence de ces pathogènes viraux. Nos travaux se concentrent sur le ciblage de la latence du VIH-1, et sur le ciblage de la réplication des coronavirus HCoV-229E et SARS-CoV-2. Dans le cadre du ciblage de la latence et réactivation du VIH-1, nous avons quantifié la présence du provirus dans les cellules T CD4+ et dans les monocytes de patients sous traitement antirétroviraux combinés. Nous avons évalué l'effet des différentes classes de traitements sur la taille des réservoirs de latence et sur la réactivation. Nous avons également testé l'effet de la protéine virale Nef sur la réactivation, ainsi que l'impact de la voie de signalisation Akt dans la réactivation virale. Nous avons montré que les inhibiteurs de protéases permettent une réduction de la taille des réservoirs

de latence du VIH, dans les lymphocytes T CD4+ et dans les monocytes/macrophages. La réactivation du provirus latent est également inhibée par l'action des inhibiteurs de protéases. La réactivation induite par la protéine virale Nef est également bloquée par ces traitements. Les cellules infectées de manière latente présentent également une augmentation de l'activation de la voie de signalisation Akt. La protéine virale Nef induit également une augmentation de l'activation d'Akt. Cette activation d'Akt est réduite lors du traitement par les inhibiteurs de protéase. Dans le cadre du ciblage de la réplication des coronavirus, nous avons testé plusieurs traitements (n=7) utilisés pour d'autres pathologies et ayant précédemment montré un possible effet antiviral. Nous avons également testé de nouvelles molécules (n=10), principalement dérivées de l'acide férulique, dont la structure est proche de molécules ayant montré un effet antiviral sur les coronavirus. Nous avons montré que le resveratrol inhibe la réplication du HCoV-229E et du SARS-CoV-2. Nous avons montré que des dérivés de l'acide férulique inhibent la réplication du HCoV-229E et du SARS-CoV-2. La recherche de nouveaux traitements contre ces virus doit continuer pour permettre leur éradication, mais cela nécessite également une connaissance approfondie des mécanismes moléculaires du cycle de réplication virale afin de pouvoir les cibler plus efficacement.

Title : HIV-1 latency and Coronavirus replication : new therapeutic approaches.

Keywords : HIV, Latency, Reservoir, Coronavirus

Abstract : Viruses are cellular pathogens, responsible for diseases in different animal species, including humans. The transmission of a pathogenic virus from animals to humans leads to the emergence of zoonoses, infectious diseases that are regularly responsible for pandemics. Among the viruses responsible for pandemics, HIV continues to pose a global threat to human health due to the lack of a cure. More recently, the emergence of pandemics linked to various coronaviruses has shown that this family of viruses presents a risk for humans. The search for new antiviral treatments is necessary to face the emergence of these viral pathogens. Our work focuses on targeting HIV-1 latency, and targeting the replication of HCoV-229E and SARS-CoV-2 coronaviruses. For targeting HIV-1 latency and reactivation, we quantified the presence of provirus in CD4+ T cells and monocytes of patients undergoing combination antiretroviral therapy. We evaluated the effect of different treatment classes on the size of latency reservoirs and on reactivation. We also tested the effect of the viral protein Nef on reactivation, as well as the impact of the Akt signaling pathway on viral reactivation. We have shown that protease inhibitors allow for a reduction in the size of HIV latency reservoirs

in CD4+ T cells and in monocytes/macrophages. Reactivation of latent provirus is also inhibited by the action of protease inhibitors. Reactivation induced by the viral protein Nef is also blocked by these treatments. Latently infected cells also show increased activation of the Akt signaling pathway. The Nef viral protein also induces an increase in Akt activation. This Akt activation is reduced upon treatment with protease inhibitors. In the context of targeting coronavirus replication, we tested several treatments (n=7) used for other pathologies and having previously shown a possible antiviral effect. We also tested new molecules (n=10), mainly derived from ferulic acid, whose structure is close to molecules having shown an antiviral effect on coronaviruses. We have shown that resveratrol inhibits the replication of HCoV-229E and SARS-CoV-2. We have shown that ferulic acid derivatives inhibit the replication of HCoV-229E and SARS-CoV-2. The search for new treatments against these viruses must continue to allow their eradication, but this also requires a thorough knowledge of the molecular mechanisms of their life cycle, in order to target them more efficiently..