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Mélanie Casile

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Mélanie Casile. Relations doses / effets en radiothérapie externe des tumeurs radiorésistantes. Cancer. Université Clermont Auvergne, 2023. Français. NNT : 2023UCFA0070 . tel-04448556

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ÉCOLE DOCTORALE DES SCIENCES DE LA VIE - SANTÉ - AGRONOMIE – ENVIRONNEMENT

THÈSE DE DOCTORAT

Présentée à l'Université Clermont-Auvergne pour l'obtention du grade de
Docteur d'Université

Spécialité : Génétique, Physiologie, Pathologies, Nutrition, Microbiologie Santé, Innovation

Soutenue publiquement le 15 septembre 2023

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RELATION DOSES / EFFETS EN RADIOTHERAPIE EXTERNE DES TUMEURS RADIORESISTANTES

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REMERCIEMENTS

Je tiens tout d'abord à adresser mes sincères remerciements à la Professeure Delphine ANTONI de l'ICANS ainsi qu'à la Professeure Ulrike SCHICK du CHU de Brest d'avoir toutes les deux accepté d'être rapportrices de ce manuscrit et d'être membres de mon jury de thèse. Je tiens également à remercier la Professeure Florence HUGUET de l'Hôpital Tenon de Paris et le Professeur Xavier DURANDO du Centre Jean PERRIN de Clermont-Ferrand d'avoir accepté d'être membres de mon jury de thèse.

Je remercie le Professeur Xavier DURANDO, directeur de la Division de Recherche Clinique du Centre Jean PERRIN pour la confiance que vous m'avez accordée durant ces trois années de doctorat.

Je tiens à remercier tout particulièrement mon directeur et co-directeur de thèse, qui ont su m'accompagner dans ce projet. Julian, je te remercie pour tout le temps que tu as pu m'accorder et pour l'aide que tu m'as apporté. Emmanuel, ou bien Manu, je te remercie pour la relecture de ce manuscrit, l'organisation des différentes réunions et ton aide précieuse pour toutes les démarches administratives. Un grand merci à vous deux pour votre encadrement et votre écoute.

Je remercie bien évidemment les doctorant(e)s / ami(e)s avec qui j'ai partagé ces trois années : Hugo, Alexia et Céleste. Hugo, merci pour ton soutien. Grâce à toi, les deux premières années (et demi) m'ont parue presque courtes. Tes blagues, tes râleries, nos conversations après ton départ m'ont forcément manqué. Alexia, je suis très heureuse d'avoir appris à te connaître. Très vite rejoins par Céleste, vous m'avez toutes les deux soutenue durant cette dernière année, un grand merci ! Nous avons pu travailler dans la bonne ambiance et dans la sincérité et je vous souhaite que cela continue. Bon courage les filles !

Angeline, sans toi, je ne serais pas en train de rédiger ce manuscrit aujourd'hui. Tu m'as attribué ta confiance et ton soutien dès le début de mon stage pour que je puisse réaliser cette thèse. Travailler avec toi, sur l'étude STEP notamment, fut un vrai plaisir.

Emilie T, nous avons commencé et fini cette thèse dans les mêmes difficultés. Cette situation, très formatrice, m'a donné l'occasion de travailler avec toi, sur différents projets. Je te remercie infiniment de m'avoir partagé ton expertise en chefferie de projets.

Je remercie plus globalement l'ensemble des cheffes de projets de l'équipe, Angeline, Catherine, Émilie et Judith, pour vos conseils et le partage de vos connaissances sur le métier de chef de projets.

Ioana, merci pour ton travail sur l'étude GMaPIC. Ce projet a été si long et tu n'as jamais abandonné, malgré ta charge de travail importante. Merci pour le temps que tu m'as accordé. Merci également pour ton travail sur les analyses de l'étude RAPIDARCO et de m'avoir aidé dans la conception de l'étude PROGLIO entre autres.

Comment ne pas remercier cette fine équipe, de passage pour certains, récemment arrivé pour d'autres : Alexia, Atallah, Brian, Céleste, Cheilla, Claire-Isabelle, Clotilde, Manon, Hamza, Hugo, Julie et Mohamad. J'ai partagé de très bons moments avec vous tous. C'est toujours un grand plaisir d'aller boire un verre ou faire une bonne randonnée avec vous.

Merci à toute l'équipe de la Division de Recherche Clinique du Centre Jean PERRIN. Merci à tous les ARC d'avoir pris de votre temps, si précieux, afin de m'aider lorsque j'en avais besoin. Un grand merci à Rodolphe pour m'avoir épaulé sur le protocole DEBIO, c'était une grande étape pour ma petite expérience d'ARC. Merci Isabelle, pour ton implication que ce soit dans l'organisation des réunions et l'enregistrement des inclusions en autres.

Emilie V, mon grand regret est de ne pas avoir pu bénéficier de ton encadrement dès mon arrivée, mais je te remercie pour tout ce que tu as mis en place pour les prochains. Ton écoute et ta gentillesse font énormément de bien.

Je remercie également tous mes proches et ami(e)s qui m'ont soutenu durant ces trois années de thèse. Merci Mathilde d'être une amie aussi formidable. Merci à toute ma famille et ma belle-famille pour leur soutien et leur présence. Mamoune, merci pour ces sacrifices qui m'ont permis de réaliser toutes ces années d'études. Promis c'est fini maintenant !

Pour finir, Eddy, merci d'être présent chaque jour, depuis si longtemps maintenant. Merci pour ton soutien sans faille dans tous mes projets.

RESUME

La radiothérapie externe constitue l'une des modalités majeures dans la prise en charge en cancérologie. Malgré les progrès récents en radiothérapie externe, ce traitement reste cependant insuffisamment efficace dans certains cas, du fait d'une résistance. Ces phénomènes de radiorésistance peuvent conduire à une récurrence et ainsi à une évolution défavorable du pronostic, notamment pour les patients atteints d'un gliome de haut grade.

Ces travaux de thèse en recherche clinique ont porté principalement sur trois essais cliniques sur la radiothérapie des gliomes de haut grade. Le premier travail a été l'élaboration d'un essai clinique visant à évaluer les concentrations plasmatiques de l'hPG80, potentiellement impliquée dans la radiorésistance : L'essai clinique PROGLIO (NCT05157594). Dans cette continuité, le deuxième travail a consisté à l'analyse et à la valorisation d'un essai clinique proposant une méthode non invasive de suivi de la pression intracrânienne après radiothérapie. Les résultats de l'essai clinique GMaPIC (NCT02520492) ont mis en évidence un taux important d'augmentation de la pression intracrânienne au sein de cette population et une corrélation significative avec l'augmentation du volume T2/Flair. Pour finir, dans le but de potentialiser la radiothérapie, le troisième travail a consisté à l'élaboration d'un essai clinique visant à tester l'association de nanoparticules AGuIX[®], à une radio-chimiothérapie avec témozolomide concomitant chez des patients présentant un glioblastome nouvellement diagnostiqué : L'essai clinique NANO-GBM (NCT04881032).

Cette thèse de recherche clinique a ainsi permis d'étudier les relations doses /effets en radiothérapie externe de tumeurs radiorésistantes que sont les gliomes de haut grade dans le but d'améliorer la prise en charge des patients.

ABSTRACT

External radiotherapy is one of the major treatment methods used in oncology. Despite recent advances in external radiotherapy, this treatment remains insufficiently effective in some cases, with the emergence of resistance. This radioresistance can lead to recurrence, and thus to an unfavorable prognosis for patients with high-grade glioma.

This clinical research thesis focused on three clinical trials of radiotherapy for high-grade gliomas. The first was the elaboration of a clinical trial to evaluate plasma concentrations of hPG80, potentially involved in radioresistance, in patients with high-grade brain tumors: the PROGLIO clinical trial (NCT05157594). In the same line, the second work consisted in the analysis and evaluation of a clinical trial proposing a non-invasive method of monitoring intracranial pressure after radiotherapy. The results of the GMaPIC clinical trial (NCT02520492) demonstrated a significant rate of increase of intracranial pressure in this population, and a significant correlation with T2/Flair volume increase. Finally, with the aim of enhancing radiotherapy, this work involved setting up a clinical trial to test the association of AGuIX[®] nanoparticles with radio-chemotherapy and concomitant temozolomide in patients with newly diagnosed glioblastoma: the NANO-GBM clinical trial (NCT04881032).

The aim of this clinical research thesis was to study dose-effect relationships in external radiotherapy of radioresistant tumors such as high-grade gliomas, with the aim of improving patient management.

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COMMUNICATIONS SCIENTIFIQUES (ANNEXES 2/3)

- Sous forme de présentation orale au concours régional « Ma thèse en 180 secondes » le 06 mars 2023.
- Sous forme de poster : « Etude GMaPIC : Mesure non invasive de la pression intracrânienne pour les patients atteints de gliomes de haut grade traités par radiothérapie et/ou chimiothérapie ». Forum de la recherche en cancérologie Auvergne-Rhône-Alpes le 04 et 05 avril 2023.
- Sous forme de présentation orale : « Dose/effect relations in external radiotherapy of radioresistant tumors ». Journées de l'école doctorale Sciences de la Vie, Santé, Agronomie, Environnement le 05 juin 2023 – **Prix communication Oral**

ENSEIGNEMENTS

- Initiation aux méthodologies appliquées à la recherche clinique – Essais cliniques en cancérologie – Master 1 Sciences du Médicament – Mars 2022
- Initiation aux méthodologies appliquées à la recherche clinique – Essais cliniques en cancérologie – Master 1 Sciences du Médicament – Février 2023

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ABREVIATIONS

AGuIX® : Activation and Guidance of Irradiation by X-ray

CDKN2A/B : Inhibiteur de kinase cycline-dépendante 2A/B

CTV : Clinical anatomical target volume

DPOAE : Distorsion des otoémissions acoustiques

EGFR : Récepteur du facteur de croissance épidermique

EPR : Enhanced Permeability and Retention

FISH : Hybridation *in situ* en fluorescence

GTV : Gross tumor volume

HIF : Hypoxia inducible factor

ICRU : International commission of radiation units and measurements

IDH1 : Isocitrate déshydrogénase de type 1

IDH2 : Isocitrate déshydrogénase de type 2

NGS : Séquençage de nouvelle génération

OMS : Organisation mondiale de la santé

PARP : Poly(ADP-ribose) polymérase

PCV : Procarbazine, CCNU, Vincristine

PIC : Pression intracrânienne

PTV : Planning target volume

RANO : Response assesment in neuro-oncolgy

RCMI : Radiothérapie conformationnelle avec modulation d'intensité

TEP : Tomographie par emission de positons

TERT : Telomerase reverse transcriptase

TPF : Taxane-Cisplatine-Fluoro-uracile

VEGF : Vascular Endothelial Growth Factor

VMAT : Arcthérapie volumétrique par modulation d'intensité

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1. HISTORIQUE ET DESCRIPTION DU TRAVAIL

Cette thèse, réalisée en co-direction avec le Pr Julian BIAU (Oncologue Radiothérapeute) et le Dr Emmanuel CHAUTARD (Chercheur), est le fruit d'une collaboration entre la Division de Recherche Clinique du Centre Jean PERRIN, dirigée par la Pr. DURANDO Xavier et l'équipe « Recherche translationnelle en imagerie fonctionnelle, radiopharmaceutiques et biomarqueurs » de l'unité de recherche IMoST (UMR 1240, INSERM, UCA) dirigée par la Pr. Elisabeth MIOT-NOIRAULT.

Elle a été initiée en septembre 2020 après mon stage de deuxième année, réalisé dans le cadre du master Ingénierie de la santé, parcours Diagnostic Biomédical de l'Université Clermont Auvergne au sein de la Division de Recherche Clinique du Centre Jean PERRIN. Elle m'a permis de poursuivre ma formation en recherche clinique, dans le but de prétendre, à l'issue de celle-ci, à un poste de cheffe de projets en recherche clinique.

La radiothérapie externe est l'un des traitements majeurs en cancérologie. Malgré des progrès récents ce type de traitement peut s'avérer insuffisamment efficace dans certains cas avec l'apparition d'une résistance. Ce phénomène de radiorésistance peut conduire à une rechute et ainsi à une évolution défavorable du pronostic des patients. D'autre part, la radiothérapie externe peut être à l'origine d'effets secondaires aigus ou tardifs, parfois difficilement prévisibles.

Partant de ce constat, au cours de ce travail de thèse, j'ai ainsi pu travailler sur plusieurs projets permettant d'étudier les **relations doses/effets en radiothérapie externe afin d'améliorer l'efficacité et/ou d'identifier des paramètres biologiques et cliniques impliqués dans la radiothérapie de tumeurs radiorésistantes** que sont les gliomes de haut grade, dans le but, *in fine*, d'améliorer la prise en charge des patients. Pour parvenir à cet objectif, mon travail s'est articulé autour de trois projets de recherche clinique :

- L'élaboration d'un essai clinique visant à évaluer les concentrations plasmatiques de l'hPG80 chez des patients atteints d'une tumeur cérébrale de haut grade: L'essai clinique PROGLIO (NCT05157594) (1,2).

- L'élaboration d'un essai clinique visant à tester l'association de nanoparticules AGuIX® (Activation and Guidance of Irradiation by X-ray), à une radio-chimiothérapie avec témozolomide concomitant chez des patients présentant un glioblastome nouvellement diagnostiqué : L'essai clinique NANO-GBM (NCT04881032) (3,4)
- L'analyse et à la valorisation d'un essai clinique proposant une méthode non invasive de suivi de la pression intracrânienne pour les patients atteints d'un gliome diffus de l'adulte : L'essai clinique GMaPIC (NCT02520492) (5)

Ainsi, j'ai pu contribuer au titre de premier auteur ou deuxième auteur à la conception de 4 manuscrits dont 3 articles originaux (protocole PROGLIO, protocole NANO-GBM et résultats de l'étude GMaPIC) et une revue de la littérature. De plus, tout au long de mon parcours, le Pr BIAU et le Dr CHAUTARD m'ont honoré de leur confiance en me donnant l'opportunité de participer à plusieurs projets avec leurs collaborateurs en neuro-oncologie et cancérologie des voies aéro-digestives supérieures. Ces articles sont disponibles en [Annexe 1 : Travaux collaboratifs en neuro-oncologie](#) et [Annexe 2 : Travaux collaboratifs en cancérologie des voies aéro-digestives supérieures](#).

2. ANALYSE BIBLIOGRAPHIQUE

A. LES GLIOMES DIFFUS DE L'ADULTE

I. Les données épidémiologiques

1. L'incidence

Les tumeurs cérébrales primitives les plus fréquentes chez l'adulte sont les gliomes ou également appelés tumeurs gliales. Le nombre de nouveaux cas de tumeurs malignes du système nerveux central est estimé à 5 886 en France en 2018, dont 56% chez l'homme. Les taux d'incidence standardisés Europe sont de 8,5 cas pour 100 000 personnes-années chez l'homme et de 5,6 cas pour 100 000 personnes-années chez la femme. Les âges médians au diagnostic et au décès sont respectivement de 63 et 66 ans chez l'homme et de 67 et 68 ans chez la femme en 2018 (6).

Le taux annuel de cas a presque doublé entre 1990 et 2018, pour les deux sexes, avec une augmentation de 93% chez l'homme et de 95% chez la femme (6). Cette augmentation peut être attribuée à plusieurs facteurs. L'évolution des pratiques concernant le diagnostic grâce aux progrès de l'imagerie et le recours plus fréquemment à la biopsie ou la chirurgie. L'augmentation du vieillissement de la population a également un impact direct sur l'augmentation de l'incidence. Pour finir, il est difficile d'exclure l'apparition ou la progression de facteurs de risque aujourd'hui mal connus.

Le type histologique le plus fréquent est le glioblastome, il représente environ 56,4% et 52,5% des tumeurs cérébrales en 2015 chez l'homme et la femme, respectivement. On estime à 3 841 le nombre de nouveaux cas de glioblastome, avec confirmation histologique, en France en 2018, dont 58 % chez l'homme (6).

2. La survie

Malgré les améliorations dans le diagnostic, le nombre de décès dus aux gliomes diffus de l'adulte restent inchangés depuis les années 1980 (7). Le nombre de décès est estimé en 2018 à 2346 chez l'homme et 1782 chez la femme en France (6).

La survie à 5 ans est estimée à 50% pour les gliomes de grade 2 ; à 30% pour les gliomes de grade 3 et inférieure à 5% pour les gliomes de grade 4 (8).

La survie médiane d'un patient atteint d'un glioblastome à partir du diagnostic est légèrement supérieure à un an, estimée entre 14,6 et 16,6 mois selon les essais cliniques (9,10). La survie médiane sans progression est d'un peu moins de 7 mois (9,10). L'évolution tumorale est généralement rapide. Même dans les situations les plus favorables, la majorité des patients décèdent dans les deux ans. Ces tumeurs représentent donc l'un des plus grands défis de l'oncologie.

II. Les origines et la classification de l'OMS

1. La cancérogénèse

Les gliomes diffus de l'adulte se développent aux dépens des cellules gliales permettant le soutien fonctionnel et structural du système nerveux. Les cellules gliales sont de plusieurs types et possèdent diverses fonctions. Au niveau du système nerveux central, les cellules de la microglie jouent un rôle immunitaire (11), les oligodendrocytes permettent la myélinisation des axones et les astrocytes jouent un rôle dans la nutrition et le soutien des neurones entre autres (12).

La reprise d'une prolifération plus soutenue et anarchique de ces cellules normalement quiescentes est à l'origine de la cancérogénèse. Les gliomes ont alors plusieurs origines histologiques et sont nommés en fonction de celle-ci. Ainsi, les astrocytomes se développent à partir des cellules astrocytaires, les oligodendrogliomes à partir des oligodendrocytes et les glioblastomes à partir des cellules astrocytaires. Il existe également des gliomes dits mixtes comme les oligoastrocytomes.

2. La classification de l'OMS

La classification des gliomes a évolué au fil du temps. Elle est actuellement basée sur la classification de l'Organisation mondiale de la santé (OMS) des tumeurs du système nerveux central. Celle-ci a été publiée initialement en 1979 (13) et révisée en 1993, 2000, 2007, et 2016 (14–17). La classification de l'OMS la plus récente date de 2021 (18). Depuis 2016, les gliomes sont classés en fonction non seulement de leur histologie mais également en fonction de paramètres moléculaires.

La mutation de l'isocitrate déshydrogénase de type 1 (IDH1) et de type 2 (IDH2) est une caractéristique moléculaire déterminante des tumeurs oligodendrogiales et astrocytaires. Cette mutation confère de manière significative un meilleur pronostic par rapport aux

tumeurs de type IDH sauvage (19) (**Figure 1**). Une coloration immunohistochimique est donc réalisée sur les échantillons afin de pouvoir mettre en évidence la présence ou non de la mutation IDH1. En revanche, les mutations moins courantes IDH1 et les mutations IDH2 non identifiées par immunohistochimie pourront être détectées à l'aide d'un séquençage de l'ADN.

La codélétion 1p/19q est également la caractéristique moléculaire déterminante des tumeurs oligodendrogiales. La perte des régions chromosomiques 1p et 19q est associée à une meilleure réponse à la chimiothérapie et à une meilleure survie globale (20) (**Figure 1**). Le statut 1p/19q est réalisé à l'aide d'une hybridation *in situ* en fluorescence (FISH) qui peut être complétée par une approche moléculaire telle que le séquençage de nouvelle génération (NGS).

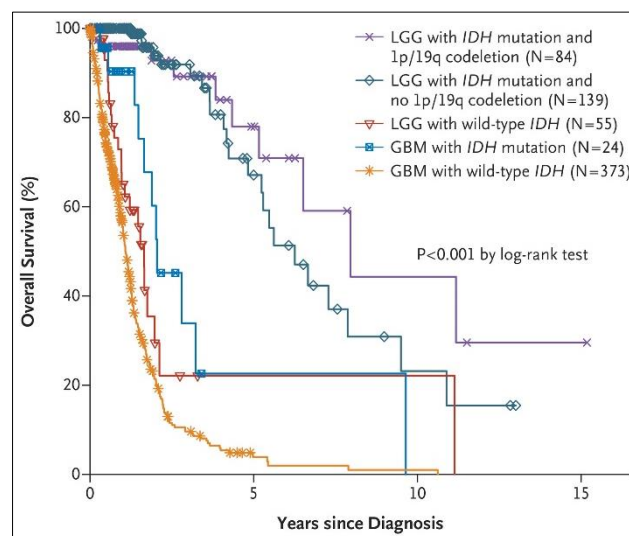


Figure 1 : Survie globale de patients atteints d'un gliome diffus en fonction du statut de la mutation IDH et de la codélétion 1p/19q

D'après The Cancer Genome Atlas Research Network (19)

Les patients atteints de gliomes de bas grade (LGG) avec une mutation IDH avaient une survie médiane de 1,7 an alors que les patients atteints de gliomes de bas grade avec une mutation IDH et une codélétion 1p/19q avaient une survie médiane de 8 ans, et ceux avec une mutation IDH et sans codélétion avaient une survie médiane de 6,3 ans. De plus, les patients atteints de glioblastomes (GBM) avec IDH de type sauvage avaient une survie médiane de 1,1 an alors que les patients atteints de glioblastomes avec IDH mutée avaient une survie médiane, 2,1 ans.

Une fois le statut IDH et la codélétion 1p/19q déterminés, plusieurs types de mutations ou délétions peuvent aider au diagnostic. La mutation somatique faux-sens du gène TP53 et la mutation du gène régulateur de la chromatine ATRX sont présentes en majorité chez les patients atteints d'un astrocytome IDH muté. Les tumeurs astrocytaires non mutées IDH sont diagnostiquées comme un glioblastome (grade 4) s'il existe une amplification d'EGFR (Récepteur du facteur de croissance épidermique), ou une mutation du promoteur de la télomérase (TERT), ou un gain simultané du chromosome 7 et une perte du chromosome 10. L'amplification d'EGFR et le génotype +7/-10 peuvent être détectés par FISH, et les mutations du promoteur de TERT peuvent être détectées par NGS.

La classification des gliomes diffus de l'adulte se base donc sur le statut de mutation de l'IDH et la codélétion 1p/19q. De cette base émergent alors trois types de tumeurs : les astrocytomes IDH muté, les oligodendrogliomes IDH muté et codélétés 1p/19q et pour finir les glioblastomes IDH sauvage **Tableau 1**.

Pour déterminer le grade, la classification de l'OMS de 2021 incorpore des critères histologiques ainsi que moléculaires. Concernant les astrocytomes IDH muté, l'OMS reconnaît 3 grades. Le grade 2 est défini par une activité mitotique non significative, sans prolifération endothéliale ou nécrose. Le grade 3 se distingue par une activité mitotique plus importante. Le grade 4 est histologiquement similaire au glioblastome avec une activité mitotique importante accompagnée d'une prolifération microvasculaire et/ou d'une nécrose. La délétion homozygote de l'inhibiteur de kinase cycline-dépendante 2A/B (CDKN2A/B), associée à un mauvais pronostic permet également le diagnostic d'un astrocytome IDH muté de grade 4, indépendamment des résultats histologiques. Cette délétion peut être détectée notamment par une FISH ou par NGS. Concernant les oligodendrogliomes IDH muté l'OMS reconnaît seulement 2 grades. La distinction entre les 2 s'identifie par l'activité mitotique, la prolifération microvasculaire et également la présence de la délétion CDKN2A/B **Tableau 1**.

Tableau 1 : Classification OMS 2021 des gliomes diffus de l'adulte

Les gliomes diffus de l'adulte			
Type de tumeur	ASTROCYTOME	OLIGODENDROGLIOME	GLIOBLASTOME
Altérations génétiques moléculaires caractéristiques			
IDH1, IDH2	X	X	
Codélétion 1p/19q		X	
TP53	X		
ATRX	X		
EGFR Ou TERT Ou +7/-10			X
Grade histologique et moléculaire			
2			
3	Index mitotique important	Prolifération microvasculaire Index mitotique important CDKN2A/B	
4	CDKN2A/B ou Index mitotique important Prolifération microvasculaire +/- Nécrose		Grade 4 uniquement

D'après la classification OMS 2021 des tumeurs du système nerveux central

CDKN : Inhibiteur de kinase cycline-dépendante ; IDH : isocitrate déshydrogénase ; TERT : télomérase transcriptase inverse ; EGFR : récepteur du facteur de croissance épidermique

3. Les facteurs de risques

La majorité des gliomes diffus de l'adulte est sporadique, sans aucun facteur de risque retrouvé. Il existe, tout de même, une forte association entre ces tumeurs et certaines maladies héréditaires rares dans moins de 5% de l'ensemble des gliomes diffus de l'adulte (8). Les différents syndromes de prédisposition sont énumérés dans le **Tableau 2**.

Tableau 2 : Principaux syndromes familiaux de prédisposition aux gliomes diffus de l'adulte.

Syndrome de prédisposition	Gène impliqué	Type de tumeur
Neurofibromatose de type 1	NF1	Astrocytome
Neurofibromatose de type 2	NF2	Astrocytome
Syndrome de Li-Fraumeni	P53	Glioblastome
Syndrome de Lynch	MLH1, MSH2, MSH6, PMS2	Glioblastome
Sclérose tubulaire de Bourneville	TSC2, TSC1	Astrocytome
Syndrome de Rubinstein-Taybi	CREBBP	Oligodendrogliome

D'après Pouchieu 2016 et Davis 2018 (8,21).

L'exposition à des doses élevées de radiations ionisantes est le seul facteur de risque environnemental confirmé par de nombreuses études. Une augmentation du nombre de gliomes a été observée chez les survivants d'Hiroshima et de Nagasaki, avec également un risque plus élevé pour les personnes exposées durant leur enfance (22). Le risque de développer une tumeur du système nerveux central secondaire chez les enfants traités par irradiation cérébrale pour une première tumeur est 7 fois plus élevé (23). Cette association entre l'âge au moment de l'exposition aux radiations ionisantes et le risque de développer un gliome a également été observée dans une cohorte de 11 000 adultes traités dans leur enfance en Israël pour la teigne du cuir chevelu (24).

De nombreux facteurs de risques environnementaux possibles ont été étudiés concernant le développement du gliome diffus de l'adulte. En 1999, le Centre international de Recherche sur le Cancer a coordonné une étude internationale, cas-témoin, afin de déterminer si l'utilisation du téléphone portable augmenterait le risque de développer une tumeur cérébrale. L'étude INTERPHONE a inclus 10 500 personnes dans 13 pays différents (Australie, Canada, Danemark, Finlande, France, Allemagne, Israël, Italie, Japon, Nouvelle-Zélande, Norvège, Suède et Royaume-Uni). Il n'a été identifié aucune augmentation du risque de gliome lié à l'utilisation du téléphone portable. Un risque accru de gliome a été suggéré dans le cas des temps d'utilisation les plus élevés de manière ipsilatérale (25).

Dans la catégorie des facteurs de risques environnementaux, le régime alimentaire a longuement été étudié. Les nitrates et les nitrites utilisés comme additifs alimentaires dans la viande transformée et la charcuterie sont reconnus comme cancérigènes par l'Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail. Ces composés N-nitroso ont été détectés post-mortem dans le tissu cérébral, indiquant que le passage de la barrière hémato-encéphalique est possible (26). Une première méta-analyse datant 2016, sur 9 études incluant au total 2264 patients, a conclu que la consommation de nitrites ou nitrates alimentaires n'était pas associée à un risque de gliome diffus de l'adulte (27). En revanche, deux autres méta-analyses présentent des résultats contradictoires. La première datant de 2015, sur 17 études représentant une population totale de 4797 patients, suggère que la consommation de viande transformée pourrait augmenter le risque de gliome (28). Et la seconde datant de 2022, portant sur 41 études, met en évidence qu'un apport élevé en nitrite est associé à un risque accru de gliome (29). Ces résultats sont basés sur des analyses observationnelles rétrospectives. Ils devront donc être confirmés par des études de cohorte prospectives afin de pouvoir confirmer ce risque.

L'exposition aux pesticides, aux solvants et aux engrais a également été étudiée comme facteur de risque environnemental auprès des agriculteurs. Une méta-analyse, synthétisant 42 ans d'études épidémiologiques sur l'agriculture et le gliome a relevé que l'agriculture serait associée à un risque accru de gliome (30). La validité des études peut être discutable. En effet, de nombreuses études regroupaient des ouvriers agricoles et des directeurs d'exploitation, deux professions différentes, n'impliquant pas le même risque d'exposition. Le risque accru de développement de gliome en fonction de l'exposition aux pesticides et autres produits chimiques utilisés dans l'agriculture n'est pas totalement établi. De plus, il ne faut pas exclure la contribution d'autres facteurs de risques. Il convient pour autant, de limiter ces expositions en attendant des études plus robustes.

Plusieurs hypothèses concernant le rôle des facteurs de risque des gliomes diffus de l'adulte ont été testées au cours des dernières décennies. Seuls les rayonnements ionisants sont concluants. Des études prospectives sont nécessaires pour confirmer le rôle potentiel des facteurs de risques environnementaux tels que la consommation de nitrates et nitrites et l'exposition aux pesticides.

III. Les signes cliniques et le diagnostic

1. Les signes cliniques

Les symptômes du gliome diffus sont plutôt variés et dépendent de l'emplacement, du volume et de l'évolution de la tumeur. Les plus courants sont les céphalées, les crises d'épilepsie et les troubles fonctionnels (31).

Les céphalées sont une manifestation courante des gliomes diffus. Les céphalées sévères sont peu fréquentes, sauf en cas d'augmentation de la pression intracrânienne (PIC). La boîte crânienne contient le cerveau, des vaisseaux et du liquide cébrospinal. Cette cavité osseuse, protégeant l'encéphale est inextensible. Toute augmentation du volume de l'un de ces composants doit donc être compensée par une diminution de volume de l'un ou de plusieurs de ces composants selon la doctrine de Monro-Kellie (32). Le sang et le liquide cébrospinal sont les volumes pouvant s'adapter le plus facilement afin de maintenir une PIC normale. En revanche, si l'un des volumes augmente significativement et que la PIC ne peut être maintenue, elle augmentera jusqu'à provoquer une hypertension intracrânienne (33). Les tumeurs à croissance rapide telles que les gliomes diffus de haut grade induisent une hypertension intracrânienne, mal tolérée, ne laissant pas le temps nécessaire aux mécanismes de compensation. L'hypertension, définie par une valeur supérieure à 20 mmHg, peut être causée par un œdème cérébral provoqué par la tumeur, par la compression du cerveau en lien avec la taille de la tumeur, ou bien, être due à une accumulation de liquide cébrospinal dans les ventricules. L'étude de la PIC, mesurée à l'aide d'une méthode non invasive est la base de l'essai clinique GMaPIC dont les résultats sont présentés dans la partie **4-TRAVAUX DE RECHERCHES**.

Le développement de la tumeur peut également altérer le fonctionnement du cerveau en lui-même. Selon les zones impactées, différents troubles fonctionnels peuvent survenir comme la difficulté d'orientation, la perturbation de la vue, la difficulté de s'exprimer, le manque de coordination, le changement de personnalité, etc. (31) (**Figure 2**).

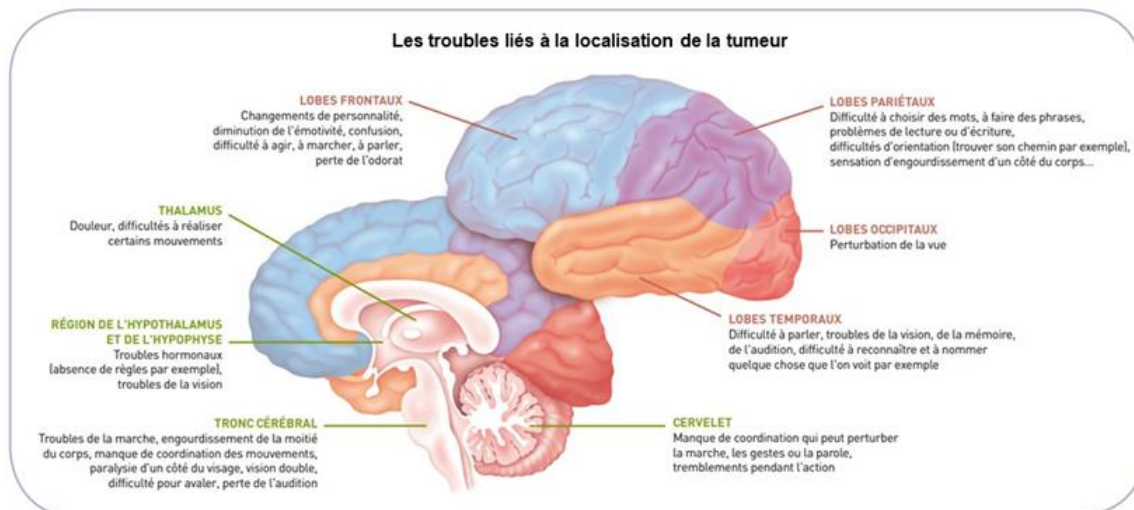


Figure 2 : Troubles liés aux gliomes diffus de l'adulte

D'après l'Institut National du Cancer

L'illustration décrit les principaux symptômes possibles, en fonction de la localisation de la tumeur.

2. Le diagnostic

Si la présence de ces symptômes ne suffit pas au diagnostic d'un gliome diffus, ils permettent de suspecter sa présence. Le degré des déficits neurologiques et l'examen clinique permettent d'évaluer l'urgence thérapeutique. Les tumeurs provoquant des symptômes quelques semaines seulement avant le diagnostic ont généralement une croissance rapide (34).

Puis, est réalisé un examen d'imagerie idéalement par résonance magnétique (IRM), afin de confirmer la présence d'une tumeur cérébrale, de la localiser et de mesurer sa taille. Ainsi une évaluation est réalisée se basant sur les critères RANO (Response Assessment in Neuro-Oncology) (35). Ces critères permettent de prendre en compte les résultats de l'imagerie, la prise de corticostéroïdes réduisant l'œdème cérébral et indirectement la pression intracrânienne, ainsi que le statut clinique du patient.

Pour finir, un examen anatomopathologique rendu possible grâce à une chirurgie ou une biopsie permet *via* différentes analyses histopathologique et moléculaire, de caractériser le grade et l'histologie tumorale, selon la classification de l'OMS en vigueur. Une fois le diagnostic établi, les traitements possibles pourront être discutés en réunion de concertation pluridisciplinaire.

IV. Les stratégies thérapeutiques

1. La chirurgie

Les décisions de traitements sont prises sur la base du diagnostic tissulaire et moléculaire rendus possibles grâce à une chirurgie. Lorsque la résection tumorale est difficilement envisageable à cause de l'emplacement de la tumeur ou l'état général du patient, une biopsie stéréotaxique peut être réalisée (34).

Le second objectif de la chirurgie est thérapeutique. Elle doit permettre de réduire au maximum le volume tumoral, tout en préservant les fonctions neurologiques du patient. En raison de la nature infiltrante de ces tumeurs, il peut être difficile de discerner la limitation entre le tissu sain et le tissu tumoral. La chirurgie guidée par l'imagerie et l'ajout d'acide 5-aminolévulinique permettent une meilleure visualisation des marges de résection. Ces outils aident à réduire le volume tumoral résiduel post-opératoire et l'apparition de nouveaux déficits neurologiques (36). Une neurochirurgie éveillée, sous anesthésie locale, peut également être envisagée pour opérer les gliomes situés dans les zones cérébrales éloquentes, responsables des fonctions motrices ou de la parole. Cette technique permet de limiter le risque de développer des déficits neurologiques en évaluant ces fonctions au cours de l'intervention chirurgicale (37). Actuellement, ce type de chirurgie est principalement réalisé pour les gliomes diffus de bas grade. L'essai clinique SAFE (NCT03861299) (38), contrôlé et randomisé, ayant pour objectif de comparer la neurochirurgie éveillée à l'anesthésie générale pour les patients atteints d'un glioblastome est en cours de recrutement.

La balance entre les bénéfices d'une résection la plus optimale et les risques de développer de nouveaux déficits neurologiques doit toujours être discutée. Une résection complète est préférable car elle améliore de manière significative la survie des patients (39,40), en revanche celle-ci n'est réalisable que dans 40% des cas si l'on prend l'exemple du glioblastome (41,42). Ainsi, dans la majorité des cas, seule une biopsie ou chirurgie partielle sera réalisée.

La chirurgie est la principale prise en charge thérapeutique des oligodendrogliomes, IDH muté, avec codélétion 1p19q de grade 2 et des astrocytomes IDH muté de grade 2. Seule une surveillance est réalisée après la chirurgie pour les patients jeunes (<40 ans) ou ayant eu une résection complète.

2. La chimiothérapie et la radiothérapie

Concernant la prise en charge des oligodendrogliomes, IDH muté, avec codélétion 1p19q de grade 2 et des astrocytomes IDH muté de grade 2, une radiothérapie suivie d'une chimiothérapie de type PCV (Procarbazine, CCNU, Vincristine) peut être envisagée après la chirurgie (43). L'essai clinique, contrôlé et randomisé de phase II/III a démontré une augmentation majeure de la survie globale, de 7,8 à 13,3 ans, des patients jeunes (18 à 39 ans) ayant réalisé une biopsie ou une résection partielle, suivie d'une radiothérapie et chimiothérapie PCV comparée à une radiothérapie seule (43).

La prise en charge des oligodendrogliomes, IDH muté, avec codélétion 1p19q de grade 3 repose sur deux essais cliniques randomisés. L'essai clinique, contrôlé et randomisé de phase III RTOG, comparant la radiothérapie seule à une chimiothérapie PCV suivie d'une radiothérapie (NCT00002569) (44,45). Et l'essai clinique, contrôlé et randomisé de phase II EORTC comparant la radiothérapie seule à la radiothérapie suivie d'une chimiothérapie PCV (NCT00002840) (46,47). La survie globale était significativement augmentée avec un traitement par radiothérapie et PCV comparativement à une radiothérapie seule chez les patients présentant une codélétion 1p19. La survie globale des patients était doublée dans l'étude RTOG (14,7 ans *versus* 7,3 ans) (44) et significativement améliorée dans l'étude EORTC (112 mois *versus* survie globale non atteinte) (46). Le traitement par radiothérapie et chimiothérapie PCV apparaît donc comme le nouveau standard des oligodendrogliomes, IDH muté, de grade 3 avec codélétion 1p19q. Une étude de phase III, contrôlée et randomisée, est en cours de recrutement afin de déterminer si l'utilisation du témozolomide, au lieu de la chimiothérapie PCV, pourrait améliorer la survie sans progression de ces patients. Le témozolomide étant moins toxique et plus simple à utiliser de par sa forme galénique, les résultats de cette étude pourraient permettre d'améliorer la qualité de vie des patients (48).

Concernant la prise en charge des astrocytomes IDH muté de grade 3, les résultats de la deuxième étude intermédiaire de l'essai CATNON (NCT00626990) ont été publiés récemment (49,50). Cet essai de phase III, contrôlé et randomisé, a étudié l'impact de l'utilisation du témozolomide en situation adjuvante et/ou concomitante à une radiothérapie. Les patients étaient randomisés en 4 groupes : Radiothérapie seule ; radiothérapie avec témozolomide (75mg/m² quotidien) ; radiothérapie suivie de 12 cycles de témozolomide (150-200 mg/m²) et radiothérapie avec témozolomide suivie du témozolomide seul. Les résultats de cette étude

ont démontré un bénéfice de survie significatif dans le groupe ayant reçu le témozolomide en situation adjuvante. Cette seconde analyse intermédiaire a pu mettre en évidence la futilité de l'utilisation du témozolomide en situation concomitante (49). La prise en charge actuelle est donc la réalisation d'une chirurgie, suivie d'une radiothérapie et d'une chimiothérapie en entretien de type témozolomide.

Pendant des décennies, la radiothérapie seule constituait la base du traitement du glioblastome après une chirurgie. La radiothérapie a pratiquement doublé la survie globale des patients passant de 14 à 35 semaines (51). Celle-ci était délivrée à la dose de 60 Gy en 6 semaines. Fractionnée sur 5 jours par semaine avec des doses de 1,8 à 2 Gy. En 2005, un essai clinique de phase III, chez des patients de moins de 70 ans, nouvellement diagnostiqués, a comparé l'utilisation de la radiothérapie seule (60 Gy en 6 semaines) à une radiothérapie associée à du témozolomide concomitant (75mg/m² par jour pendant 6 semaines) suivie de 6 cycles de témozolomide d'entretien (150-200 mg/m² pendant 5 jours tous les 28 jours) (10). Les résultats de cet essai ont démontré que l'association radio-chimiothérapie comparée à la radiothérapie seule augmentait la médiane de survie de 12,1 à 14,6 mois. Les taux de survie à 2 ans étaient respectivement de 26,5 % et 10,4 %. Cet essai clinique constitue donc la norme actuelle de soin pour les patients nouvellement diagnostiqués, de moins de 70 ans et en bon état général (**Figure 3**). Depuis 2005, le traitement de référence du glioblastome n'a pas changé et le pronostic reste toujours aussi sombre. Les patients plus âgés suivent également ce protocole ou peuvent réduire la durée de la radiothérapie (40 Gy en 15 fractions) et augmenter le nombre de cycles de témozolomide à 12 en l'absence de progression (52,53).

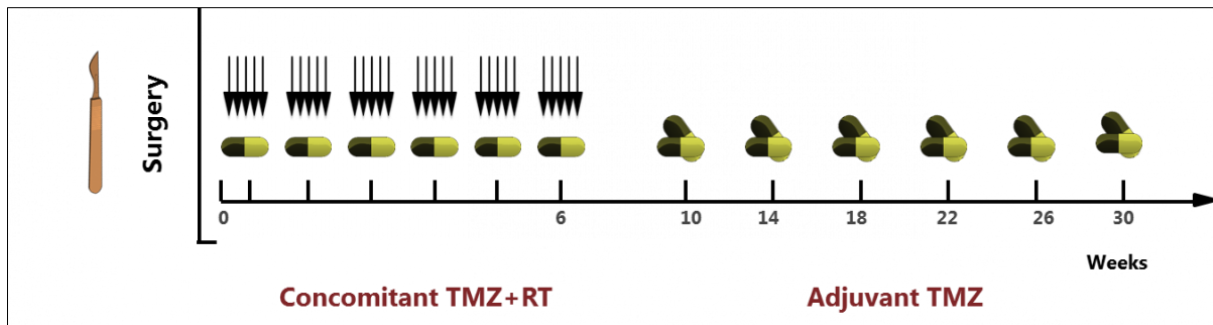


Figure 3 : Schéma de traitement de référence du glioblastome (protocole STUPP)

Source : precisioncancermedicinegroup.com

Les patients réalisent une chirurgie (partielle ou incomplète), suivie de six semaines de radiothérapie conformationnelle à la dose de 60Gy et d'un traitement concomitant par témozolomide (75mg/m²/jour) 7 jours par semaine, puis d'au moins six cycles de chimiothérapie adjuvante par témozolomide (200mg/m²/jour) pendant 5 jours tous les 28 jours.

Récemment, les résultats d'un essai clinique, multicentrique, international, ont démontré que l'association du témozolomide en entretien avec l'utilisation du dispositif médical OPTUNE comparée à l'utilisation du témozolomide en entretien seul augmentait de manière significative la médiane de survie de 16 à 20,9 mois et la médiane de survie sans progression de 4 à 6,7 mois, sans effet indésirables notables (54,55). Ce dispositif médical délivre des champs électriques (Tumor Treating Fields) de faible intensité pouvant stopper la mitose et favoriser l'apoptose des cellules tumorales sans altérer les cellules saines (56,57). Il a ainsi obtenu l'avis favorable de la commission nationale d'évaluation des dispositifs médicaux et des technologies de santé permettant son remboursement en France depuis le 15 mars 2023. La prise en charge des glioblastomes nouvellement diagnostiqués devrait donc certainement évoluer dans les prochaines années.

3. Potentialisation de la radiothérapie : L'utilisation de nanoparticules

L'utilisation de nanoparticules permettant de potentialiser la radiothérapie serait très prometteuse. En effet, cette nanotechnologie peut agir comme radiosensibilisant. Les nanoparticules métalliques avec un numéro atomique (Z) élevé permettent d'améliorer l'efficacité de l'irradiation en augmentant, au niveau des cellules tumorales, l'absorption des photons émis par le faisceau de rayonnement (58). Malgré de nombreuses études précliniques, seuls deux types de nanoparticules ont été évalués en clinique pour leurs

propriétés radiosensibilisantes. Les premières sont les nanoparticules d'oxyde d'hafnium (HfO₂) NBTXR3 qui ont montré leur efficacité pour le traitement de patients atteints d'un sarcome des tissus mous localement avancé, dans une étude internationale, randomisée, de phase II/III (NCT02379845). L'injection intra tumorale des nanoparticules NBTXR3 en amont de la radiothérapie préopératoire chez 89 patients a augmenté significativement le taux de réponse complète (16 % contre 8 %) par rapport à une radiothérapie seule chez 90 patients (58). Les secondes sont les nanoparticules AGuIX[®], à base de gadolinium, développées par la société grenobloise NH TherAguix (59).

Dans le cadre du traitement des gliomes diffus, l'administration de ces nanoparticules ne peut s'envisager que de manière intra-veineuse, ce qui est le cas des nanoparticules AGuIX[®]. Les pages suivantes présentent les mécanismes d'action, leur développement préclinique, jusqu'aux premiers essais cliniques de ces nanoparticules AGuIX[®] sous forme d'une revue de la littérature publiée, en première auteure, dans la revue Innovations & Thérapeutiques en Oncologie en 2022.

L'essai clinique NANO-GBM de phase I/II (NCT04881032), présenté dans la partie **4-TRAVAUX DE RECHERCHES** a pour objectif d'évaluer l'association des nanoparticules AGuIX[®] avec une radio-chimiothérapie avec témozolomide concomitant chez des patients atteints d'un glioblastome nouvellement diagnostiqué (4).

Nanoparticules AGuIX[®] et radiothérapie : du développement préclinique aux premiers essais chez l'homme

AGuIX[®] nanoparticles and radiotherapy: from the bench to the bedside

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RÉSUMÉ

Depuis quelques années, l'utilisation des nanotechnologies est au cœur des recherches thérapeutiques. L'utilisation en cancérologie de nanoparticules ouvre un vaste champ d'applications cliniques. En effet, les nanoparticules ont la capacité d'agir comme des radiosensibilisants. Elles permettent d'améliorer l'efficacité de l'irradiation au niveau des cellules tumorales. Leur utilisation pourrait donc être une approche prometteuse pour le traitement local des tumeurs par radiothérapie externe.

Dans ce contexte, la société de biotechnologies NH TherAguix (Grenoble, France) développe les nanoparticules AGuIX[®] (*Activation and Guidance of Irradiation by X-ray*). Ce sont des nanoparticules de très petite taille, de l'ordre de 5 nm, constituées d'une matrice de polysiloxane et de chélates de gadolinium. Elles possèdent des propriétés radiosensibilisantes très importantes ainsi que des propriétés d'agent de contraste, grâce à la présence de gadolinium. D'après les différentes études réalisées, aucun signe de toxicité n'a été observé sur deux espèces animales (rongeurs et singes) après une administration intraveineuse. La biodistribution sur différents modèles animaux a prouvé une accumulation passive et sélective dans les tumeurs grâce à l'effet EPR (*Enhanced Permeability and Retention effect*) et une élimination rénale des nanoparticules après administration. Un effet radiosensibilisant a également été observé avec différents types d'irradiations *in vitro* et *in vivo*, sur plusieurs types de cancers, y compris des modèles radiorésistants. Ces nanoparticules prometteuses sont actuellement en cours d'évaluation dans des essais cliniques en association avec la radiothérapie dans plusieurs centres en France et aux États-Unis.

Cette revue résume ainsi les principaux résultats précliniques qui ont conduit à la première administration chez l'homme des nanoparticules AGuIX[®].

● **Mots clés** : nanoparticules ; radiothérapie ; nanomédecine ; théranostique.

ABSTRACT

For several years, the use of nanotechnology has been at the heart of research. The use of nanoparticles in oncology opens a vast field of clinical applications. Indeed, nanoparticles have the ability to act as radiosensitizers. They enhance the effectiveness of irradiation in tumor cells, and may represent a promising approach for the local treatment of tumors by external radiotherapy.

In this context, the biotechnology company, NH TherAguix (Grenoble, France), has developed AGuIX[®] (Activation and Guidance of Irradiation by X-ray) nanoparticles. These ultra-small nanoparticles, around 5 nm, are

Pour citer cet article : Casile M, Thivat E, Durando X, Chautard E, Khalil T, Biau J. Nanoparticules AGuIX[®] et radiothérapie : du développement préclinique aux premiers essais chez l'homme. *Innov Ther Oncol* 2021 ; 7 : 1-5. doi : 10.1684/ito.2021.0278

Remerciements et autres mentions :

Financement : aucun.

Liens d'intérêts : Essai clinique NANO-GBM promu par le Centre Jean Perrin pour lequel le Dr. Julian Biau est l'investigateur principal et le Pr. Xavier Durando le co-investigateur.

composed of a polysiloxane matrix and gadolinium chelates. The nanoparticles have very high radiosensitizing properties as well as contrast agent properties due to the presence of gadolinium. According to the various studies carried out, no signs of toxicity were observed in two animal species (rodents and monkeys) after intravenous administration. The biodistribution in different animal models has shown that the nanoparticles accumulate passively and selectively in tumours due to the EPR effect (Enhanced Permeability and Retention effect) and are cleared via renal elimination. A radiosensitizing effect has also been observed with different types of irradiation in vitro and in vivo on several types of cancers, including radio-resistant models. These promising nanoparticles are therefore currently undergoing clinical trials combined with radiotherapy in several centres in France and in the USA. This review summarizes the main preclinical results that have led to the first administration of AGuIX[®] nanoparticles in humans.

● **Key words:** nanoparticles; radiotherapy; nanomedicine; theranostic.

Tirés à part : M. Casile

La radiothérapie est un traitement locorégional majeur en cancérologie. Plus de 50 % des patients atteints de cancer auront recours à des séances de radiothérapie durant leur prise en charge [1]. De nombreux progrès ont été réalisés au cours des dernières années. En effet, les techniques telles que la radiothérapie par modulation d'intensité, l'arcthérapie volumétrique par modulation d'intensité et la radiothérapie guidée par l'imagerie ont largement amélioré le ciblage des tumeurs et réduit les toxicités.

Les nanoparticules métalliques avec un numéro atomique (Z) élevé ont la capacité d'agir comme des radiosensibilisants. Elles permettent d'améliorer l'efficacité de l'irradiation en augmentant, au niveau des cellules tumorales, l'absorption des photons émis par le faisceau de rayonnement [2]. L'utilisation de nanoparticules pourrait ainsi être une approche prometteuse pour le traitement local des tumeurs par radiothérapie.

« Deux nanoparticules évaluées en clinique »

Malgré de nombreuses études précliniques, seuls deux types de nanoparticules ont été évalués en clinique pour leurs propriétés radiosensibilisantes. Les premières sont les nanoparticules d'oxyde d'hafnium (HfO₂) NBTXR3 qui ont montré leur efficacité pour le traitement de patients atteints d'un sarcome des tissus mous localement avancé, dans une étude internationale, randomisée, de phase 2/3. L'injection intratumorale des nanoparticules NBTXR3 en amont de la radiothérapie préopératoire a augmenté significativement le taux de réponse complète (16 % contre 8 %, p = 0,044) par rapport à une radiothérapie seule [3]. Les secondes sont les nanoparticules AGuIX[®] (Activation and Guidance of Irradiation by X-ray) à base de gadolinium développées par la société NH TherAguix (Grenoble, France) [4]. Ces nanoparticules administrées par voie intraveineuse possèdent des propriétés radio-

sensibilisantes ainsi que des propriétés d'agent de contraste, grâce à la présence de gadolinium [5].

Biodistribution et pharmacocinétique

Les nanoparticules AGuIX[®] sont des nanoparticules de très petite taille, de l'ordre de 5 nm, composées d'une matrice de polysiloxane et de chélates de gadolinium. Plusieurs études de biodistribution ont été réalisées avec différentes modalités d'administration afin d'étudier le comportement des nanoparticules AGuIX[®] dans l'organisme.

Après administration, notamment intraveineuse chez le rongeur, les nanoparticules AGuIX[®] sont rapidement éliminées par voie rénale. Ainsi, les nanoparticules sont détectées dans les reins dans les premières minutes après l'injection, puis disparaissent complètement et ne sont plus détectées deux semaines après l'administration [2]. Ces données comparables ont été obtenues chez le singe *cynomolgus* [6]. Ces deux études ont confirmé que l'élimination rénale est le mécanisme de la clairance des nanoparticules AGuIX[®] et qu'il n'y a quasiment pas de captation dans les autres organes sains.

En parallèle, des études réglementaires de toxicités et de pharmacocinétique ont été réalisées conformément aux bonnes pratiques de laboratoire sur deux espèces animales : les rats (société Wil Research) et les singes *cynomolgus* (société Wil Research) avec des administrations intraveineuses répétées [6]. Aucune toxicité n'a été observée aux doses testées. Seules des vacuolisations transitoires des tubules rénaux ont été rapportées, notamment liées à l'élimination physiologique des particules (et comparables à celles observées pour les agents de contraste à base de chélates de gadolinium utilisés en clinique), et n'altérant pas les fonctions rénales. Ces études ont permis de déterminer la dose d'AGuIX[®] à administrer lors des premiers essais cliniques réalisés chez l'homme.

Grâce à la présence de gadolinium, les nanoparticules offrent des propriétés d'agent de contraste pour l'imagerie par résonance magnétique (IRM). Ces propriétés ont permis d'évaluer la pharmacocinétique et l'absorption des nanoparticules AGuIX[®] au niveau tumoral. Les nanoparticules ont été testées sur différents modèles animaux orthotopiques de tumeurs cérébrales (gliosarcome [7], glioblastome [8] et métastases cérébrales de mélanome [4]), mais également sur des modèles de tumeurs hétérotopiques de différents types de cancers. Ces études ont montré une accumulation sélective et persistante dans le temps des nanoparticules dans les tumeurs, grâce à l'effet EPR (*Enhanced Permeability and Retention effect*) [5]. En effet, les nanoparticules sont encore détectables par IRM 24 heures après leur administration par voie intraveineuse. Ce temps d'accumulation est parfaitement adapté à la clinique. En effet, les protocoles de radiothérapie incluent un fractionnement sur plusieurs jours. De plus, aucune preuve d'extravasation dans le tissu cérébral sain n'a été apportée. Les nanoparticules ont rapidement été éliminées de la circulation. Ces résultats ont également été confirmés sur des modèles animaux de tumeurs du pancréas [2, 9], du côlon-rectum [10], des poumons [5], du sein [2] et des chondrosarcomes [11].

Voies alternatives d'administration des nanoparticules AGuIX[®]

Des voies alternatives d'administration (intra-tumorale, intrapéritonéale et intratrachéale) ont été explorées. Comme pour les nanoparticules NBTXR3, l'administration intra-tumorale a été testée *in vivo* sur des modèles murins de tumeurs des voies aérodigestives supérieures SQ20B. Cette voie d'administration a l'avantage de délivrer la quantité exacte de nanoparticules au niveau de la tumeur et permet donc une optimisation des doses délivrées [12]. En revanche, des études supplémentaires sont nécessaires afin de montrer que ce type d'administration conduit à une distribution bien homogène des nanoparticules dans la tumeur.

L'administration intrapéritonéale a été étudiée dans un modèle de carcinome hépatocellulaire. Une accumulation avec une rétention importante a été observée dans la tumeur, sans accumulation significative dans les autres tissus normaux (à l'exception des reins et de la vessie qui constituent la voie d'élimination) [13].

L'administration par aérosol a également été évaluée sur un modèle murin de tumeur du poumon et montre un passage rapide du tissu pulmonaire vers la circulation sanguine après administration intratrachéale des nanoparticules [14]. Après le passage dans la circulation sanguine, les nanoparticules se concentrent dans les tumeurs *via* l'effet EPR. Cette voie d'administration serait plus intéressante pour les tumeurs pulmonaires, car des quantités plus faibles de nanoparticules sont nécessaires pour atteindre des concentrations thérapeutiques.

Radiosensibilisation

Des études *in vitro* ont été réalisées en collaboration avec différentes équipes de recherche, prouvant ainsi l'intérêt des nanoparticules AGuIX[®] non pas seulement comme agents de contraste, mais également comme radiosensibilisants. Ces études ont été effectuées avec différents types de rayonnements et différentes lignées cellulaires, y compris des lignées radorésistantes. Dans toutes les conditions testées, une augmentation de l'efficacité de la dose d'irradiation a été observée en présence des nanoparticules AGuIX[®] par rapport à l'irradiation seule [15].

Des études ont été réalisées afin de comprendre le mécanisme de sensibilisation de la radiothérapie induit par les nanoparticules AGuIX[®] [15, 16]. Il a été montré que les nanoparticules se localisent principalement dans les lysosomes et qu'aucune nanoparticule n'a été détectée dans les noyaux des cellules. De plus, après irradiation, on constate une nette augmentation de la production de ROS (*Reactive Oxygen Species*) ainsi qu'une augmentation significative de la mort cellulaire par autophagie.

De plus, les propriétés radiosensibilisantes des nanoparticules AGuIX[®] ont été étudiées *in vivo*, chez des animaux porteurs de tumeur cérébrale [7, 17], pulmonaire [5], des voies aérodigestives supérieures [12], du pancréas [9], de mélanome [15] et de métastases cérébrales [4]. Dans toutes les conditions testées, un bénéfice en survie a été observé avec la combinaison de nanoparticules AGuIX[®] et de radiothérapie, par rapport à l'irradiation seule. Les nanoparticules semblent agir localement en augmentant l'efficacité de la dose délivrée.

Ainsi, les nanoparticules AGuIX[®] sont devenues une source d'espoir afin d'améliorer la réponse tumorale, mais également de réduire la dose délivrée et d'épargner les tissus sains dans le cas du traitement des tumeurs et des métastases radorésistantes.

« Un espoir pour lutter contre l'antibiorésistance »

Les nanoparticules AGuIX[®] peuvent être modifiées afin d'améliorer les propriétés ou d'explorer de nouvelles applications.

Le gadolinium agit comme un agent de contraste radiosensibilisant grâce à son numéro atomique élevé ($Z = 64$), mais d'autres métaux ayant un Z plus élevé comme l'or ($Z = 79$) ou le bismuth ($Z = 83$) peuvent s'avérer plus efficaces. Après une administration intraveineuse, la pharmacocinétique des nanoparticules Bi@AGuIX[®] et AGuIX[®] a été évaluée par spectrométrie de masse par plasma à couplage inductif chez des souris porteuses d'un adénocarcinome pulmonaire. Les résultats étaient similaires, montrant l'intégrité des nanoparticules, leur fixation rapide dans la tumeur, suivie de leur élimination rénale, sans rétention dans les autres organes [2]. À la suite de l'irradiation réalisée dans les 30 minutes après l'administration des nanoparticules Bi@AGuIX[®],

une nette réduction de la croissance tumorale et une augmentation de la survie ont été observées [2]. L'ajout de différents métaux peut alors fournir d'autres propriétés aux nanoparticules AGuIX[®] sans changer leur taille et leur pharmacocinétique.

Les nanoparticules AGuIX[®] peuvent également être utilisées pour d'autres approches thérapeutiques, comme la thérapie photodynamique. Cette technique implique l'utilisation d'un agent photosensibilisant qui génère, sous activation lumineuse, des espèces réactives de l'oxygène cytotoxiques [18]. Les propriétés d'agent de contraste d'AGuIX[®] permettent de délimiter la tumeur et ainsi de guider le positionnement des fibres optiques lors de cette thérapie. Cette technique a été étudiée pour le traitement du glioblastome chez des rats, après greffage d'un agent photosensibilisant sur les nanoparticules AGuIX[®] (AGuIX[®]-PS). Cinq minutes après l'administration par voie intraveineuse aux souris, les nanoparticules ont été détectées dans les tumeurs, la position de la fibre optique a été confirmée par IRM et le traitement photodynamique a été réalisé une heure après l'administration [19]. Pour le groupe ayant reçu les nanoparticules AGuIX[®]-PS et le traitement photodynamique, des effets inflammatoires ont été observés un jour après le traitement. En revanche, aucun effet n'a été observé pour le groupe témoin ayant reçu des nanoparticules AGuIX[®] et le traitement photodynamique. De manière intéressante, deux profils de réponse ont été observés dans le groupe traité, avec des répondeurs et des non-répondeurs. L'étude de dosimétrie de la lumière, réalisée par simulation Monte-Carlo sur la base du positionnement de la fibre optique et de la diffusion des photons, a permis de mettre en évidence que les répondeurs recevaient une illumination suffisante pendant le traitement photodynamique [20]. Ces données illustrent le potentiel de fonctionnalisation des nanoparticules avec des agents photosensibilisants.

Premières administrations chez l'homme

Actuellement, l'utilisation des nanoparticules AGuIX[®] est au stade de la recherche clinique. La première administration chez l'homme a été réalisée dans le cadre de l'essai clinique NANO-RAD (NCT02820454). Cet essai de phase Ib, en escalade de doses, réalisé avec des patients présentant des métastases cérébrales, a été piloté par la société NH TherAguix et le CHU de Grenoble Alpes, qui en était le promoteur. Cette étude a permis d'évaluer la tolérance de l'injection intraveineuse de nanoparticules AGuIX[®] en association avec une irradiation pan-encéphalique, grâce à une escalade de doses (15 mg/kg, 30 mg/kg, 50 mg/kg, 75 mg/kg et 100 mg/kg) chez 15 patients présentant quatre types différents de métastases cérébrales issues de plusieurs cancers primaires : mélanomes (n = 6), cancers du poumon non à petites cellules (n = 6), du côlon (n = 1) et du sein (n = 2). Cet essai clinique a montré une bonne

tolérance de l'injection intraveineuse des nanoparticules AGuIX[®], ainsi que leur capacité à s'accumuler sélectivement dans les métastases cérébrales et à permettre leur détection par IRM jusqu'à une semaine après l'administration. De plus, une corrélation entre la concentration en AGuIX[®] dans les tumeurs (mesurable par IRM) et la réduction du volume tumoral ainsi qu'un bénéfice clinique pour 13 patients sur 14 évaluables ont été démontrés [21].

Les résultats prometteurs de la phase I ont permis de poursuivre le développement clinique d'AGuIX[®] dans cette indication avec deux essais de phase II. Le premier essai NANORAD 2 (NCT03818386), issu d'une collaboration entre la société NH TherAguix et le CHU de Grenoble Alpes, est un essai multicentrique et randomisé, qui évalue l'efficacité de la combinaison de trois injections intraveineuses de nanoparticules AGuIX[®] avec une radiothérapie pan-encéphalique dans le traitement des métastases cérébrales multiples [22].

Le second essai NANOBRAINMETS (NCT04899908), conduit par le Dana Farber/Brigham and Women's Cancer Center (Boston, États-Unis), en collaboration avec la société NH TherAguix, a pour but d'évaluer l'efficacité de la combinaison de deux ou trois injections intraveineuses de nanoparticules AGuIX[®] avec une irradiation stéréotaxique pour le traitement de métastases cérébrales [23].

« D'autres indications cliniques possibles »

Les nanoparticules AGuIX[®] sont également évaluées dans d'autres indications, notamment le cancer avancé du col de l'utérus dans le cadre de l'étude de phase I NanoCOL (NCT03308604) en collaboration avec l'Institut Gustave Roussy (Villejuif, France) [24] ou le cancer du pancréas et le cancer du poumon dans l'étude NanoSMART (NCT04789486), en collaboration avec le Dana Farber/Brigham and Women's Cancer Center [25].

Enfin, en collaboration avec le Centre Jean Perrin (Clermont-Ferrand), un essai de phase I/II NANO-GBM (NCT04881032) va être initié et aura pour objectif d'évaluer l'association des nanoparticules AGuIX[®] avec une radiochimiothérapie avec témozolomide concomitant chez des patients atteints d'un glioblastome nouvellement diagnostiqué. L'objectif de la phase I sera de déterminer la dose optimale des nanoparticules AGuIX[®] à utiliser en association avec la radiochimiothérapie pour la phase II qui, elle, évaluera l'efficacité et la tolérance de cette association.

Conclusion

Les nanoparticules AGuIX[®] présentent un potentiel radiosensibilisant élevé, une accumulation sélective et assez longue au niveau tumoral et une bonne élimination rénale. Tous ces arguments permettent de limiter la dose

ionisante délivrée et d'épargner les organes sains. Elles sont généralement administrées par voie intraveineuse, mais pour des applications spécifiques peuvent également être administrées localement. Leur effet thérapeutique a été prouvé *in vivo* sur huit modèles de tumeurs différents, y compris des modèles radiorésistants. De plus, l'étude NANO-RAD, de première administration chez l'homme, a donné des résultats prometteurs en termes de tolérance de l'administration intraveineuse d'AGuIX[®], de distribution tumorale et d'efficacité d'AGuIX[®] en association avec une radiothérapie pan-encéphalique pour le traitement de patients ayant des métastases cérébrales. Ainsi, les nanoparticules AGuIX[®] pourraient offrir de nouvelles perspectives théranostiques en cancérologie, en permettant de détecter, d'imager et de traiter les cellules cancéreuses.

Take home messages

Les nanoparticules AGuIX[®] :

- ne présentent pas de toxicités après administration ;
- possèdent des propriétés radiosensibilisantes très élevées ;
- s'accumulent sélectivement dans les tissus tumoraux en épargnant les tissus sains.

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4. La prise en charge des récurrences

L'évaluation de la réponse tumorale se base sur les critères RANO. Une IRM doit être réalisée dans les 3 mois après la fin du traitement. Une prise de contraste de la tumeur dans les 12 semaines suivant la fin de la radiothérapie n'est pas forcément liée à une progression tumorale. En effet, cela peut être lié à l'effet du traitement, on parle alors de pseudo-progression (60). Une attention particulière est nécessaire lors de l'interprétation de ces imageries pendant cette période. En cas de doutes, un nouvel examen sera reprogrammé rapidement. Une évaluation sera par la suite réalisée environ tous les 3 mois environ afin de déterminer la réponse tumorale.

En cas de progression, l'augmentation de la taille de la tumeur pourra induire un changement de la PIC selon la doctrine de Monro-Kellie (32,33). En effet, si la tumeur continue de croître, les principaux mécanismes compensatoires vont s'épuiser jusqu'à ce que l'équilibre de pression soit rompu, pouvant être à l'origine d'une hypertension intracrânienne. Cette PIC peut être monitorée par un capteur intra parenchymateux invasif ou une dérivation ventriculaire. De par son caractère invasif, ces méthodes ne peuvent être utilisées pour suivre en continu la PIC des patients atteints d'un gliome diffus de l'adulte (61). Ainsi, les oncologues s'appuient uniquement sur les signes cliniques et les imageries réalisées dans le cadre du suivi pour monitorer la PIC.

Les normes de soins en cas de progression tumorale ou de récurrence sont bien moins définies. Les traitements peuvent comprendre une chirurgie supplémentaire, une ré-irradiation, le recours à des traitements systémiques voir à des soins de soutien. Le type de traitement envisagé dépendra de l'état général du patient et des thérapies précédemment réalisées (34,62).

Une seconde chirurgie est envisagée 6 mois après la première intervention chirurgicale ou en cas d'une récurrence précoce chez des patients présentant une tumeur symptomatique. Une ré-irradiation peut être discutée en prenant en compte la zone précédemment irradiée, la dose délivrée et le délai. Les principales options de traitements systémiques comprennent les nitroso-urées, le témozolomide et le bevacizumab. Malheureusement, leur efficacité reste modeste avec une survie sans progression à 6 mois d'environ 20% (63,64). L'utilisation du bevacizumab, en association avec l'irinotecan, est approuvée en Amérique Nord sur la base

de deux essais cliniques non contrôlés (65,66). Il n'est pas approuvé dans l'union européenne, n'ayant pas démontré son efficacité à l'aide d'essais cliniques contrôlés et randomisés.

5. Les soins de support et soins palliatifs

Les patients atteints d'un gliome diffus souffrent de déficits neurologiques qui peuvent être importants et progressifs tout au long de la maladie. Les convulsions sont l'un des symptômes courant avant ou après la chirurgie. Les patients ayant des antécédents cliniques épileptiques ou ayant présenté des convulsions doivent donc être traités avec un médicament antiépileptique. Le lévétiracétam est recommandé pour sa sécurité et parce qu'il présente peu d'interactions avec les médicaments utilisés en routine (67).

Les corticostéroïdes peuvent être prescrits avant la chirurgie afin de réduire un œdème cérébral, pouvant augmenter la pression intracrânienne, améliorer les céphalées et les déficits neurologiques (34). Le dexaméthasone est le corticostéroïde le plus utilisé (68). Une diminution rapide et l'arrêt des corticostéroïdes est recommandée afin d'éviter les toxicités importantes liées à ce traitement comme la lymphopénie ou le syndrome de Cushing.

Le risque d'événements thromboemboliques chez ces patients est accru (jusqu'à 20% des patients à 1 an) (69). Les anticoagulants ne sont pas recommandés, mais afin d'exclure tout risque d'une thrombose veineuse profonde ou d'une embolie pulmonaire, les héparines de bas poids moléculaire sont indiquées en cas de symptômes suspects (62).

Les soins palliatifs et les soins de soutien sont parfois les traitements les plus appropriés selon les patients. La prise en charge des symptômes, tels que la fatigue, les troubles d'humeur et de comportements et les troubles cognitifs doit être primordiale pour améliorer la qualité de vie de ces patients. Faire face à de tels changements neurologiques et physiques peut être accablant pour les patients et l'entourage entraînant de l'anxiété, de la dépression et de l'isolement (70).

B. LA RADIORESISTANCE

I. La radiothérapie externe

La radiothérapie est aujourd'hui une spécialité médicale incontournable dans la prise en charge oncologique. Plus de 200 000 patients atteints de cancer sont traités chaque année en France par radiothérapie (71,72), devenant ainsi l'un des traitements majeurs en cancérologie. En effet, plus de la moitié des patients atteints d'un cancer sont traités avec de la radiothérapie au cours de leur parcours de soins (73). Son utilisation peut être curative, mais également palliative ou symptomatique. Si la source de rayonnement est au plus proche de la tumeur, à l'intérieur du corps du patient, on parlera alors de curiethérapie. A l'inverse, lorsque celle-ci est à l'extérieur du patient, *via* l'utilisation d'un accélérateur linéaire de particules, on parlera de radiothérapie externe. La radiothérapie externe est un traitement locorégional qui peut être utilisé de manière exclusive ou en complément d'un traitement chirurgical soit de façon adjuvante ou néo-adjuvante. La radiothérapie externe fait partie intégrante des modalités de prises en charge de nombreux cancers et notamment des gliomes diffus de l'adulte.

1. Les mécanismes d'actions

La première étape, en radiothérapie externe, est la production de radiations ionisantes à l'aide d'un accélérateur linéaire de particules. Les électrons sont accélérés à grande vitesse et envoyés sur une cible de tungstène qui génère des photons de haute énergie. Ceux-ci sont ensuite modelés par les lames d'un collimateur, permettant d'ajuster la forme du faisceau à la cible thérapeutique (**Figure 4**). Cet ajustement permet de préserver au maximum le tissu sain environnant.

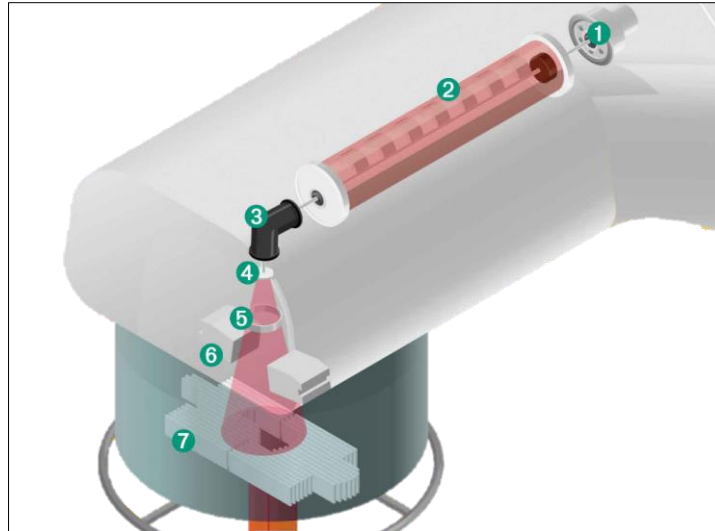


Figure 4 : Schéma d'un accélérateur linéaire de particules utilisé pour la radiothérapie externe

Source : www.cea.fr

L'accélérateur linéaire est composé d'une source d'électrons (1), qui seront ensuite accélérés au niveau d'une section linéaire (2). Un aimant de déviation des électrons (3) permet de sélectionner et filtrer l'énergie pour la focaliser sur une cible en tungstène (4), transformant le faisceau d'électrons en photons. Puis, le collimateur primaire (5) va permettre de mettre en forme ce faisceau, dont le débit sera contrôlé par le dosimètre (7). Pour finir, un collimateur multi-lames permet d'adapter la forme du faisceau au volume de la tumeur, en temps réel, au cours de la séance de radiothérapie externe.

Les radiations ionisantes sont des rayonnements capables de générer des ionisations dans la matière en ôtant des électrons aux atomes des molécules du milieu. Ces rayonnements sont constitués de flux de particules chargées (électrons, protons) ou neutres (neutrons) ou bien de particules sans masse non chargées, les photons, qui sont des rayonnements électromagnétiques. L'effet biologique de l'irradiation résulte de la dose utilisée, du débit de dose, mais également de l'absorption d'énergie dans les structures cellulaires à l'intérieur desquelles l'énergie est distribuée de manière non uniforme.

Les radiations ionisantes exercent leurs effets biologiques de façon directe et indirecte via la radiolyse de l'eau. Elles exercent leurs effets biologiques sur l'ADN, en premier lieu, de façon directe. Les cellules sont altérées très rapidement, à l'échelle atomique et moléculaire conduisant à la formation de radicaux libres comme les cations H_2O^+ , ADN^+ , protéines⁺ en

autres. Dans un second temps, interviennent des actions physico-chimiques qui sont majoritaires et dites indirectes. La radiolyse de l'eau forme des radicaux libres très réactifs tels que les radicaux hydroxyles ($\text{OH}\cdot$), les radicaux superoxydes ($\text{O}_2\cdot^-$) et l'eau oxygénée (H_2O_2). Ces derniers vont ensuite interagir et altérer les biomolécules environnantes dont l'ADN. Ces radicaux libres issus de la radiolyse de l'eau s'ajoutent aux radicaux libres déjà présents dans la cellule et issus notamment de l'activité mitochondriale. Les radiations ionisantes sont donc à l'origine d'un stress oxydatif supplémentaire pouvant endommager toute la cellule et majoritairement l'ADN (74) (**Figure 5**)

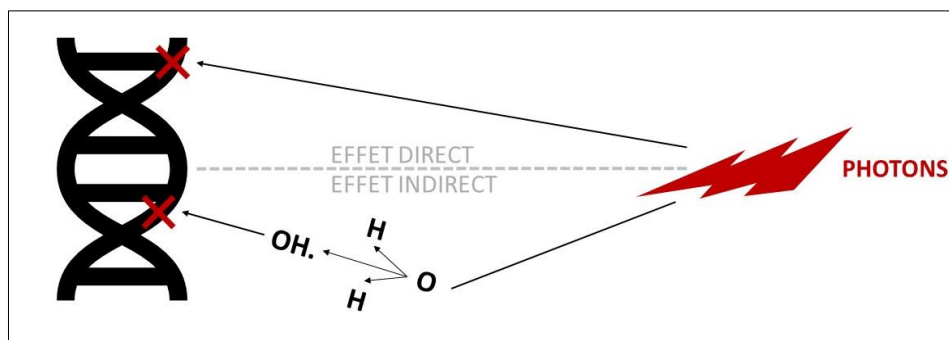


Figure 5 : Effets directs et indirects des radiations ionisantes sur l'ADN

Source : Création personnelle

Schéma illustrant les effets biologiques des radiations ionisantes sur l'ADN.

Les photons agissent soit directement sur l'ADN soit en ionisant la molécule H_2O , ce qui produira des radicaux libres qui pourront endommager l'ADN.

Au niveau de celui-ci, les radiations ionisantes induisent principalement des modifications de bases, des cassures simple brin et / ou des cassures doubles brins (75). Pour une dose de 1 Gy délivrée à une cellule, on considère induire 1 000 à 2 000 lésions de bases, 500 à 1 000 lésions simple brin, environ 200 pontages/protéines et 40 lésions double brin. La plupart des cellules ne manifestent pas de signes de lésions immédiatement après la séance de radiothérapie. Celles-ci se manifestent lorsque que la cellule entre en mitose. En effet, c'est au moment de la duplication de l'ADN que la cellule va détecter les altérations et conduire, s'il lui est impossible de réparer ces dommages, à la mort cellulaire. Il s'agit de la mort cellulaire mitotique. Ceci est donc intrinsèquement lié à la capacité de division de chaque type cellulaire.

Enfin, les radiations ionisantes peuvent également activer des mécanismes obligeant la cellule à entrer en apoptose ou mort cellulaire programmée (74).

2. Les 5 R de la radiothérapie

Depuis 1975, les radiothérapeutes se basaient essentiellement sur le principe des « 4 R's de la radiothérapie » publié par Withers, à savoir la réparation cellulaire, la redistribution cellulaire dans le cycle, la repopulation tumorale et la réoxygénation tumorale (76). La radiosensibilité intrinsèque a été ajoutée quelques années plus tard. On parle alors des « 5 R's de la radiothérapie » (77).

Le premier « R » évoque la réparation cellulaire. Les cellules saines, grâce à des mécanismes de réparations de l'ADN fonctionnels, sont plus à même de réparer les lésions causées par les radiations ionisantes. En revanche, la plupart des cellules tumorales présentent des altérations importantes de leurs systèmes de réparation, les rendant inactifs et empêchant ainsi la survie de ces cellules tumorales. Certaines cellules peuvent toutefois échapper à la mort cellulaire malgré un taux de cassures de l'ADN important et être la source d'une rechute tumorale. Le deuxième « R » correspond à la redistribution cellulaire dans le cycle engendrée par les arrêts induits par les radiations ionisantes bloquant la progression du cycle cellulaire. Ainsi après une exposition à des radiations ionisantes, les cellules ont tendance à se bloquer à la transition G1-S et G2-M du cycle cellulaire permettant aux mécanismes de réparation de se mettre en place et d'éviter la division cellulaire qui pourrait être fatale (78). De plus, sachant que la radiosensibilité des cellules est différente selon les phases du cycle cellulaire, cette redistribution va également impacter le devenir de ces cellules (79,80). La phase S présente notamment une forte capacité de réparation des cassures doubles brin grâce à une recombinaison homologue très active. Elle est donc associée à une moindre sensibilité aux radiations ionisantes. En effet, la capacité des cellules tumorales à réparer, fidèlement ou non, les lésions induites par les radiations ionisantes, influence la réponse tissulaire globale (81). Si la prolifération cellulaire prend l'ascendant sur la mort cellulaire radio-induite, il y aura une croissance tumorale. Le troisième « R » se rapporte à la repopulation tumorale entre deux fractions d'irradiation. Après une exposition à des rayonnements ionisants, la vitesse de croissance du nombre de cellules tumorales est accélérée, influençant la réponse tumorale (82). Cette capacité de croissance cellulaire sera donc déterminante pour l'efficacité du traitement. Le quatrième « R » correspond à la réoxygénation tumorale. La sensibilité de

chaque tissu aux radiations ionisantes dépend du taux d'oxygène car il permet la création des radicaux libres par la radiolyse de l'eau. Les tumeurs plus radiosensibles présenteraient une réoxygénation supérieure aux tumeurs plus résistantes. Le dernier « R » est le concept de radiosensibilité intrinsèque. Les caractéristiques intrinsèques des cellules tumorales (altérations génétiques, altérations des voies de signalisation, capacité de réparation des lésions radio-induites, capacité de prolifération) constituent le déterminant principal de la radorésistance. En effet, il a été démontré que les lignées cellulaires issues de tumeurs répondant à la radiothérapie étaient radiosensibles *in vitro* alors que des lignées dérivées de tumeurs ne répondant que faiblement à la radiothérapie présentaient une résistance élevée à l'irradiation *in vitro* (83). L'efficacité des radiations ionisantes est dépendante de la dose délivrée. En théorie, plus cette dose est élevée, plus l'efficacité sera élevée. En revanche, en pratique un seuil de tolérance doit être déterminé afin d'éviter des effets secondaires importants. Cette dose seuil, dépend du type cellulaire et est dépendante de chaque individu. En routine, les doses sont standardisées et calculées en fonction de la population générale. L'un des enjeux majeurs de la radiothérapie externe est donc de pouvoir déterminer cette radiosensibilité intrinsèque afin de pouvoir délivrer un traitement personnalisé, améliorant ainsi l'efficacité et la diminution des effets secondaires importants (84). La recherche de facteurs cellulaires ou tumoraux intrinsèques comme des biomarqueurs de substitution prédictifs de la réponse aux rayonnements ionisants est donc l'un des défis majeurs de la radiothérapie externe et de la médecine personnalisée.

Ces cinq principes mettent en évidence un effet différentiel de la radiothérapie sur les cellules saines et tumorales en raison notamment de différentes capacités de réparation. Ces différences sont alors mises à profit dans le fractionnement des doses en radiothérapie externe. Cette technique permet de laisser suffisamment de temps aux cellules saines pour réparer les altérations mais pas suffisamment de temps pour que les cellules tumorales puissent y parvenir complètement. Elle permet d'exploiter ces différences en réalisant un compromis entre le nombre de fractions suffisants afin de protéger les cellules saines et un temps réduit pour éviter la repopulation des cellules tumorales.

3. Le processus de traitement par radiothérapie externe

En premier lieu, un scanner dosimétrique est réalisé. Celui-ci permet d'acquérir des images anatomiques tridimensionnelles en position de traitement. Des outils comme des masques

thermoformés, des cales ou d'autres systèmes de contentions permettent la reproductibilité du positionnement entre le scanner dosimétrique et les séances de radiothérapie. Cet examen d'imagerie tridimensionnel permet également au radiothérapeute d'établir un plan de radiothérapie en déterminant les volumes cibles et des organes à risques. D'autres imageries comme une IRM ou une tomographie par émission de positons (PET) pourront être fusionnées, si besoin, avec le scanner dosimétrique.

Pour définir les volumes, il existe des recommandations disponibles dans les différents rapports de la commission internationale des unités et mesures de rayonnement (ICRU) (85–88). Pour les tumeurs en place, le premier volume à définir est le GTV (gross tumor volume) correspondant à toute lésion maligne macroscopique, que l'on peut détecter à un examen clinique ou qui se visualise sur les imageries selon l'ICRU 50. Quand cela est approprié, il peut être divisé en volume tumoral (GTV-T) et volume nodale (GTV-N). Le second volume à déterminer est le CTV (clinical-anatomical target volume). Il englobe le GTV ainsi que les extensions microscopiques non visibles à l'imagerie autour de la tumeur mais pouvant être potentiellement atteintes. Comme pour le GTV, celui-ci peut être divisé en CTV-T et CTV-N. Le dernier volume décrit par l'ICRU 50 est le PTV (planning target volume) qui englobe le CTV plus une marge de sécurité. Cette marge permet de prendre en compte d'éventuelles incertitudes notamment liées au repositionnement du patient (**Figure 6**).

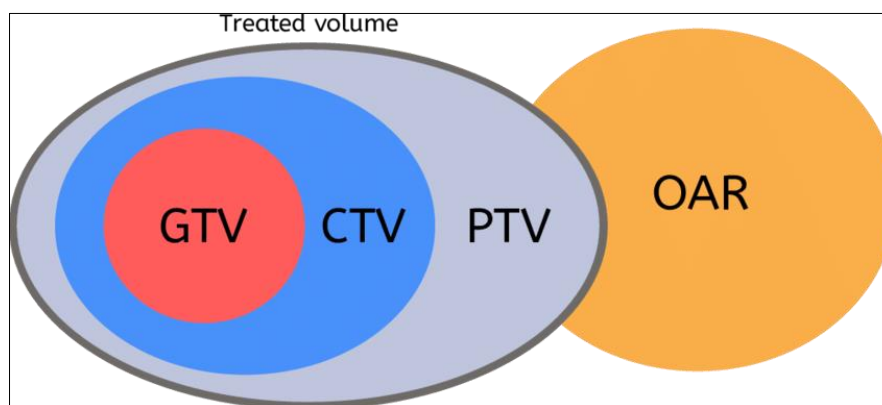


Figure 6 : Volumes définis par la commission internationale des unités et mesures de rayonnement

Source : Semanticscholar.org

Les volumes à définir sont le GTV (gross tumor volume) correspondant à toute lésion maligne macroscopique, le CTV (clinical-anatomical target volume) englobant le GTV ainsi que les extensions microscopiques et le PTV (planning target volume) englobant le CTV plus une marge de sécurité.

Pour finir, les conditions de délinéation des organes à risque sont également décrites dans ces rapports. Les organes ont une architecture soit en série, soit en parallèle et n'ont donc pas la même tolérance fonctionnelle. L'irradiation d'organes en série comme la moelle épinière entraînera des conséquences sur les organes en aval. Alors qu'un organe en parallèle comme le poumon est indépendant. Il faut donc le prendre en compte lors de la délinéation des organes à risque dont les fonctions doivent être préservées après irradiation.

Une fois les volumes établis, le radiothérapeute en collaboration avec les dosimétristes et les médecins planifie les doses et les contraintes dose-volume pour les organes à risque. Celles-ci sont enregistrées dans un compte-rendu conformément aux recommandations disponibles dans les rapports de l'ICRU.

4. Les techniques en radiothérapie externe

La radiothérapie conformationnelle tridimensionnelle utilise des faisceaux fixes conformés à la forme de la tumeur pour le traitement grâce à l'utilisation d'images en trois dimensions obtenues à l'aide d'un scanner, une IRM ou une TEP (Tomographie par émission de positons). Ces images tridimensionnelles permettent de visualiser le volume cible et le volume des organes à risques. Au cours de la séance de radiothérapie, la forme de chacun des faisceaux est complexe mais reste fixe, délivrant une dose homogène (89). On constate depuis plusieurs années une diminution de l'utilisation de celle-ci (73).

Contrairement à la radiothérapie conformationnelle tridimensionnelle, la radiothérapie conformationnelle avec modulation d'intensité (RCMI) utilise les variations d'intensité du faisceau pour optimiser l'irradiation du tissu tumoral en diminuant l'irradiation absorbée par les tissus sains environnants. L'intensité des faisceaux est modulée pendant la séance grâce aux lames du collimateur qui filtrent plus ou moins l'intensité du rayonnement modulant ainsi la dose délivrée point par point (89).

De cette dernière technique a émergé plus récemment l'arcthérapie volumétrique par modulation d'intensité (VMAT). Cette technique de RCMI rotationnelle délivre l'irradiation de manière hélicoïdale. Cela permet de varier la forme du faisceau, le débit de la dose délivrée et la vitesse de rotation du bras autour du patient (89).

La radiothérapie stéréotaxique permet de réaliser une irradiation à forte dose avec une précision millimétrique grâce à l'utilisation de faisceaux de petites dimensions. La dose est

délivrée lors d'une seule séance ou de façon hypofractionnée sur plusieurs séances. Cette précision nécessite un repositionnement extrêmement précis du patient. Cette technique est utilisée pour de petits volumes comme les métastases cérébrales. La radiothérapie stéréotaxique est dans la pratique courante réalisée après la chirurgie des métastases. L'utilisation de cette technique en pré-opératoire, afin d'avoir une meilleure visualisation des contours des métastases est en cours d'essai clinique dans 7 centres en France. L'étude de phase II intitulée STEP, évaluant la radiothérapie stéréotaxique hypofractionnée préopératoire des métastases cérébrales, présentée dans la partie [Annexe 1 : Travaux collaboratifs en neuro-oncologie](#), est promue par le Centre Jean PERRIN (NCT04503772) (90,91).

II. Les origines de la radiorésistance

Malgré les avancées technologiques et thérapeutiques, la réponse à la radiothérapie peut être affectée par de nombreux facteurs. On parle de radiorésistance lorsque la réponse au traitement est défavorable, se manifestant par une récurrence.

1. Les facteurs cliniques tumoraux

Les tumeurs infiltrantes ou ayant un grand volume présentent une charge tumorale plus élevée à l'origine, généralement, d'un pronostic plus défavorable. Plus le volume tumoral est important, plus il est difficile d'endommager toutes les cellules tumorales. Et cela, malgré une dose ionisante élevée. Ainsi une influence pronostique significative du volume tumoral sur le résultat de la radiothérapie a été retrouvée pour différentes tumeurs (92).

2. Les facteurs biologiques ou intrinsèques

Les cellules tumorales mettent à profit leurs caractéristiques intrinsèques (capacité de réparation de lésions, activation de voies de survie cellulaire, modifications métaboliques *etc.*) dérivant de leurs altérations génétiques afin de survivre aux différents traitements, puis continuer à proliférer et potentiellement migrer vers d'autres organes. Parmi ces facteurs intrinsèques de résistance à la radiothérapie on retrouve différentes voies de signalisation.

2.1. La voie de signalisation EGFR

Le récepteur du facteur de croissance épidermique (EGFR) est à l'origine de plusieurs voies de signalisation induisant la prolifération cellulaire, l'angiogenèse, la migration cellulaire et l'inhibition de l'apoptose. Ces voies participent au développement normal de l'organisme. En

revanche, à la suite de mutations activatrices, ces voies sont activées de façon aberrante dans de nombreux cancers. Ainsi, la voie PI3K/AKT/mTOR et la voie RAS/RAF/MEK/ERK sont impliquées dans la cancérogenèse. Cette activation se réalise à la suite de la liaison de facteurs de croissance ou d'interleukines au récepteur EGFR. Cette liaison induit une dimérisation du récepteur, activant sa fonction tyrosine kinase à l'origine de sa transphosphorylation. Un certains nombres de protéines vont par la suite s'associer au domaine intracellulaire du récepteur pour finalement activer différents effecteurs, en cascade, jusqu'au noyau cellulaire (93,94) (**Figure 7**). La repopulation tumorale entre deux fractions d'irradiation est l'un des facteurs critique de la radiorésistance. A chaque fraction de radiations ionisantes, les cellules tumorales sont qu'en partie détruites. Les cellules tumorales survivantes vont quant- à -elles proliférer. Un compromis entre le nombre de fractions suffisants protégeant les tissus sains et un temps court pour éviter au maximum cette repopulation tumorale est essentiel. L'un des gènes le plus fréquemment altéré chez les patients atteints d'un glioblastome code pour le récepteur EGFR qui est amplifié dans environ 50% des glioblastomes (95). En plus de cette amplification, les patients atteints d'un glioblastome peuvent présenter également une mutation fréquente qui est la délétion des exons 2-7 appelée EGFRvIII. C'est une forme constitutivement active du récepteur, de faible intensité, sans domaine de liaison (95,96). Malheureusement, aucun traitement ciblant ce récepteur n'a apporté à ce jour de bénéfice clinique pour les patients. A titres d'exemples, l'étude ACT IV (NCT01480479) portant sur l'ajout du vaccin rindopepinut à la chimiothérapie a dû être arrêté pour futilité (97). Cet essai clinique de phase III, randomisé, en double aveugle portant sur 745 patients atteints de glioblastome exprimant EGFRvIII n'a pas mis en évidence d'effet significatif chez ces patients (98). Tout comme l'étude INTELLANCE-1 (NCT02573324), portant sur l'utilisation du depatuxizumab mafodotin en association à une radiothérapie et une chimiothérapie de type témozolomide (99). Cet essai de phase 3, randomisé, réalisé sur 639 patients a également été stoppé pour futilité, ne prouvant aucun bénéfice (100). Une meilleure compréhension de la signalisation cellulaire entre le récepteur EGFR et EGFRvIII et la clarification des mécanismes de résistance sont nécessaires afin d'améliorer la recherche de nouvelles thérapies ciblées (96).

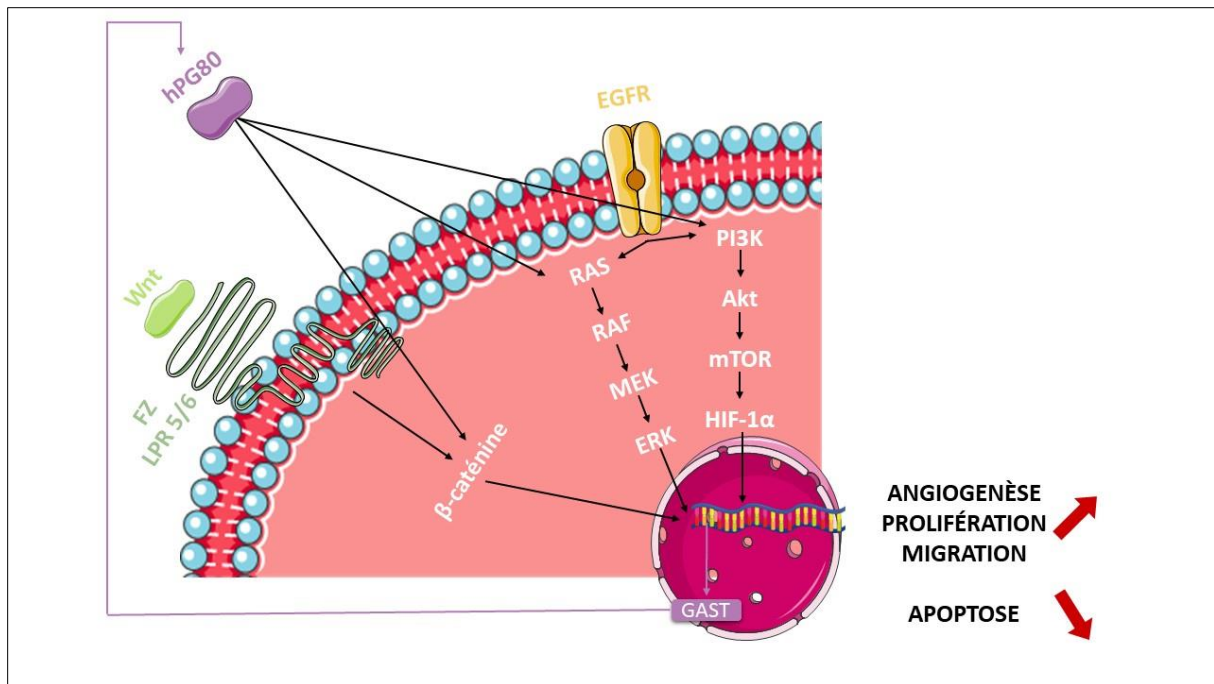


Figure 7 : Voies de signalisation cellulaire impliquées dans la radiorésistance

Source : Création personnelle grâce à smart.servier.com

Les voies de signalisation PI3K/AKT/mTOR ; RAS/RAF/MEK/ERK et Wnt/ β -caténine sont impliquées dans la cancérogenèse. L'activation aberrante de ces voies de signalisation dans les cellules cancéreuses augmente l'angiogenèse, la prolifération, la migration cellulaire et diminue l'apoptose favorisant le développement du cancer.

2.2. L'hypoxie

L'hypoxie influence également la réponse à la radiothérapie, jouant un rôle majeur dans le pronostic. La privation d'oxygène au niveau des cellules tumorales conduit à une baisse des actions physico-chimiques des radiations ionisantes. Le manque d'oxygène dans la cellule va diminuer la production des radicaux libres à l'origine des dommages à l'ADN (101). Une tumeur en situation d'hypoxie sera donc plus résistante aux radiations ionisantes en évitant l'apparition d'altérations ou de cassures à l'origine de l'arrêt du cycle cellulaire voir de l'apoptose. L'hypoxie est également à l'origine d'un pH légèrement acide. Dans ces conditions, les membranes cellulaires sont sujettes à un courant de sodium *via* l'activation de canaux ioniques permettant aux cellules tumorales de modifier leur forme et leur volume favorisant ainsi leur caractère infiltrant (102). De plus, cette privation d'oxygène va également induire des modifications au niveau de la voie PI3K/AKT/mTOR et de l'expression de certains gènes (**Figure 7**). Le facteur de transcription HIF1 α (Hypoxia Inducible Factor) s'accumule dans la

cellule dans des conditions hypoxiques. Puis, il migre vers le noyau où il se dimérise avec la sous-unité HIF1 β . Pour finir, cet hétérodimère va se lier aux éléments de réponse à l'hypoxie présents au niveau des régions régulatrices de gènes impliqués dans l'angiogénèse, le métabolisme et le pouvoir métastatique (103,104) retrouvés notamment dans les gliomes diffus de haut grade (105) (**Figure 8**). De ce fait, l'hypoxie réduit considérablement l'efficacité de la radiothérapie et diminue le contrôle local et la survie globale des patients (106). C'est un facteur clé de l'angiogénèse et du développement tumoral raison pour laquelle il est au cœur de la recherche en cancérologie. A titre d'exemple, l'utilisation de la TEP combinée à un scanner est toujours en cours d'étude. Cette technique d'imagerie permettrait d'identifier les zones hypoxiques et ainsi permettre une adaptation des doses de radiations ionisantes (107).

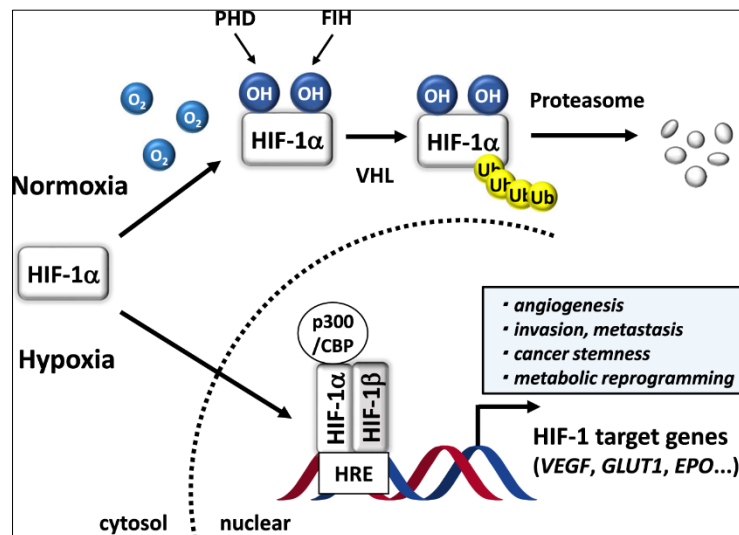


Figure 8 : Voie de signalisation du facteur de transcription HIF1 α en normoxie et hypoxie

Source : Hiroaki IKEDA (108)

En situation de normoxie, le facteur de transcription HIF1 α est ubiquitinylé puis dégradé par le protéasome.

En situation d'hypoxie, le facteur de transcription HIF1 α s'accumule et migre vers le noyau où il se dimérise avec la sous-unité HIF1 β et se lie à aux éléments de réponse à l'hypoxie (HRE). Ceux-ci sont présents au niveau des régions régulatrices de gènes impliqués dans l'angiogénèse, le métabolisme et le pouvoir métastatique.

2.3. La voie de signalisation Wnt/ β -caténine/ hPG80

En se liant aux récepteurs FZ (Frizzled) et LRP5/6 (LDL-related protein), la protéine Wnt active la voie de signalisation Wnt/ β -caténine et induit l'arrêt de la phosphorylation de la β -caténine. De ce fait, cette protéine ne se conjugue plus à l'ubiquitine et n'est pas dégradée par le

protéasome. C'est ainsi que la β -caténine, libre dans le cytoplasme, entre dans le noyau et s'associe à des facteurs de transcription présents au niveau des régions régulatrices de gènes impliqués dans la prolifération cellulaire (109) (**Figure 7**). Cette voie participe également au développement normal de l'organisme, mais est activée de façon aberrante dans de nombreux cancers.

Le dérèglement de la voie Wnt/ β -caténine et de la voie RAS/RAF/MEK/ERK induisent l'activation de l'expression du gène GAST (**Figure 7**). Il code pour un peptide précurseur de 101 acides aminés, la pré-progastrine, qui génère la progastrine après plusieurs modifications post-traductionnelles. Cette pro-hormone de 80 acides aminés est ensuite clivée par des pro-hormone convertases et des carboxypeptidases. Ainsi sont formées deux gastrines à extension glycine (G34-Gly et G17-Gly). Ces extensions glycine servent ensuite de substrats pour les peptidyl- α -amidating mono-oxygénases. Cela entraîne la formation des deux formes majoritaires biologiquement actives de la gastrine présentes dans l'antré de l'estomac : G34-NH₂ pour la gastrine à 34 acides aminés et G17-NH₂ pour la gastrine à 17 acides aminés (110,111) (**Figure 9**). Ces deux formes actives permettent de contrôler les sécrétions acides pendant la digestion.

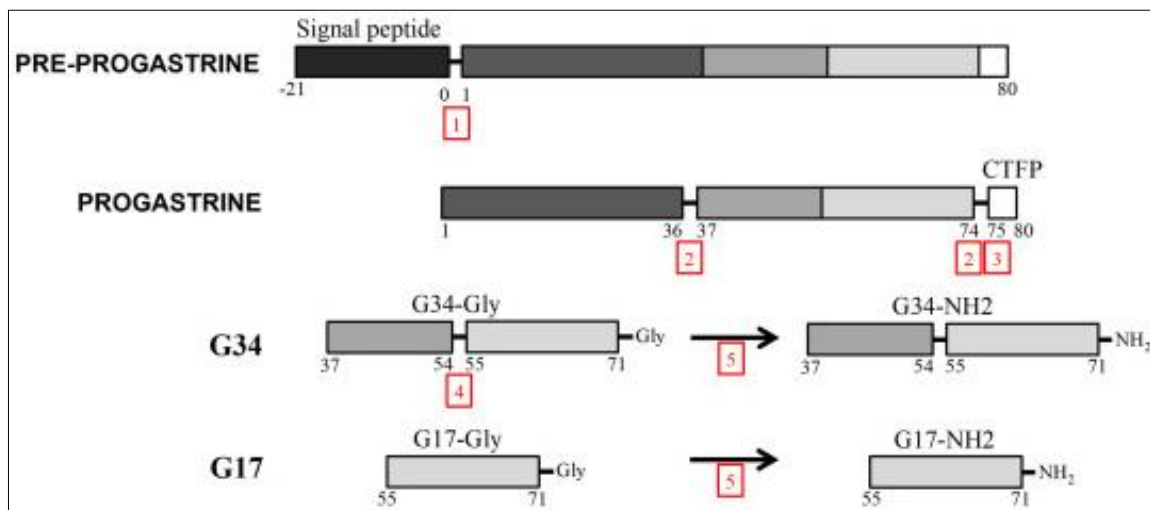


Figure 9 : Maturation de la pré-progastrine en gastrine

Source : Benoit YOU (112)

Les chiffres en rouge indiquent les enzymes de maturation : 1 = signal peptidase, 2 = pro-hormone convertase 1/3, 3 = carboxypeptidase E, 4 = pro-hormone convertase 2, et 5 = peptidyl- α -amidating-mono-oxygénase.

G34 pour gastrine-34 et G17 pour gastrine-17

En situation pathologique, le dérèglement de la voie Wnt/ β -caténine dans les cancers induit l'activation de l'expression du gène GAST codant pour la progastrine et sa sécrétion sous la forme circulante hPG80 (41). Son rôle a longtemps été étudié dans le cancer colorectal. Ainsi, son implication dans la formation des jonctions cellulaires, l'inhibition de l'apoptose, la régulation des cellules souches cancéreuses, l'angiogénèse et dans la régulation de la voie de signalisation Wnt a été prouvé dans la littérature (113–118) (**Figure 10**). De plus, sur différentes lignées cellulaires de cancers colorectales, il a été observé tout d'abord une augmentation de l'expression du gène progastrine après une irradiation. Puis, il a été constaté que l'inhibition de l'expression de ce gène augmenterait significativement la sensibilité de ces cellules à l'irradiation, l'apoptose radio-induite et le niveau de dommages à l'ADN induits par les radiations ionisantes (119).

L'essai clinique PROGLIO (NCT05157594) présenté dans la partie **4-TRAVAUX DE RECHERCHES** a pour objectif d'évaluer les concentrations plasmatiques de l'hPG80 chez des patients atteints d'une tumeur cérébrale de haut grade (1,2).

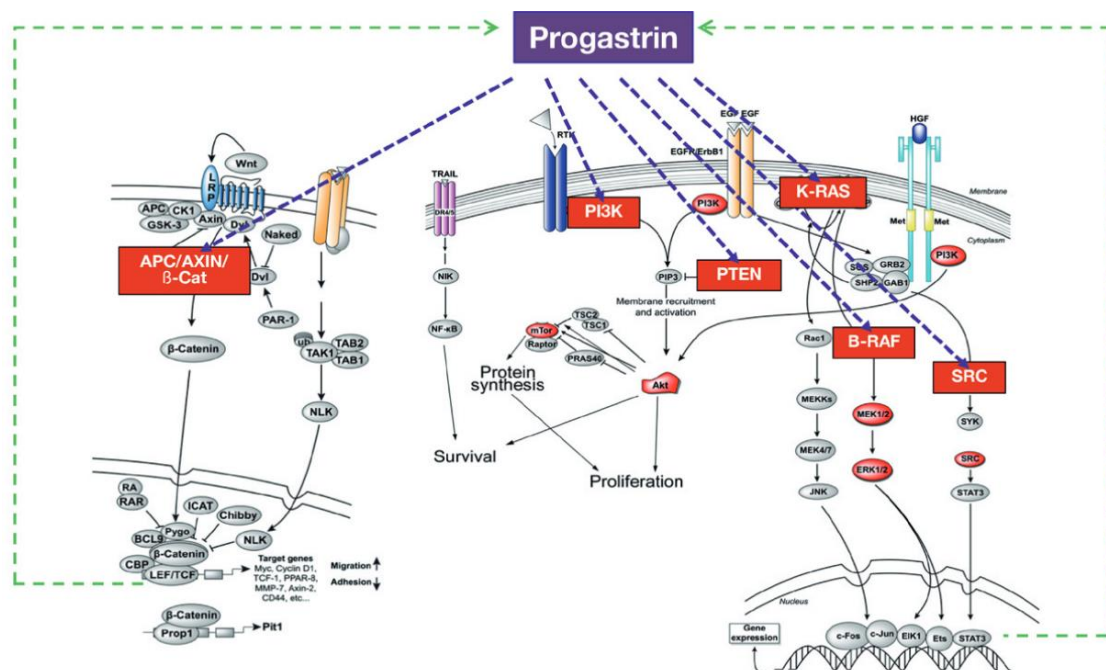


Figure 10 : La progastrine, pierre angulaire des voies oncogénique

Source : progastrin-cancer-control.org

L'hPG80 et son implication dans les différentes voies oncogénique à l'origine de la formation des jonctions cellulaires, l'inhibition de l'apoptose, la régulation des cellules souches cancéreuses, l'angiogénèse et la régulation de la voie de signalisation Wnt.

2.4. La voie de signalisation p53

En réponse à différents types de stress tels que l'hypoxie ou des dommages causés à l'ADN, la protéine p53 codée par le gène suppresseur de tumeurs TP53 se libère de la ligase MDM2 lui permettant alors de se déplacer vers le noyau. C'est ainsi que le gène TP53, souvent appelé « gardien du génome », régule l'expression de gènes intervenant dans le contrôle du cycle cellulaire et l'apoptose (120) (**Figure 11**) Malheureusement, c'est l'un des gènes les plus fréquemment altérés dans les cellules tumorales. Sa mutation active ce processus de façon aberrante et favorise alors la croissance et la survie cellulaire dans les cellules tumorales. Ce gène a ainsi été associé à une radiorésistance chez des patients atteints de tumeurs cérébrales pédiatriques, de l'endomètre ou des VADS (121–123).

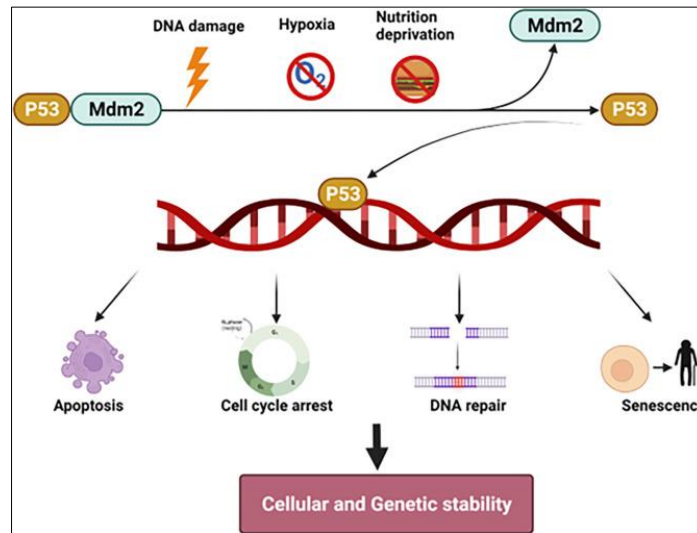


Figure 11 : Réponses intra-cellulaires de P53 face à différents stress cellulaires

Source : D'après Elmira ROSHANI ASL (120)

Lors d'un stress cellulaire tels que l'hypoxie, la privation de nutriment et les dommages causés à l'ADN, p53 se libère de la ligase MDM2, lui permettant de se déplacer dans le noyau. Une fois arrivé au niveau de l'ADN, p53 peut induire l'expression de gènes impliqués dans l'apoptose, l'arrêt du cycle cellulaire, la réparation de l'ADN et la prolifération cellulaire. Le but étant d'empêcher l'accumulation de mutations et d'altérations génomiques.

C'est ainsi que le gène TP53 est décrite comme étant le gardien du génome.

2.5. Les cellules souches tumorales

La présence d'une sous-population de cellules souches tumorales ayant des propriétés d'auto-renouveau et de multipotence est également à l'origine de l'échappement thérapeutique. Concernant les gliomes diffus, plusieurs études ont identifié des cellules souches tumorales (124,125). La recherche portant sur ces cellules est particulièrement importante car elles jouent un rôle essentiel dans la radiorésistance. Elles stimulent l'angiogenèse et contribuent à la croissance tumorale (126). De plus, elles possèdent une capacité supérieure de réparation des dommages causés à l'ADN, leur permettant de se renouveler et d'échapper au traitement. Malheureusement, ces cellules possèdent les mêmes marqueurs de surface et sont impliquées dans les mêmes voies de signalisation que les cellules souches neuronales. Cibler un marqueur de surface ou ces voies de signalisation s'avère donc difficile. Des études supplémentaires sont nécessaires pour tout d'abord arriver à une meilleure compréhension de la biologie tumorale mais également permettent le développement de nouvelles thérapies spécifiques (127). De plus, l'identification de biomarqueurs associés à ces cellules souches tumorales est essentielle afin de pouvoir les identifier à des fins diagnostiques, pronostiques et thérapeutiques (128).

3. Les facteurs environnementaux

Le tabagisme durant la radiothérapie est également un facteur de radiorésistance. Il a été démontré, dans plusieurs types de cancers, qu'une consommation tabagique maintenue durant la radiothérapie avait un impact significatif sur le contrôle loco-régional et la survie globale par rapport aux patients non-fumeurs (129). En revanche, aucune étude n'a prouvé d'impact sur la survie globale et le contrôle tumoral chez des patients atteints d'un gliome diffus de l'adulte. Les mécanismes sous-jacents restent incertains. L'hypoxie tumorale, facteur de radiorésistance, pourrait être favorisée par la consommation tabagique en augmentant la concentration de carboxyhémoglobine dans le sang. Les taux de carboxyhémoglobine chez les non-fumeurs sont généralement de 1 à 2 %, alors que taux chez les fumeurs peut atteindre 15 à 20 % (130). Elle pourrait également être favorisée par une insuffisance respiratoire induite par le tabagisme. Ce facteur étant sûrement le plus contrôlable, l'information au patient doit être exhaustive, afin de proposer au patient un sevrage tabagique.

3. OBJECTIFS DE LA THESE

La radiothérapie externe constitue l'une des modalités majeures dans la prise en charge en cancérologie. Les progrès récents en radiothérapie externe tels que la radiothérapie stéréotaxique ou la RCMI ont permis un meilleur ciblage des tumeurs. Si elle peut être curative pour de nombreuses localisations tumorales, elle reste cependant insuffisamment efficace dans certains cas avec l'apparition d'une résistance au traitement. Ces phénomènes de radiorésistance peuvent conduire à une récurrence et ainsi à une évolution défavorable du pronostic des patients atteints d'un gliome diffus de l'adulte. De plus, la radiothérapie externe peut être à l'origine d'effets secondaires aigus ou tardifs, parfois difficilement prévisibles, pouvant entraîner des toxicités sévères.

Ma thèse a donc pour principal objectif **d'étudier les relations doses /effets en radiothérapie externe afin d'améliorer l'efficacité et/ou d'identifier des paramètres biologiques et cliniques impliqués dans la radiothérapie de tumeurs radiorésistantes** que sont les gliomes de haut grade, dans le but, *in fine*, d'améliorer la prise en charge des patients. Ce travail s'inscrit dans la continuité des projets élaborés au sein de la Division de Recherche Clinique du Centre Jean PERRIN (131,132). Pour parvenir à cet objectif, mon travail s'est articulé autour de trois projets de recherche sur les gliomes diffus de l'adulte :

- **L'essai clinique PROGLIO**

La protéine circulante, hPG80, n'a pas encore été étudiée dans les gliomes diffus de l'adulte malgré son implication connue dans la formation des jonctions cellulaires, l'inhibition de l'apoptose, la régulation des cellules souches cancéreuses, l'angiogenèse, la régulation de la voie de signalisation Wnt et dans la sensibilité des cellules aux radiations ionisantes. La recherche de cette protéine dans les gliomes diffus de l'adulte pourrait ainsi permettre **l'identification d'un nouveau paramètre biologique impliqué dans la radiothérapie de tumeurs radiorésistantes**. Mes travaux de recherche ont alors consisté à l'élaboration d'un essai clinique visant à évaluer les concentrations plasmatiques de l'hPG80 chez des patients atteints d'une tumeur cérébrale de haut grade: L'essai clinique PROGLIO (NCT05157594) (1,2). Afin d'élaborer cet essai clinique, j'ai réalisé une revue de la littérature me permettant ainsi de rédiger le protocole. J'ai également eu la charge de la budgétisation de l'essai, des

soumissions réglementaires aux autorités compétentes et à la conduite de l'essai en réalisant la mise en place de l'étude, l'inclusion des patients et le recueil des données.

- **L'essai clinique GMaPIC**

Les patients atteints d'un gliome diffus, à haut risque de récives, le plus souvent à l'intérieur du volume tumoral irradié, risquent de développer une hypertension intracrânienne. A ce jour, aucune méthode non-invasive ne permet de mesurer la PIC en continu. La recherche d'une méthode non invasive de suivi de la PIC pourrait ainsi permettre **l'identification d'un nouveau paramètre clinique impliqué dans la radiothérapie de tumeurs radiorésistantes**. Mes travaux de recherche ont alors consisté aux analyses et à la valorisation d'un essai clinique proposant une méthode non invasive de suivi de la pression intracrânienne pour les patients atteints d'un gliome diffus de l'adulte : L'essai clinique GMaPIC (NCT02520492) (167).

- **L'essai clinique NANO-GBM**

Les patients atteints d'un glioblastome présentent malheureusement un mauvais pronostic et sont à haut risque de récives, en grande majorité à l'intérieur du volume tumoral irradié. **Améliorer l'efficacité de la radiothérapie** par l'utilisation de nanoparticules permettrait d'identifier une nouvelle combinaison **pouvant améliorer la prise en charge de ces patients**. Mes travaux de recherche ont alors consisté à l'élaboration d'un essai clinique visant à tester l'association de nanoparticules AGuIX®, à une radio-chimiothérapie avec témozolomide concomitant chez des patients présentant un glioblastome nouvellement diagnostiqué : L'essai clinique NANO-GBM (NCT04881032) (3,4). En collaboration avec une cheffe de projets sénior, j'ai réalisé une revue de la littérature sur les nanoparticules AGuIX® (133), participé à l'élaboration de l'étude, participé à la création de l'eCRF de l'étude et rédigé l'article du protocole.

4. TRAVAUX DE RECHERCHES

A. L'étude PROGLIO

I. Introduction

Malgré les améliorations dans le diagnostic, le pronostic à long terme et le taux de survie des patients atteints d'un gliome diffus de l'adulte de haut grade restent inchangés depuis les années 1980 (52). La survie à 5 ans est estimée à 30% pour les gliomes de grade 3 et est inférieure à 5% pour les gliomes de grade 4 (53).

Une étude prospective, analysée rétrospectivement, a été réalisée sur un total de 1 546 patients et 557 donneurs de sang sains, permettant le dosage de l'hPG80, avec le kit DxPG80 IVD ELISA développé par BIODENA CARE (134). Dans cette étude, les niveaux de l'hPG80 (progastrine circulante) ont été déterminés dans 11 cancers épithéliaux et non épithéliaux différents (colorectal, sein, utérus, ovaire, prostate, rein, pancréas, œsophage/estomac, foie, mélanome et poumon). Les concentrations médianes de l'hPG80, dans tous les types de cancers, étaient toutes supérieures à la limite de détection (1,2 pM) et à la limite de quantification (2,3 pM). Lorsque tous les types de cancers étaient combinés, la concentration médiane de l'hPG80 était de 4,88 pM et la concentration médiane de l'hPG80 chez les donneurs de sang sains était de 1,05 pM. Cette étude a démontré que l'hPG80 est exprimée dans le sang des patients à des concentrations significativement plus élevées que dans un groupe témoin de donneurs de sang sains (112). Depuis ces résultats ont été confirmés dans différents types de cancer grâce à plusieurs études rétrospectives. Début 2021, une étude rétrospective de 143 patients atteints d'un cancer du rein métastatique a révélé que l'hPG80 était détectée chez 94% des patients (135). Puis début 2022, une étude rétrospective portant sur 168 patients atteints de carcinome hépatocellulaire a révélé que la hPG80 était détectée chez 84 % des patients (136). Plus récemment, une étude rétrospective, dont les résultats en ont été publiés en avril 2023, portant sur 464 patientes atteintes d'un cancer du sein a révélé qu'un taux élevé d'hPG80 mesuré au diagnostic est significativement associé à un risque de récurrence ou de décès (137).

Comme l'hPG80 est impliquée dans de nombreuses voies de signalisation, qui sont elles-mêmes impliquées dans la tumorigenèse de nombreux types de cancers différents, il est justifié de supposer que l'hPG80 serait mesurable chez les patients atteints de gliomes diffus

de l'adulte de haut grade. De plus, l'entreprise BIODENA CARE dispose de données préliminaires sur le glioblastome IDH sauvage traité par radiochimiothérapie, présentée à l'aide d'un poster cette année lors de la réunion annuelle de l'ASCO (138). Après une chirurgie, l'hPG80 a été détecté chez 48 (69%) patients (hPG80+) avec une concentration médiane de 9,52 pM (IQR 5,21–21,20). Ces données confirment la détection de l'hPG80 dans ces tumeurs et une diminution de ce marqueur après résection totale de la tumeur. Elles fournissent une base solide pour émettre raisonnablement l'hypothèse que l'hPG80 est mesurable dans le sang des patients atteints de gliomes diffus de haut grade. De plus, aucun biomarqueur sanguin n'est actuellement utilisé en routine pour ce type de tumeur. Ceci justifie l'intérêt de mener une étude évaluant les concentrations plasmatiques d'hPG80 chez des patients atteints de gliomes diffus de haut grade.

L'étude PROGLIO est une étude pilote visant à évaluer les concentrations plasmatiques de l'hPG80 au fil du temps chez des patients atteints de tumeurs cérébrales de haut grade. Cette étude est monocentrique et interventionnelle, à risques et contraintes minimales de catégorie 2. Elle a reçu l'avis favorable du comité de protection des personnes Ouest III (CHU La Milétrie de Poitiers) le 26 octobre 2021. Cette étude a été enregistrée sur Clinicaltrials.gov (NCT05157594). Il est prévu d'inclure un maximum de 30 patients. L'étude a débuté en février 2022 avec une période de recrutement de 18 mois et une date de fin estimée à février 2025. A ce jour, 16 patients sont inclus dans cette étude. Les détails concernant le protocole sont présentés ci-dessous dans l'article du protocole publié, en 1^{ère} auteure, dans la revue *Frontiers in Neurology*.

II. Publication du protocole



OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to
Neurological Biomarkers,
a section of the journal
Frontiers in Neurology

RECEIVED 18 October 2022
ACCEPTED 28 December 2022
PUBLISHED 11 January 2023

CITATION
Casile M, Passildas J, Vire B, Molnar I
and Durando X (2023) hPG₈₀
(circulating progastrin) as a blood
biomarker for high-grade glial tumors:
A pilot study.
Front. Neurol. 13:1073476.
doi: 10.3389/fneur.2022.1073476

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hPG₈₀ (circulating progastrin) as a blood biomarker for high-grade glial tumors: A pilot study

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Background: Currently, the long-term prognosis and survival rate of patients with high-grade glial tumors remains poor and there are no biomarkers. hPG₈₀ (circulating progastrin) secreted into the blood by tumor cells has been widely studied in colorectal cancer. Its involvement in tumorigenesis has been demonstrated in the literature. Moreover, according to a recent study, hPG₈₀ is expressed in the blood of cancer patients at a significantly higher concentration than in the control group composed of healthy blood donors.

Methods: The PROGLIO study is a pilot, single-center, longitudinal study that primarily seeks to evaluate circulating plasma hPG₈₀ concentrations over time in patients with high-grade glial tumors. A fasting blood sample will be taken on the start and end day of radiotherapy and during the adjuvant chemotherapy (every 3 cycles). Follow-up monitoring will be performed for 9 months, with a blood sample taken every 3 months on the day of the follow-up MRI. The study plans to recruit 30 patients and recruitment started in February 2022.

Trial registration: [ClinicalTrials.gov](#), ID NCT05157594; registered on October 27, 2021.

KEYWORDS

progastrin, hPG₈₀, high grade glial tumors, biomarker, brain cancer

1. Introduction

Since 2016, glial tumors have been classified *via* a new approach, which combines histo-pathological and molecular features (1). This classification has been recently updated. The 2021 fifth edition introduces major changes that advance the role of molecular diagnostics in central nervous system tumor classification (2). In addition, this classification enables the aggressiveness of the tumor to be assessed. The most aggressive tumors are classified as high-grade gliomas, i.e., WHO grade 3 and 4, and have a poor prognosis with an estimated 5-year survival of 30% for grade 3 and < 5% for grade 4 (3). Indeed, the long-term prognosis and survival rate for patients with high-grade glial tumors remains poor (4).

The *GAST* gene codes for a 101-amino acid precursor peptide, progastrin, which generates progastrin after several post-translational modifications. This 80-amino acid pro-hormone is then cleaved by pro-hormone convertases and carboxypeptidases to form glycine extended gastrins (G34-Gly and G17-Gly). These glycine ends then serve as substrates for peptidyl- α -amidating monooxygenases, resulting in the formation of the two biologically active majority forms of gastrin in the G-cells of the antrum of the stomach, controlling acid secretions during digestion: G34-NH₂ for 34-amino acid gastrin and G17-NH₂ for 17-amino acid gastrin (5, 6). In a pathological situation, the disruption of the Wnt/ β -catenin pathway in cancer induces the activation of the expression of the *GAST* gene coding for progastrin and its secretion in circulating form known as hPG₈₀ (7). Its role has been widely studied in colorectal cancer. Thus, its involvement in the formation of cell junctions, in the inhibition of apoptosis, in the regulation of cancer stem cells, in angiogenesis and in the regulation of the Wnt signaling pathway, has been demonstrated in the literature (8–13). Furthermore, progastrin has been shown to be a factor in radio-resistance (14). An increase in *GAST* gene expression has been observed after irradiation. On the other hand, targeting hPG₈₀ induces an increase in radiation-induced apoptosis. A decrease in hPG₈₀ expression increases radiation-induced DNA damage and leads to the inhibition of the AKT and ERK pathways, induced by irradiation (14).

A prospective study, which was analyzed retrospectively, was performed on a total of 1,546 patients and 557 healthy blood donors, enabling the determination of hPG₈₀, with the DxPG80 IVD ELISA kit developed by BIODENA CARE (15). In this study, the levels of hPG₈₀ (circulating progastrin) were determined in 11 different epithelial and non-epithelial cancers (colorectal, breast, uterus, ovary, prostate, kidney, pancreas, esophagus/stomach, liver, melanoma and lung). Median hPG₈₀ concentrations across cancer types were all above the limit for detection (1.2 pM) and the limit for quantification (2.3 pM). When all the cancer types were combined, the median hPG₈₀ concentration was 4.88 pM. The median hPG₈₀ concentration in the healthy blood donors was 1.05 pM. This study demonstrates that hPG₈₀ is expressed in the blood of patients with all types of cancers at significantly higher concentrations than in a control group of healthy blood donors (7). In addition, a retrospective study of 143 patients with metastatic kidney cancer found that hPG₈₀ was detected in 94% of the patients (16). More recently, a retrospective study of 168 patients with hepatocellular carcinoma found that hPG₈₀ was detected in 84% of the patients (17).

As hPG₈₀ is involved in many signaling pathways, which are themselves involved in the tumorigenesis of many different types of cancers, it is justifiable to assume that hPG₈₀ will be measurable in patients with high-grade brain tumors. In addition, BIODENA CARE has preliminary data on IDW-wild type glioblastoma treated with radio-chemotherapy, published

on a poster at the annual ASCO meeting this year (18). After surgery, hPG₈₀ was detected in 48 (69%) patients (hPG₈₀+) with a median concentration of 9.52 pM (IQR 5.21–21.20). This data confirms the detection of hPG₈₀ in these tumors and a decrease in that marker after total tumor resection. This data provides a strong basis to reasonably hypothesize that hPG₈₀ is measurable in the blood of patients with high-grade brain tumors. Furthermore, there is no routine use of a blood biomarker for glial tumors. This justifies the interest of conducting a study assessing plasma hPG₈₀ concentrations in patients with high-grade brain tumors.

2. Methods and analysis

2.1. Study design

The PROGLIO study is a pilot, single-center, longitudinal study aiming to evaluate plasma hPG₈₀ concentrations over time in patients with high-grade brain tumors.

This study has been registered on [Clinicaltrials.gov](https://clinicaltrials.gov) (NCT05157594). A maximum of thirty patients are expected to be enrolled. The study was started in February 2022 with an 18-month enrolment period and an estimated completion date by February 2025.

2.2. Study objectives and endpoints

2.2.1. Main objective and endpoint

The main objective of the study is to evaluate the plasma levels of hPG₈₀ (circulating progastrin) during therapeutic follow-up and relapse monitoring among patients with high-grade glial tumors.

The primary endpoint is hPG₈₀ plasma concentrations measured by BIODENA CARE using their DxPG80 IVD ELISA kit, from inclusion to the last follow-up.

2.2.2. Secondary objectives

The secondary objectives are:

- To study hPG₈₀ concentrations according to tumor progression using the Response Assessment in Neuro-Oncology (RANO) criteria, which take progression objectified by imagery, clinical status and level of steroid therapy into account.
- To evaluate the sensitivity to irradiation of hPG₈₀ concentration variations.
- To compare hPG₈₀ plasma levels in patients, and to study inter-individual variations before, after and during disease management.

TABLE 1 Section criteria.

Inclusion criteria	Non-inclusion criteria
Female or male 18 years of age or older.	Patient under guardianship or curatorship.
Patients with a high-grade primary glial tumor (Astrocytoma grade 3; Glioblastoma; Anaplastic Oligodendroglioma).	Psychological disorder (<i>cognitive disorders, vigilance disorders, etc.</i>) or social reasons (<i>deprivation of liberty by judicial or administrative decision</i>) or geographical reasons that could compromise the medical follow-up of the trial.
Patients scheduled to begin radiotherapy and/or chemotherapy.	Refusal to participate.
Able to give informed consent to participate in the research.	Pregnant or breastfeeding woman.

2.3. Participant eligibility

The inclusion and non-inclusion criteria are presented in [Table 1](#).

Patients with a high-grade glial tumor (glioblastomas IDH-wt, astrocytomas IDH mutated grade 3 and 4, oligodendrogliomas grade 3 and 4), who need to start radiotherapy and/or chemotherapy, will be eligible for the protocol.

2.4. Intervention

Eligible patients will be included during a consultation with an investigator after verification of their compliance with the inclusion and non-inclusion criteria (and signing of informed consent).

A fasting blood sample will be taken on the start and end day of radiotherapy and during adjuvant chemotherapy (every 3 cycles). Follow-up monitoring will be performed for 9 months, with a blood sample taken every 3 months on the day of the follow-up MRI.

Pseudonymized blood samples will be stored at the Centre Jean PERRIN and then sent to the Hospices Civils de Lyon (France) for analysis. The blood samples will be analyzed to evaluate the plasma concentrations of hPG₈₀ in pmol/L using the ELISA DxPG80 kit (BIODENA CARE, France).

The risks or constraints appear minimal for the subjects participating in the study since the only interventions are blood tests.

2.5. Measures of hPG80 levels in blood samples

The ELISA DxPG80 lab kit (BIODENA CARE, France) will be used to measure hPG₈₀ levels in all plasma EDTA samples according to the manufacturer's instructions. The analytical performances of the kit have been described by Cappellini et al. (15). In brief, the limit of detection (LoD) is a hPG₈₀ concentration of 1 pM and the limit of quantitation (LoQ)

is a hPG₈₀ concentration of 3.3 pM. The inter- and intra-assay coefficients of variation (CV%) are below 10%. No cross-reactivity has been detected with gastrin-17, Gastrin-Gly or CTFP (C-Terminus Flanking Peptide). No cross-reactivity has been detected with other blood biomarkers such as CA125, CEA or PSA. No interference has been detected with chemicals such as SN-38, 5-FU or triglycerides, cholesterol or hemoglobin.

2.6. Study procedures and participant timeline

The overview of study assessments and procedures are presented in [Table 2](#).

2.7. Sample size

The PROGLIO study is a pilot, single-center, longitudinal study whose primary objective is to describe plasma hPG₈₀ concentrations over time, in patients with high-grade brain tumors. At the present time, there are no published results on the expected distributions of plasma hPG₈₀ concentrations during patient follow-up in the study population, hence the pilot aspect of our study, thus no power analysis is possible for the necessary sample size estimation. A sample size of 30 patients seems generally appropriate considering the state of recent literature. On the basis of preliminary data (currently confidential being consolidated for publication), we can at least estimate that the sample size of 30 patients should enable us to ensure 80% power (5% alpha risk, two-tailed test) to show an effect size of 0.75 of the difference between high-grade brain tumors and control values (healthy subjects) in the literature (16). The preliminary results were stratified by type of surgery (biopsy, partial resection, and total resection), and no gender differences were observed.

2.8. Data analysis

The main analysis consists of the descriptive analysis of plasma progestin concentration values at each time point.

TABLE 2 Data collection schedule.

	Inclusion	Active treatment phase			Follow-up		
		Radiotherapy (1st session)	Radiotherapy (Last session)	Adjuvant chemotherapy (Every 3 cycles)	3 months	6 months	9 months
Information and consent	✓						
Clinical evaluation							
Medical & surgical history	✓						
History of the disease	✓						
Previous and concomitant treatments	✓	✓	✓	✓	✓	✓	✓
Toxicity evaluation (NCI CTCAE v5.0)	✓	✓	✓	✓	✓	✓	✓
Biological examination							
hPG80 plasma level assessment		✓	✓	✓	✓	✓	✓
Tumor evaluation							
MRI (rano evaluation)		✓	✓*	✓	✓	✓	✓

* Optional: If performed in standard practice.

For the third secondary objective, this analysis will be further developed by taking into account the longitudinal character of the data and by modeling data using mixed effects regression models, associated with graphical representations of individual trajectories.

The study of the relationship between hPG₈₀ concentrations and tumor evaluation according to the RANO criteria (secondary objective 1) at different time points will be performed using generalized linear mixed models with response as the dependent variable, with hPG₈₀ concentration as a fixed effect (as well as possibly other covariates), and with random effect calculations enabling the modeling of repeated data during follow-up.

The impact of radiotherapy on the variations in hPG₈₀ concentrations (secondary objective 2) will be investigated using linear mixed models, followed, if relevant, by a *post-hoc* analysis by paired sample comparison tests.

2.9. Data management and monitoring

The data collected for the study will be recorded on an eCRF (Ennov Clinical software). The users with access

to the data will be the investigators, the clinical research associates, the project leaders and the biostatisticians. They are authorized professionals and are subject to professional secrecy. The investigator will ensure the accuracy, completeness, and consistency of the data recorded (pseudonymized patient data) and of the provision of answers to data queries.

A clinical research associate mandated by the sponsor will perform regular monitoring reviews. The objectives will be to ensure the proper conduct of the study, the recording of the data generated in writing, and its documentation, storage and reporting, in accordance with the legislative and regulatory provisions in force. The follow-up reports will ensure traceability.

2.10. Consideration of risk of bias

The risk of selection bias should be limited by an exhaustive inclusion of eligible patients treated at the Centre Jean PERRIN during the study period. As recruitment is consecutive, a list of potentially eligible patients will be kept, in order to verify a posteriori the representativeness of the sample.

The existence of another undiagnosed cancer pathology also represents a risk of bias. In the event that this pathology is detected during the follow-up, a statistical analysis will be performed to evaluate the impact of the inclusion of these patients on the results obtained.

2.11. Trial status

The PROGLIO trial is currently recruiting. Participant recruitment began in February 2022 and recruitment is expected to finish in August 2023. The approved protocol is version 2, 20/10/2021.

3. Discussion

The investigation of blood biomarkers for high-grade glial tumors remains a major challenge in order to predict and monitor treatment response. Identify an accurate circulating biomarker is a highly rewarding area of interest in neuro-oncology. Different methods have been examined (circulating tumor cells, cell-free DNA, microRNA, exosomes, and proteins) but no single biomarker test seemingly optimal at this time (19). This study, still at the proof-of-concept stage, could confirm that hPG₈₀ is measurable in patients with high-grade glial tumors. If the main hypothesis of the PROGLIO trial is confirmed, further research will be required to demonstrate the clinical value of this blood biomarker. A study with a larger, multicentric enrolment would then enable its prognostic and diagnostic roles to be assessed, and to potentially highlight the usefulness of hPG₈₀ as a blood biomarker.

Ethics statement

The studies involving human participants were reviewed and approved by French Ethics Committee (Comité de protection des personnes Ouest III). The patients/participants provided their written informed consent to participate in this study.

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Author contributions

Conception and design: MC, XD, and IM. Principal investigators of the study: XD. Revision of the study design and protocol: MC, JP, and XD. Study coordination: MC and JP. Acquisition of data and patient recruitment and obtaining funding and supervision: MC and XD. Statistical analysis: IM. Drafting the manuscript: MC. Revision, adaptation and final approval of the manuscript: MC, JP, XD, and BV. All authors contributed to the article and approved the submitted version.

Funding

BIODENA CARE has undertaken to finance the costs related to the analytical part and the transport of the samples. The research is carried out with the support of BIODENA CARE as a partner and supplier of the DxPG80 solution. Centre Jean PERRIN has undertaken funding related to costs of the staff involved in the study. The funding parties are not involved in the design and conduct of the study, nor in the collection, management, analysis, and interpretation of the data.

Conflict of interest

BV is a Medical Science Liaison of BIODENA CARE.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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III. Conclusion

L'étude de biomarqueurs sanguins pour les gliomes diffus de haut grade reste un défi majeur en oncologie pour prédire et surveiller la réponse au traitement. Identifier un biomarqueur circulant précis est un domaine d'intérêt très gratifiant en neuro-oncologie. Différentes méthodes ont été examinées (cellules tumorales circulantes, ADN cellulaire, microARN, exosomes et protéines), mais aucun biomarqueur ne semble optimal à ce jour (139). L'étude PROGLIO, encore au stade de preuve de concept, pourrait confirmer que l'hPG80 est mesurable chez les patients atteints de gliomes diffus de haut grade. Si l'hypothèse principale de l'essai PROGLIO est confirmée, d'autres recherches seront nécessaires pour démontrer la valeur clinique de ce biomarqueur sanguin notamment en termes de répétabilité, spécificité, sensibilité et robustesse. Une étude avec un recrutement plus important et multicentrique permettrait alors d'évaluer ses rôles pronostique et diagnostique comme biomarqueur sanguin.

L'identification d'un biomarqueur, pour les gliomes diffus de haut grade pourrait améliorer la prise en charge de ces patients. En revanche, les informations fournies par un biomarqueur ne peuvent à elles seules être suffisantes. L'utilisation de biomarqueur doit être associée à une réflexion plus globale, intégrant des paramètres cliniques. Dans ce sens, mon travail s'est également articulé autour du projet de recherche clinique GMaPIC.

B. L'étude GMaPIC : L'identification de paramètres cliniques

I. Introduction

Bien que la radiothérapie associée au témozolomide concomitant et adjuvant ait augmentée la survie des patients, le pronostic à long terme des patients atteints de gliome diffus de haut grade reste médiocre, avec des risques élevés de progression tumorale se produisant le plus souvent à l'intérieur du volume tumoral irradié (10,53). Dans ce contexte, les patients atteints d'un gliome diffus de haut grade sont à risque de développer une hypertension intracrânienne au cours de leur suivi. A ce jour, aucune méthode non-invasive ne permet de mesurer la PIC en continu. Les recherches portant autour de nouvelles méthodes non-invasives sont basées soit sur les propriétés des structures intracrâniennes soit sur les propriétés des structures extra-crâniennes. Elles sont du types ultrasonores, vasculaires, ophtalmologiques ou audiologiques (140). En revanche, aucune de ces techniques, bien que non-invasives, ne semblent pouvoir remplacer, à elle seule, l'utilisation d'un capteur intra-parenchymateux ou d'une dérivation ventriculaire. Elles présentent effectivement des limites. Elles ne sont pas suffisamment précises, sont limitées par différents facteurs dépendant de l'opérateur et ne sont pas toutes applicables en pratique clinique (141).

La cochlée, en réponse à une stimulation acoustique, émet des sons appelés otoémissions acoustiques (OEA) (142). Elles résultent du mouvement mécanique des cellules ciliées présentes dans la cochlée. Ce mouvement crée des vibrations sonores qui se propagent de façon rétrograde de l'oreille interne à l'oreille externe.

L'aqueduc cochléaire est un petit canal osseux permettant la communication entre l'espace périlymphatique de la cochlée et l'espace sub-arachnoïdien contenant le liquide cérebrospinal. Il maintient l'équilibre de la PIC avec la pression intra cochléaire (143).

Dans le cas d'une variation de la PIC, qui peut être constatée chez des patients atteints d'un gliome diffus, on constate un changement de la pression intra cochléaire *via* l'aqueduc cochléaire. Ce changement de pression, dans l'oreille interne, modifie l'impédance acoustique ce qui provoque également un changement des OEA. Les vibrations sonores émises à la sortie de l'oreille externe qui sont les OEA sont ainsi modifiées à chaque variation de la PIC. Leurs modifications permettent une évaluation non invasive des changements de la PIC, même si la grande variabilité interindividuelle des propriétés de base de l'oreille interne empêche

l'évaluation de la PIC absolue. Ce phénomène a été étudié et validé dans deux études grâce aux produits de distorsion des otoémissions acoustiques (DPOAE) (144,145).

Les PDA sont des OEA que l'on dit être provoquées. En effet, lorsque l'on envoie simultanément deux sons purs aux fréquences f_1 et f_2 , les cellules ciliées en se mouvant amplifient les sons reçus dans l'oreille interne et provoquent, par le déplacement des stéréocils, une distorsion des ondes acoustiques. Il en résulte des PDA de fréquence $mf_1 - nf_2$ qui sont renvoyés à l'oreille externe. Chez l'Homme, le PDA le plus facile à détecter, à l'aide d'un microphone, correspond à la fréquence $2f_1 - f_2$ (146).

Ce concept a été exploité et testé par le laboratoire de biophysique neurosensorielle (INSERM UMR 1107), en collaboration avec la société ECHODIA, tous deux situés à Clermont-Ferrand. Une technique de mesure des DPOAE a été développée et brevetée afin d'observer les variations pressionnelles isolées ou reproductibles de la cochlée, dans le but de mettre en évidence un hydrops cochléaire ou une augmentation de la PIC (145,147).

En supposant que les mesures DPOAE non invasives sont un marqueur de substitution pour la PIC et que la variation de la PIC reflète l'aggravation de la tumeur, nous avons émis l'hypothèse que le dispositif médical ECHODIA pourrait être un moyen non-invasif de suivre l'évaluation de la tumeur dans le cas d'un gliome diffus de haut grade. Le protocole de recherche impliquant la recherche humaine GMaPIC est la première évaluation de ce dispositif médical non-invasif, permettant la surveillance de la PIC chez des patients traités par radiothérapie pour un gliome diffus de haut grade. L'objectif est de comparer la variation de la PIC détectée par cette méthode avec les volumes mesurés grâce à l'IRM tels que le volume T1/Gadolinium et le volume T2/Flair.

Les détails concernant cette étude et les résultats sont présentés ci-dessous, dans l'article soumis, en 1^{ère} auteure, dans la revue *Radiotherapy and Oncology*.

II. Article

Non-invasive intracranial pressure monitoring for high-grade gliomas patients treated with radiotherapy: results of the GMaPIC trial

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ABSTRACT

Purpose

Patients with high-grade gliomas are at risk of developing increased intracranial hypertension (ICHT) in relation to the increase in volume of their tumor. ICP change cannot be measured by invasive method but can be estimated by using routine clinical signs, in combination with a standard imaging method (MRI). ICP could be measured non-invasively through the cochlear response to audio stimulation with distortion-product otoacoustic emissions (DPOAE) monitoring, using echodia medical device enabling the detection of changes as small as 4 mmHg. A non-invasive monitoring of ICP with echodia could be of interest in high-grade glioma, in particular after radiotherapy treatment with as major side effect a cerebral oedema.

Patients and methods

This prospective clinical study aimed to compare the ICP changes (estimated by a non-invasive method based upon DPOAE monitoring) with volume changes observed on magnetic resonance imaging (MRI) in patients with high-grade gliomas treated with radiotherapy.

DPOAE measurements were performed one month after the end of radiotherapy and then every 3 months for one year. At each visit, the patient also underwent MRI as well as an evaluation of clinical signs.

Results

Forty patients were enrolled from April 2015 to December 2018. The variation in the estimate of intracranial pressure readout measured at each follow-up visit (in absolute value with respect to the baseline measurements) was significantly associated with the variation of T2/FLAIR volume (n=125 ; $p<0.001$) with a cut off value of change ICP readout of 40.2 degrees (e.i. an estimated change of 16 mm Hg).

Conclusion

The GMaPIC trial confirm the hypothesis that the ICP change estimated by DPOAEs measurement using a non-invasive medical device is correlated with the change of the tumor or edema in high grade glioma after radiotherapy. The device could thus become an easy-to-use and non-invasive intracranial pressure monitoring tool for these patients.

INTRODUCTION

High-grade gliomas account for the great majority primary malignant brain tumors in adults (1). Radiotherapy is, with surgery, one of the mainstay treatment of these tumors. Intracranial hypertension (ICHT) is one of the major issue for the management of patients with high-grade gliomas.

Indeed, the cranium contains the brain, vessels and cerebrospinal fluid (CSF) in an inextensible bony cavity. The pressure inside this cavity i.e. intracranial pressure (ICP) is relative to these different components. In normal conditions, an increase in the volume of one of these components is compensated by a decrease in the volume of one or more of the components (2). The physiological value of ICP is between 5 and 15 mmHg in adults, but its value can oscillate up to 20 mmHg (3). Blood and CSF are the two components whose volume adapts most readily to maintain a normal ICP. However, if their volume cannot decrease enough, then ICP cannot be maintained causing ICHT (4). In the presence of high-grade glioma, the effect of mass specific to the tumor and/or the appearance of edema, in particular due to inflammatory

reaction around the tumor, can be the cause of ICHT. It is defined as an ICP value higher than 20 mmHg (5).

The use of direct measurement of ICP by invasive intra-parenchymal sensor or a ventricular shunt (gold standard method) is not an option for clinical practice in the management of high-grade glioma patients (6). Currently, clinicians rely on indirect clinical and imaging signs to evaluate ICHT.

Over the last ten years, non-invasive measurement methods using auditory sensors have been developed to detect variations in ICP by way of sensitive and rapid measurements (7–13). The cochlea, in response to acoustic stimulation, emits sounds called otoacoustic emissions (14). The mechanical movement of the hair cells in the cochlea creates sound vibrations that propagate back from the inner ear to the outer ear. The cochlear aqueduct provides communication between the perilymphatic space of the cochlea and the subarachnoid space containing the CSF. It therefore maintains the balance of ICP with the intracochlear pressure (15). Therefore, any change in ICP will result in a change in intracochlear pressure. This then causes a change in the acoustic impedance of the inner ear which also leads to a change in otoacoustic emissions. The sound vibrations emitted at the outer ear are thus modified with each variation in ICP. Their modifications provide non-invasive readouts of ICP changes, even though the huge inter-individual variability in baseline inner-ear properties precludes absolute ICP to be assessed. This phenomenon has been investigated and validated in three studies using distortion product otoacoustic emissions (DPOAE) (8,16,17) enabling the detection of changes as small as 4 mmHg (10).

A DPOAE measurement technique has been developed (ECHODIA[®], Clermont-Ferrand, France) making it possible to observe isolated or reproducible pressure variations in the cochlea, with the aim of revealing cochlear hydrops or increased ICP (16,18). The GMaPIC exploratory study was the first-in-man evaluation of this medical device to monitor estimate ICP in patients treated with radiotherapy for a high-grade glioma. The main objective was to compare the estimate variation as detected by a non-invasive method based upon DPOAE monitoring with magnetic resonance imaging (MRI) outcomes.

PATIENTS AND METHODS

Study Design

The GMaPIC study is a prospective, interventional, medical device, non-randomized, single patient group study to test a medical device ECHODIA® in a longitudinal cohort. This study has been registered on Clinicaltrials.gov (NCT02520492), approved by the Committee for the Protection of Persons of Sud-Est VI, and authorized by the National Agency for the Safety of Medicines and Health Products (ANSM) in November 2014.

Patients eligibility

The study population consisted of patients aged 18-65 years with histologically confirmed high-grade glioma (according to the 2016 WHO Classification of Tumors of the Central Nervous System) for whom surgery consisted in stereotactic biopsy or incomplete resection and treated with adjuvant radiotherapy. Eligible patients were included at the initiation of radiotherapy. All patients provided written informed consent prior enrollment.

Interventions

Radiotherapy consisted in a total dose of 60Gy using a volumetric modulated arctherapy (VMAT; Rapidarc®, Varian Medical Systems, Palo Alto, CA, USA) technique. Contouring was done according to current guidelines (19). Concomitant and adjuvant temozolomide was prescribed according to recommendations (19). One month after the end of radiation therapy and then every 3 months for one year, each patient underwent: DPOAE measurements, MRI, and an evaluation of clinical signs (Figure 1).

MRI

MRI included T1, Flair and post-gadolinium T1 sequences. Disease assessment was determined using the response assessment in neuro-oncology (RANO) criteria (20,21). On each MRI, Flair volumes and post-gadolinium volumes were delineated.

ICP estimation by DPOAE measurements

DPOAE measurements were performed using the ELIOS® device (ECHODIA®, Clermont-Ferrand, France). First, an otoscopy was performed to inspect the eardrum and the external auditory canal. If this was well cleared, a tympanometry measure was carried out to check the mobility of the tympanic membrane and middle ear in response to calibrated changes in the air pressure in the ear canal. A normal tympanogram is centered on 0 daPa, i.e. when the

pressure on both sides of the tympanum is balanced. Performing this tympanometry ensures that the measurements are not confounded by a change in middle ear pressure.

Once these two tests were performed, a sound transmitter was placed in the patient's external ear canal to send two continuous pure tones to the cochlea. A microphone, placed in the patient's outer ear, recorded the distortion DPOAE received through the middle ear corresponding to the frequency $2f_1-f_2$ (22). This enabled real-time monitoring of the cochlear pressure, and indirectly the ICP. This device was connected to the ELIOS® device, capable of calculating the phase shift between the emitted and received sound waves, as a function of time, known as shift-OAE.

To collect one shift-OAE data point, the ELIOS® device calculates over a few seconds the degree of phase shift between the sound waves emitted and received. The average of 4-5 phases data provides a stable readout of shift-OAE (in degrees). Past comparisons between shift-OAE measurements and concomitant invasive ICP measurements in neurosurgery have shown that a change in readout between two measuring sessions of plus or minus 10 degrees relates to a variation in ICP of about 4 mmHg (8). In typical recording sessions, the shift-OAE of a subject at rest in a quiet room remains stable within less than 10 degrees, fluctuations of a few degrees being attributed to acoustic noise, so that a variation of 10 degrees is considered clinically significant.

To ensure the quality of the data, all measurements were examined to determine if they were analyzable. Indeed, in a situation where the earmold has moved during the measurement, the phase average may vary by more than 10 degrees and could be mistakenly considered as a variation of the ICP estimate. In this case, the frequency levels of f_1 and f_2 will be affected. A difference between f_1 and f_2 varying by more than 3 decibels during the measurement is considered to be a phase shift. In this situation, the results are unpredictable, so the data obtained are unfortunately not usable (23). Finally, if the ear canal is not sealed against surrounding noise or if the noise is too high during the measurement, the signal of interest will be confused with the background noise. This is considered to be the case when the signal to noise ratio is less than 2 decibels. The results obtained in this situation are also unpredictable. The ECHOSOFT® software connected to the device allows access to this data.

This software also has a functionality that allows an automatic correction, neutralizing the variation of the phase average that can be related to a minor displacement of the earmold. This functionality has been tested and validated to be applied to ELIOS® devices in order to provide more consistent, repeatable and accurate values (23).

Statistical Analysis

R software was used for the statistical analysis. Patient characteristics were described using standard distribution parameters: mean and standard deviation or median and range for quantitative parameters and counts and frequencies (%) for categorical parameters.

To investigate the relationship between changes in ICP estimate measures and changes in MRI measurable volumes, mixed effects linear regression models with subject as random intercept were used to account for the repeated measures design. We consider absolute change with respect to baseline, where the baseline visit is the visit done one month after radiotherapy. Changes in ICP estimate measures are considered in absolute value, since we are only interested in the magnitude of the change, and not its sign. The maximally selected rank statistics with Monte Carlo approximation was used to identify the optimal cutoff value on ICP variation.

Median follow-up was computed using reverse Kaplan-Meier method, with confidence interval based on log-log transformation.

Results

Patient Characteristics

Forty patients were included from April 2015 to December 2018. One patient was wrongly included, one patient died before the first evaluation, and for 5 patients, the DPOAE measurements collected were not technically exploitable. For one patient, radiotherapy was not technically feasible due to patient non compliance, and thus the patient only received chemotherapy, but was still kept for the analysis. Thus, 33 patients were considered in this final analysis, and were evaluable for at least 2 evaluations.

The patients characteristics are presented in Table 1. There were 23 men (69.7%) and 10 women (30.3%). The median age was 49 years (18-64). The histology was as follows: 25 (75.7%) glioblastoma, 6 (18,2%) grade III astrocytoma, and 2 (6.1%) grade III

oligodendroglioma. Nineteen patients (57.6%) had partial surgery and fourteen a stereotactic biopsy (42.4%). Twenty-nine patients (90.6%) were treated with temozolomide concomitantly with radiotherapy.

Treatment outcomes and MRI analysis

The median follow-up was 11.8 months. Thirty-three patients (100%) were evaluable at 3-month follow-up, 30 (91%) patients at 6-month, 27 (82%) patients at 9-month, and 21 (64%) patients at 1-year. At the end of 12 months follow-up, 16 (48.5%) patients remained stable, 11 (33%) patients had progressed and 6 (18%) patients had a partial response, according to the RANO criteria. Most patients (n=18; 55%) progressed at least once during their follow-up.

Mean T1/Gadolinium and T2/Flair volumes were 37.5cc (4.5-114) and 125cc (12-368) respectively at baseline. 52% and 58% of patients had a >10% increased of T1/Gadolinium and T2/Flair volumes respectively during follow-up.

DPOAE measurements

In total, of the 138 visits made during the study, 4 (2.9%) measurements were not performed. In addition, 7 (5.1%) measurements were not analyzed because of a signal-to-noise ratio below 2 decibels due to either a lack of sealing or too much background noise. For another measurement (0.7%), a significant displacement of the earmold during the measurement was identified. Moreover, one last measurement (0,7%) was not analyzed because no imaging was performed on the day of the measurement. The data from these 13 measurements (9.4%), which were not exploitable, were not used for the final analysis. In the end, 90.6% of the data could be analyzed.

All patients but 2 (31/33) had at least 1 shift-OAE of 10 degrees (considered as clinically significant) during their follow-up.

Correlation between DPOAE measurements and MRI analysis

Change in DPOAE measurements in absolute value with respect to the baseline measurements in the course of the follow-up of the patients was statistically significantly associated with the change of T2/FLAIR volume as dependent variable (estimate of the fixed effect: 1.05, 95%CI (0.51, 1.59), $p < 0.001$, mixed model) (figure 3) and with the change of T1 Gadolinium volume (0.2, $p = 0.043$). The cut-off value on the change in ICP estimate associated with the best

separation of values of T2/FLAIR variation into two groups was 40.2 degrees in absolute value (estimate 60.4, 95%CI (30.1, 90.7), $p < 0.001$, multiple comparison-adjusted p -value=0.02) (figure 3).

DISCUSSION

This study is the first-in-man exploratory study to propose a non-invasive method of monitoring indirectly ICP in patients with high-grade glioma treated with radiotherapy. Indirect ICP measurement used a commercially available audiological device, ELIOS[®], with DPOAE data-processing software specially designed for providing an ICP-related readout. Interestingly, the relative change in ICP estimate between the initial and current situations in the course of the follow-up of the patients was significantly associated with the increase in the T2/FLAIR volume. We also found a significant correlation with the change in T1Gado volumes, but to a lesser degree. In addition, this study has shown that the majority of patients (93,9%) had a significant change in ICP estimate at some time during their follow-up.

The finding that the change in DPOAE measurements associated with the best separation of values of T2/FLAIR variation into two groups was 40.2 degrees, translates into an ICP difference of 16 mmHg between the compared situations, according to the calibration of the ELIOS[®] equipment. In the present context, it is the presence of an inflammatory reaction around the tumor that could be related to radiotherapy, as explored by T2/FLAIR imaging, that entails the risk of an increase in ICP. The observed 16 mmHg-limit in ICP change makes sense as in neurosurgery, ICP is considered abnormal above 20 mmHg (5). Thus, it suggests that the measurements performed with the ELIOS[®] device, calibrated in acute neurosurgical or neurointensive care procedures, remain reliable in the context of long-term follow-up of glioma patients.

The number of inclusions required in the study was knowingly based on the number of patients that could be expected to sign in in the given period in view of the center's active file. Despite the rather small sample, significant correlations were observed. These results should therefore be validated with a larger-scale study.

Of course, a better standard for ICP measurement would have been the use of an intra-parenchymal sensor or a ventricular shunt (25), but this is only acceptable in an acute context in a neurosurgical environment. Moreover, due to infectious risks, the procedure cannot be

repeated along the course of several months as required by glioma follow-up. Some non-invasive ICP methods tested recently are based on the mechanical properties of intracranial structures, and the presence of an evolutive tumor precludes their utilization. Other techniques exploit properties of extra-cranial structures, ultrasonic, vascular, ophthalmic in addition to the current audiological one used in the present study (26,27). None of the non-audiological techniques shows better accuracy, less limitations or less operator-dependency than the audiological ones (28), whereas the present method turned out to be easily applicable in clinical practice as only 5 patients of 40 had inappropriate ICP estimate data.

The present results suggest that the use of the ELIOS® equipment could complement monitoring of the disease by the usual imaging procedures. Currently, patients undergo a tumor evaluation every 3 months. With this medical device, additional measurements between follow-up visits could be planned. An increase in ICP readout associated to some pattern of clinical symptoms might enable treatment adaptation. In case a large increase would be detected, it might alert the oncologist to possible tumor progression. Overall, non-invasive measurement of ICP estimate with the tested device could improve the management of patients with high-grade brain tumors at lower cost and risk.

Before these ambitious goals can be achieved, one must examine whether the system allowing non-invasive measurements of ICP changes is reliable enough. In this study, eight measurements (5.8%) could not be analyzed because of an insufficient signal-to-noise ratio, due either to a poor seal of the measuring probe in the ear canal or to too much background acoustic noise. A non-repeatable positioning of the measuring probe from one session to the next can also yield results that are unreliable. A study conducted at the University Medical Center Groningen on 17 volunteers with no history of hearing, vestibular or neurological disorders tested a modified version of the ELIOS® device (version 2) and demonstrated improved robustness, stability and accuracy of measurements (23). This study proposed the use of foam ear plugs that better fit the ear canal, despite their single size, an advantage since it relieves the operator of having to choose a proper ear plug. Software improvements also provide real-time information on probe positioning and quality of seal (23). This enables the user to be guided in positioning the probe in the same position as for past measurements in the same ear. Overall, this improved version could reduce the number of unusable data and provide better reproducibility.

CONCLUSION

The GMaPIC trial demonstrated that the ICP changes for patients with high-grade glioma through the use of a non-invasive medical device based on the measurement of DPOAEs, between the baseline visit and the follow-up visits, were mainly associated with an increase in the T2/FLAIR volume. The device, developed by the company ELIOS could thus become an easy-to-use ICP monitoring tool for patients with high-grade glioma, making it possible to adapt treatments and possibly control tumor progression.

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STATEMENTS & DECLARATIONS

Funding

This study was supported by the National Research Agency of France (ANR-13-TECS-0005-04)

Competing Interests

The authors have no relevant financial or non-financial interests to disclose

Author Contributions

Melanie CASILE interpreted the data and drafted the manuscript. Emilie THIVAT, Fabrice GIRAUDET, Blandine LOURENCO and Paul AVAN designed the study. Ioana MOLNAR provided the statistical analysis. Fabrice GIRAUDET, Angeline GINZAC, Ioana MOLNAR, Julian BIAU and Xavier DURANDO contributed to the analysis of data and critically revised the article for important intellectual content. All authors have contributed to the manuscript and approved the submitted version.

Data Availability Statement

The datasets presented in this article are not readily available for reasons of privacy. Requests to access the datasets should be addressed to the corresponding author.

Ethics Statement

The study protocol obtained approval from the French Ethics Committee (Comité de protection des personnes Sud-Est 6) in January 2015. The study was conducted in accordance with the Helsinki

Declaration, the Good Clinical Practice (GCP) guidelines of the International Conference on Harmonisation (ICH–E6, 17/07/96), General Data Protection Regulation (GDPR) and local regulatory requirements.

Consent to participate

Written informed consent was obtained from each patient by the investigator before any study-related assessment starts.

TABLES

Table 1: Patient baseline characteristics

BASELINE CHARACTERISTICS	VALUES NO. (%)	
GENDER	Female	10 (30.3%)
	Male	23 (69.7%)
AGE (YEARS)	Median [min-max]	49 [18-68]
HISTOLOGY	Grade III Astrocytoma	6 (18.2%)
	Grade III Oligodendroglioma	2 (6.1%)
	Glioblastoma	25 (75.7%)
PRIOR TREATMENT BEFORE INCLUSION	No	31 (93.9%)
	Yes	2 (6.1%)
PARTIAL SURGERY AND BIOPSY	Partial surgery	19 (57.6%)
	Biopsy only	14 (42.4%)
THERAPEUTIC MANAGEMENT	Radiotherapy only	3 (9.1%)
	Radiotherapy and chemotherapy	29 (90.6%)
	Chemotherapy only	1 (0.3%)

FIGURES

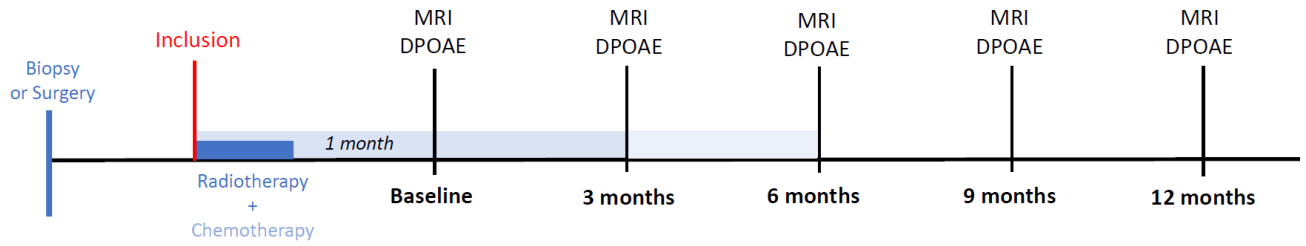


Figure 1: Study interventions

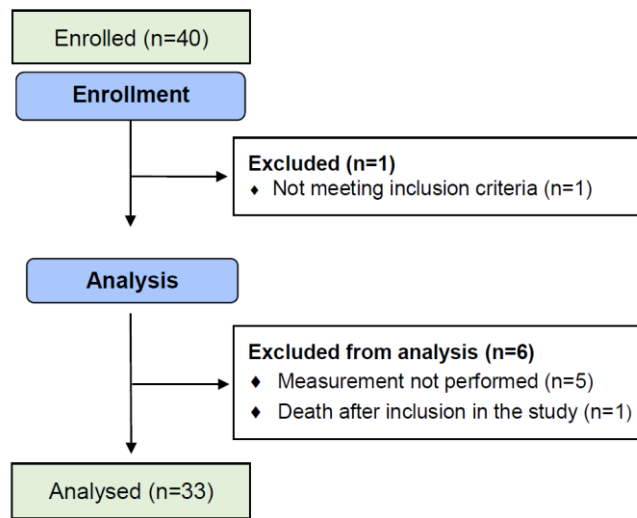


Figure 2: Flow diagram of the progress of the trial

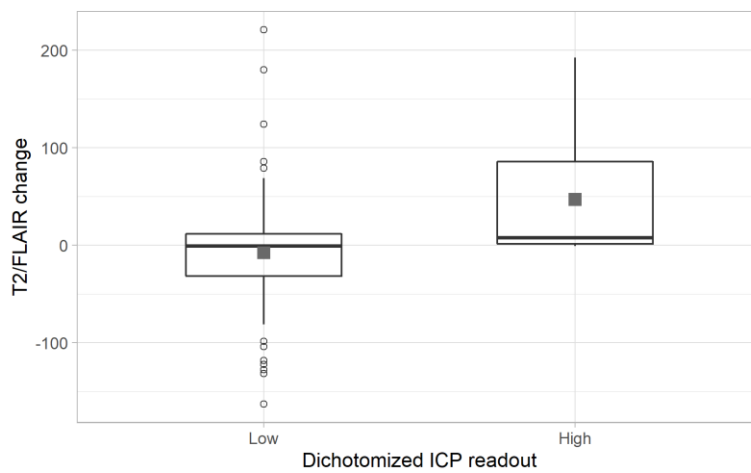


Figure 3: Boxplots of T2/FLAIR volumes according to the variation of dichotomized intracranial pressure estimate

The solid squares represent the means.

III. Conclusion

Les résultats de l'étude clinique GMaPIC mettent en évidence l'intérêt de développer une méthode de suivi de la PIC en continu. En effet, la majorité des patients inclus dans cette étude ont eu un changement significatif de leur PIC durant leur suivi correspondant à une variation de 4 mmHg. De plus, ils mettent en avant que la variation relative de la PIC au cours du suivi du patient, par rapport à la baseline, est significativement associée à une augmentation du volume T2/Flair ($p < 0,001$).

Ce dispositif médical, facile à utiliser, pourrait ainsi devenir un outil de surveillance de la PIC en complément des signes cliniques et des IRM réalisés en routine tous les 3 mois. Des mesures supplémentaires pourraient être planifiées entre les visites de suivi. Ainsi, une augmentation significative de la PIC permettrait d'alerter l'oncologue sur une éventuelle progression de la tumeur dans le but d'adapter les traitements le plus rapidement possible afin d'améliorer la qualité de vie de ces patients.

Ainsi, les résultats de cette étude ont permis l'identification d'un nouveau paramètre clinique prédictif d'efficacité qui devrait être confirmé dans une étude à plus large échelle, multicentrique. De plus, l'utilisation de la dernière version du dispositif médical permettait une meilleure reproductibilité des mesures. En effet, dans le cadre de l'étude GMaPIC, la version du dispositif médicale utilisée ne disposait pas des améliorations suivantes, à l'origine de données non analysables, estimées à environ 9% : des bouchons d'oreille en mousse adaptable à tous les conduits auditifs et le partage d'information en temps réel sur le positionnement de la sonde et la qualité de l'étanchéité.

La dernière version du dispositif médical, développée par la société ELIOS, pourrait alors devenir un outil de surveillance de la PIC pour les patients atteints de gliome de haut grade. Elle permettrait ainsi d'améliorer la prise en charge de ces patients dont le pronostic à long terme reste médiocre, avec des risques élevés de progression tumorale liés à une radorésistance. Dans ce sens, mon travail s'est également articulé autour du projet de recherche clinique NANO-GBM.

C. L'étude NANO-GBM :

I. Introduction

Les nanoparticules métalliques ayant un numéro atomique élevé (Z) ont la capacité d'agir comme radiosensibilisants. Elles permettent d'améliorer l'efficacité de l'irradiation en augmentant l'absorption des photons émis par le faisceau de rayonnement dans les cellules tumorales. L'utilisation de nanoparticules pourrait constituer une approche prometteuse pour le traitement local des tumeurs par radiothérapie externe. L'agent radiosensibilisant évalué dans cette étude est la nanoparticule AGuIX[®], une nanoparticule à base de polysiloxane chélaté au gadolinium, administrée par voie intraveineuse (59). Cette nanoparticule est un agent théranostique qui possède de très grandes propriétés de radiosensibilisation ainsi que des propriétés d'agent de contraste, grâce à la présence de gadolinium. Les nanoparticules ont été testées sur différents modèles de tumeurs orthotopiques du système nerveux central (gliosarcome (148), glioblastome (149) et métastases cérébrales de mélanome (59)) et sur des souris présentant des tumeurs hétérotopiques. Grâce à l'effet EPR (Enhanced Permeability and Retention) (150), une longue accumulation et une rétention des nanoparticules dans les tissus tumoraux est observée. Elles s'accumulent dans la tumeur après leur administration intraveineuse et restent encore détectables à l'aide d'une IRM 24h après l'administration. Les nanoparticules se caractérisent par une élimination rapide, un rapport d'affinité élevé entre la tumeur et les tissus sains, sans aucun signe d'extravasation dans le tissu cérébral. Des études *in vivo* ont démontré cette efficacité, avec un bénéfice en termes de survie, en combinant les nanoparticules AGuIX[®] avec de la radiothérapie chez des animaux atteints de glioblastome (148,151,152), de tumeurs pulmonaires (150), de tumeurs de la tête et du cou (153), de tumeurs pancréatiques (154), de mélanome (155) et de métastases multifocales de mélanome cérébral (59). Dans un modèle de gliome orthotopique chez le rat, l'accumulation sélective des nanoparticules AGuIX[®] dans les gliomes de haut grade a été démontrée, ainsi qu'un effet potentiel en termes de survie lorsqu'elles sont associées à une chimio-radiothérapie avec du témozolomide (156).

La première administration chez l'homme a été réalisée au cours de l'essai clinique NANO-RAD (NCT02820454) (157). Cet essai de phase I a évalué la tolérance et la dose maximale tolérée de l'injection intraveineuse de nanoparticules AGuIX[®] (15, 30, 50, 75 ou 100 mg/kg) en association avec une radiothérapie pan-encéphalique chez des patients présentant des

métastases cérébrales multiples. Aucune toxicités dose limitantes (TDL) n'a été observée jusqu'à 100 mg/kg. Cet essai clinique a démontré une bonne tolérance de l'injection intraveineuse des nanoparticules AGuIX® et une élimination rénale rapide en cohérence avec les résultats précliniques. Parmi les 29 événements indésirables graves, quatre ont été considérés comme possiblement liés aux nanoparticules et seraient des effets retardés de la radiothérapie, éventuellement renforcés par les nanoparticules (158). Pour finir, cet essai clinique a également démontré l'accumulation sélective d'AGuIX® dans les métastases cérébrales de différents types de tumeurs grâce à l'effet EPR. Ceci avec une persistance dans la tumeur pendant quelques jours après l'injection, une absorption des nanoparticules corrélée à la dose, une réduction du volume tumoral corrélée à la dose et un bénéfice clinique pour 13 patients sur 14 lors de l'association des nanoparticules AGuIX® à la radiothérapie (159).

Quatre études cliniques, utilisant les nanoparticules AGuIX®, de phase Ib et/ou II sont en cours. A ce jour, plus de 100 patients ont déjà reçu des injections de nanoparticules AGuIX®.

- Les études NANORAD 2 (NCT03818386) (160) et NANOBRAINMETS (NCT04899908) (161) sont des études randomisées de phase II évaluant l'efficacité de l'association des nanoparticules AGuIX® (100 mg/kg) avec une radiothérapie pan-encéphalique ou une radiochirurgie/radiothérapie stéréotaxique, respectivement, dans le traitement des métastases cérébrales.
- L'essai NANOSMART (NCT04789486) (162) est un essai de phase I/II évaluant la sécurité et l'efficacité des nanoparticules AGuIX® en association avec une radiothérapie adaptative guidée par résonance magnétique stéréotaxique pour les tumeurs pulmonaires et les cancers du pancréas localement avancés.
- Pour finir, l'essai NANOCOL (NCT03308604) (163) est un essai de phase Ib évaluant les nanoparticules AGuIX® en association avec la radio-chimiothérapie dans le cancer du col de l'utérus avancé.

Elles pourraient ainsi constituer une approche prometteuse pour accroître l'efficacité du traitement standard chez les patients atteints de glioblastome. L'essai clinique NANO-GBM est un essai de phase I/II visant à tester l'association des nanoparticules AGuIX® à une radio-chimiothérapie avec du témozolomide concomitant chez des patients atteints d'un glioblastome nouvellement diagnostiqué, avec une résection incomplète.

Cette étude multicentrique est une étude interventionnelle de catégorie 1. Elle a reçu l'avis favorable de l'Agence Nationale de sécurité du Médicament en janvier 2021 et du comité de protection des personnes Ile de France X en février 2021. Cette étude a été enregistrée sur Clinicaltrials.gov (NCT04881032). Il est prévu d'inclure un maximum de 66 patients : 12 patients dans la phase I, dont 6 traités à la dose recommandée, 34 patients supplémentaires dans le bras expérimental de la phase II et 20 patients dans le bras de référence de la phase II. L'étude a débuté en mars 2022 avec une période de recrutement de 24 mois et une date de fin estimée à mars 2026. A ce jour, 8 patients ont été inclus dans la phase I.

Les détails concernant le protocole sont présentés ci-dessous dans l'article du protocole publié, en 2^{ème} auteure, dans la revue BMC Cancer.

II. Publication du protocole

Thivat et al. *BMC Cancer* (2023) 23:344
<https://doi.org/10.1186/s12885-023-10829-y>

BMC Cancer

STUDY PROTOCOL

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Phase I/II study testing the combination of AGuIX nanoparticles with radiochemotherapy and concomitant temozolomide in patients with newly diagnosed glioblastoma (NANO-GBM trial protocol)

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Abstract

Background Despite standard treatments including chemoradiotherapy with temozolomide (TMZ) (STUPP protocol), the prognosis of glioblastoma patients remains poor. AGuIX nanoparticles have a high radiosensitizing potential, a selective and long-lasting accumulation in tumors and a rapid renal elimination. Their therapeutic effect has been proven in vivo on several tumor models, including glioblastoma with a potential synergetic effect when combined with TMZ based chemoradiotherapy, and they are currently evaluated in 4 ongoing Phase Ib and II clinical trials in 4 indications (brain metastases, lung, pancreatic and cervix cancers) (> 100 patients received AGuIX). Thus, they could offer new perspectives for patients with newly diagnosed glioblastoma. The aim of this study is to determine the recommended dose of AGuIX as a radiosensitizer in combination with radiotherapy and TMZ during the concurrent radio-chemotherapy period for phase II (RP2D) and to estimate the efficacy of the combination.

Methods NANO-GBM is a multicenter, phase I/II, randomized, open-label, non-comparative, therapeutic trial. According to a dose escalation scheme driven by a TITE-CRM design, 3 dose levels of AGuIX (50, 75 and 100 mg/kg) will be tested in phase I added to standard concomitant radio-chemotherapy. Patients with grade IV glioblastoma, not operated or partially operated, with a KPS \geq 70% will be eligible for the study. The primary endpoints are i) for phase I, the RP2D of AGuIX, with DLT defined as any grade 3–4 NCI-CTCAE toxicity and ii) for phase II, the 6-month progression-free survival rate. The pharmacokinetics, distribution of nanoparticles, tolerance of the combination, neurological status, overall survival (median, 6-month and 12-month rates), response to treatment, and progression-free survival (median and 12-month rates) will be assessed as secondary objectives. Maximum sixty-six patients are expected to be recruited in the study from 6 sites.

Discussion The use of AGuIX nanoparticles could allow to overpass the radioresistance to the reference treatment of newly diagnosed glioblastomas that have the poorest prognosis (incomplete resection or biopsy only).

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Trial registration Clinicaltrials.gov: NCT04881032, registered on April 30, 2021. Identifier with the French National Agency for the Safety of Medicines and Health Products (ANSM): N°Eudra CT 2020-004552-15. Protocol: version 3, 23 May 2022.

Keywords Glioblastoma, Nanoparticles, AGuIX, Nanomedicine, Radiotherapy, Radiosensitization

Background

Glioblastoma (GBM) is the most common malignant brain tumor in adults. Despite the significant improvement with the current standard treatment combining the largest possible resection, followed by concomitant radio-chemotherapy 60 Gy/30 fractions with temozolomide (TMZ) and then adjuvant chemotherapy by several cycles of TMZ [1] the prognosis of these patients remains poor. The median overall survival is 14.6 months (18.8 months, 13.5 months, and 9.4 months for patients with complete resection, partial resection, and biopsy alone, respectively) [2] and the progression-free survival at 6 months is 53.9% [1] with a poorer prognostic for the patient with unresectable GBM [2]. Moreover, GBMs are radioresistant tumors and recurrence occurs in approximately 90% of cases within the radiation fields [3]. Since there are few therapeutic solutions in case of recurrence, a radiosensitization strategy would permit to increase the chances of local control, from the first line of treatment on.

Metal nanoparticles with a high atomic number (Z) have the ability to act as radiosensitizers. They allow improving the efficiency of irradiation, by increasing the absorption of photons emitted by the radiation beam, in tumor cells. The use of nanoparticles could be a promising approach for the local treatment of tumors by external radiotherapy (RT). The radiosensitizing agent evaluated in this study is AGuIX (Activation and Guidance of Irradiation by X-ray) nanoparticles, a gadolinium chelated-polysiloxane based-nanoparticles administered intravenously [4]. This nanoparticle is a theranostic agent with very high radiosensitizing properties as well as contrast agent properties, thanks to the presence of gadolinium. The nanoparticles were tested on different orthotopic tumor models of the central nervous system (gliosarcoma [5], glioblastoma [6] and melanoma brain metastases [4]) and on mice with heterotopic tumors. Thanks to the EPR (Enhanced Permeability and Retention) effect [7], a long accumulation and retention of nanoparticles in tumor tissues is observed and they are still detectable by MRI 24 h after their intravenous administration. The nanoparticles are characterized by rapid clearance by normal tissue, high tumor-to-healthy tissue affinity ratio, with no evidence of extravasation into the brain tissue. In vivo studies have demonstrated such efficacy when combining AGuIX nanoparticles with

radiation in animals with GBM [5, 8, 9] lung tumors [7], head and neck tumors [10], pancreatic tumors [11], melanoma [12] and multifocal brain melanoma metastases [4] with a survival benefit with the combination of AGuIX nanoparticles and RT, compared to RT alone. In an orthotopic glioma rat model, Dufort et al. demonstrated the selective accumulation of AGuIX in high-grade glioma as well as the potential survival benefits when combined with standard of care TMZ and radiotherapy [13].

The first-in-human phase I NANO-RAD (NCT02820454) evaluated the tolerance and the maximum tolerated dose of the intravenous injection of AGuIX nanoparticles, (15, 30, 50, 75 or 100 mg/kg) in combination with whole brain radiotherapy in patients with multiple brain metastases not suitable for stereotactic radiotherapy (from primary cancers of melanoma type $n=6$, non-small cell lung cancer $n=6$, colon cancer $n=1$, and breast cancer $n=2$). No dose-limiting toxic effects were observed up to AGuIX 100 mg/kg. This clinical trial demonstrated a good tolerance of the intravenous injection of AGuIX nanoparticles, and a rapid elimination by the kidney in correspondence with pre-clinical results. Among the 29 SAEs, four were considered possibly related to AGuIX and were delayed effects of RT, possibly enhanced by AGuIX [14]. Moreover, this clinical trial also demonstrated (i) the selective accumulation of AGuIX in brain metastases from different types of primary tumors due to the EPR effect, with a persistence in the tumor lasting few days after injection, (ii) a dose-correlated uptake of AGuIX in brain metastases, (iii) a dose-correlated tumor volume reduction and (iv) a clinical benefit for 13 patients over 14 when combination of AGuIX with radiotherapy [15].

Four clinical phase Ib and/or II trials are currently underway with AGuIX, with more than 100 patients already injected with AGuIX nanoparticles. The NANO-RAD 2 (NCT03818386) and the NANOBRAINMETS (NCT04899908) trials are randomized phase II trials evaluating the efficacy of the combination of intravenous injections of AGuIX nanoparticles (100 mg/kg) with whole brain radiotherapy or stereotactic radiosurgery/radiotherapy, respectively, in the treatment of brain metastases. The NANOSMART trial (NCT04789486) is a phase I/II trial evaluating the safety and the efficacy AGuIX in combination with stereotactic magnetic resonance-guided adaptive radiation therapy for centrally located lung tumors and locally advanced pancreatic

cancers, and the NANOCOL trial (NCT03308604) is a phase Ib evaluating AGuIX in combination with radio-chemotherapy in advanced cervical cancer.

According to preclinical and clinical results, the AGuIX nanoparticles could be a promising approach to increase the effectiveness of the standard of care (TMZ and RT) in GBM patients.

Our phase I/II trial aims to test the combination of AGuIX nanoparticles with radio-chemotherapy and concomitant TMZ in patients with newly diagnosed GBM with incomplete resection.

Methods / design

Study design

NANO-GBM trial is a multicenter, phase I/II, randomized, open-label, non-comparative, therapeutic study.

The phase I part consists of a dose escalation with 3 dose levels of AGuIX: 50, 75 and 100 mg/kg, driven by a Time-to-event Continuous Reassessment Method (TITE-CRM) [16] with a dose-toxicity relationship given by a one-parameter power model and a priori risks of 5%, 10% and 25% for the 3 doses, respectively. The first patient will receive the first dose level, and the next cohorts will consist of 1 patient at a time, with a minimum of 6 treated at the recommended dose and a maximum of 12 patient treated in phase I.

The phase II part consists of a randomized open-label study. Patients will be randomized at baseline via a central system on eCRF, in a 2:1 ratio stratifying for the site and the age: one third of patients in the reference arm treated with radio-chemotherapy with concomitant TMZ (STUPP protocol), and two thirds in the experimental arm combining radio-chemotherapy with concomitant TMZ with the administration of AGuIX nanoparticles at the recommended dose determined in the phase I part. The randomization sequence is known only to the statistician. For the experimental arm, a single-stage Fleming design will be used. While the design does not allow for a formal comparison between the two arms, it may reinforce the value of the conclusions on the potential efficacy of the experimental treatment by validating the hypothesis on the control arm.

An interim analysis will be performed without interruption of inclusions for the independent data monitoring committee (IDMC) review, after 20 patients are treated in the experimental arm at the recommended dose (i.e. approximately 30 patients in phase II).

A maximum of sixty-six patients are expected to be enrolled: 12 patients in phase I, of which (minimum) 6 are treated at the recommended dose, 34 additional patients in the phase II experimental arm and 20 patients in the phase II reference arm. The study was started in

March 2022 with a 2 years enrollment period and an estimated completion date by March 2026. This study has been registered on Clinicaltrials.gov (NCT04881032).

Coordination and participating institutions

The Centre Jean PERRIN is the sponsor and is responsible for coordination, trial management, data management, trial monitoring and statistical analysis. This multicenter study will be conducted in 6 sites in France.

The list of the study sites is available on <https://clinicaltrials.gov/ct2/show/NCT04881032>

Study objectives and endpoints

Mains objectives and endpoints

The primary objectives of the NANO-GBM study are i) in the phase I part, to determine the recommended dose of AGuIX in combination with radiotherapy and TMZ during the concurrent radio-chemotherapy period for phase II (RP2D); ii) in the phase II part, to estimate the efficacy of the combination radio-chemotherapy + AGuIX (at the RP2D).

The first primary endpoint (phase I) is the RP2D of AGuIX nanoparticles corresponding to the highest dose tested for which the percentage of dose-limiting toxicities (DLT) is less than 33%. DLT is defined as any grade 3–4 toxicity according to the NCI-CTCAE classification v5.0, except for alopecia, nausea and vomiting, or fever, which can be managed by symptomatic treatment. Only toxicities occurring during the concomitant radio-chemotherapy (i.e. during 6 weeks) will be considered for DLT assessment.

The second primary endpoint (phase II) is 6-month progression-free survival rate (6-month PFS) to estimate the efficacy of the combination of radio-chemotherapy with AGuIX. Disease assessment throughout the study will be based on RANO (Response Assessment in Neuro-Oncology) criteria that take into account imaging progression (MRI) and also clinical status and level of steroid therapy [17].

Secondary objectives

The secondary objectives are to evaluate:

- The pharmacokinetics parameters (AUC, Tmax et Cmax) of AGuIX nanoparticles (phase I only) will be measured on blood samples and urinary excretion at D0, D7, D14
- The distribution of nanoparticles and the sparing of healthy tissue by MRI (W0 and D14)
- The tolerance of the combination: acute (< 90 days) and late toxicity as well as changes in dose and spread of radiotherapy (phase I and II) will be graded according to the Common Terminology Criteria for

Adverse Events (CTCAE, version 5.0). Reporting of serious adverse events and suspected unexpected serious adverse reaction will be carried out according to the local regulations.

- The neurological status will be evaluated by clinical assessment and Mini-Mental State Examination (MMSE)
- Overall survival (median, 6-month and 12-month rates), response to treatment, and progression-free survival (median and 12-month rates) according to RANO criteria (phase II)

Exploratory objective

A FFPE block of the tumor will be collected for each patient, unless they oppose it on the informed consent form. This block will be stored by the study sponsor and will be used to conduct exploratory translational research. The objective is to study potential predictive biomarkers and exploration of the tumor microenvironment.

Participant eligibility

The inclusion and non-inclusion criteria are presented in Table 1.

Patients with grade IV glioblastoma, not operated or partially operated, with a KPS \geq 70% will be eligible for the study. However, patients with a history of cerebral radiotherapy, chemotherapy or with a contraindication to TMZ or MRI or gadolinium injection, will not be eligible for the trial.

Intervention

Experimental group

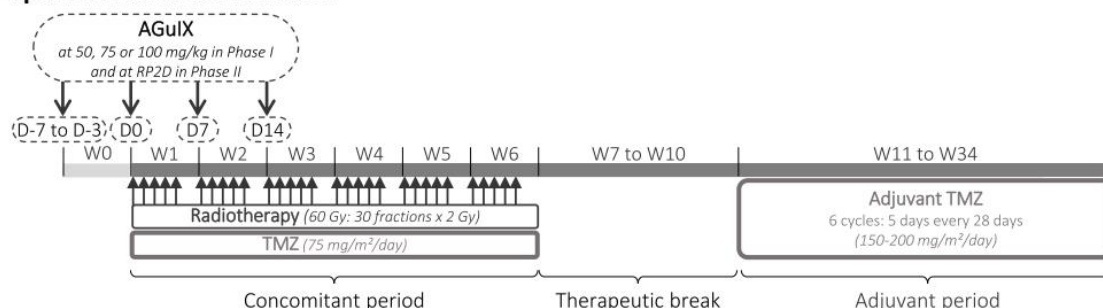
Patients in the phase I and phase II experimental arms will be treated with concomitant TMZ radio-chemotherapy (STUPP protocol) in combination with AGuIX nanoparticles during the concomitant radio-chemotherapy period (Fig. 1).

Nanoparticles AGuIX (experimental treatment) provided by NH TherAguix, will be administered in 4 intravenous injections. The first one will be administered 3 to 7 days before the start of the radio-chemotherapy and at least 48 h after dosimetry MRI. The next three injections

Table 1 Section criteria

Inclusion criteria	Non-inclusion criteria
Histological diagnosis of grade IV glioblastoma (biopsy or partial surgery)	History of cerebral radiotherapy
Patient not operated or in partial excision	History of chemotherapy (including carmustine (Gliadel®) implants) or immunotherapy (including vaccination)
Karnofsky performance status \geq 70%	Any contraindication to TMZ listed in the multidisciplinary consultation meeting
Age \geq 18 years and $<$ 75 years	History of major bowel resection that may affect oral drug absorption in the judgment of the investigator
Life expectancy \geq 6 months	Diagnosed inflammatory bowel disease (Crohn's disease or ulcerative colitis)
Platelets \geq 100 000/mm ³	Diarrhea \geq CTCAE grade 2 (regardless of cause)
PNN \geq 2000/mm ³	Current or recent treatment with another investigational drug or participation in another therapeutic clinical trial (within 30 days of inclusion)
Hemoglobin \geq 10 g/dL	History of other cancer within 5 years prior to inclusion except basal cell carcinoma of the skin and carcinoma in situ of the cervix
Creatinine $<$ 1.5 times the upper normal limit or Cockcroft-Gault clearance \geq 50 mL/min	Pregnant or breastfeeding women
Liver function (GGT, PAL, AST, ALT, bilirubin) $<$ 1.5 times the upper normal limit	Contraindication to MRI or gadolinium injection
For patients receiving corticosteroid therapy, corticosteroid therapy must be at a stable or decreasing dose for at least 14 days prior to inclusion	History of severe anaphylactic reactions due to the injection of gadolinium-based contrast material (Dotarem®, etc.)
Patient able to swallow and maintain oral therapy	Patient under guardianship or curatorship
Negative serum pregnancy test within 7 days prior to first administration of therapy for women	History of nephropathy
Women of childbearing potential and men whose partners are of childbearing potential must agree to use an approved method of contraception for themselves or their partners for the duration of treatment and at least 6 months after the last study treatment	Psychological disorder or social or geographical reasons that could compromise the medical follow-up of the trial or compliance with the treatment
Obtaining signed informed consent from the patient	
Patient enrolled in a social security plan	

Experimental treatment arm



Reference treatment (Stupp protocol): Control arm

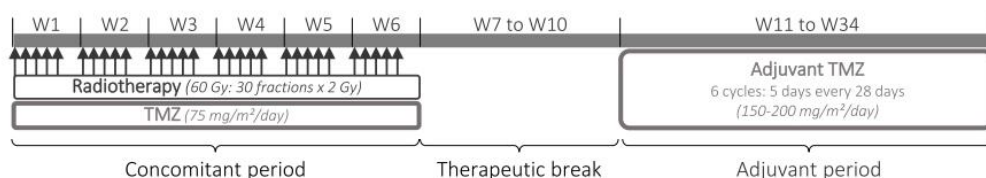


Fig. 1 Overview of the treatment period of the NANO-GBM study

will be administered during the radio-chemotherapy at the first day of the week 1, week 2 and week 3. The injection will be performed 4 h (± 1 h) before RT and 3 h before TMZ. For phase I, the dose administered at each injection will be 50, or 75 or 100 mg/kg (tested doses). For phase II, the dose administered at each injection will be the dose recommended after phase I.

Control group

Patients in the phase II reference arm will be treated with the standard STUPP protocol: RT at a dose of 60 Gy (30 fractions/6 weeks) and concomitant treatment with daily oral TMZ (75 mg/m²/day) from the first day of RT until the end of RT. Then, adjuvant TMZ will be reintroduced 4 weeks after the end of RT according to a 5-days schedule every 28 days (150 mg/m²/day for the first cycle and then at 200 mg/m²/day in the absence of toxicity).

Study procedures and participant timeline

The overview of study assessments and procedures is presented in Table 2.

Statistical analysis

Sample size

A maximum of 12 patients will be treated in phase I (sample size obtained by simulation, and in agreement

with the bound obtained by Cheung’s formula, 2013), minimum 6 patients to be treated at the recommended dose, who will then be included and analyzed in phase II.

The phase II trial consists of a randomized study with a 2:1 ratio in favor of the experimental arm. For the experimental arm, a single-stage Fleming design will be used, based on an acceptable success rate of 70% and a 50% unacceptable success rate. In order to limit the one-sided alpha risk (of wrongly concluding that the treatment is effective) to 5% and the beta risk (of wrongly failing to conclude that the treatment is effective) to 20%, the minimum number of patients to include is 37. The treatment will be considered effective (and therefore the phase II positive) as soon as 24 successes out of 37 are obtained. In this case, the estimate of the probability of success will be of around 65%, with a 90% confidence interval (90% CI) of 51.4%-76.7% (Jeffreys interval).

It was decided to include 40 patients in the experimental arm, to compensate for a rate of about 10% of missing data (all reasons combined). Therefore, the number of patients in the reference arm will be 20.

Consequently, the total number of patients to be included is 66 (maximum): 12 patients in phase I, of which (minimum) 6 were treated at the recommended dose, 34 additional patients in the experimental arm of phase II, and 20 patients in the reference arm of phase II.

Table 2 Data collection schedule

Timepoint	Screening / inclusion		Concomitant chemoradiotherapy phase										Therapeutic break					Adjuvant phase				
	Week	Day	W0	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11	W12	W13	W14	W15	W16	W17	W18	
Informed consent				0	7	14	21	28	35	42	49	56	63	70±4d	77	84	91	98±4d	105	112	119	
Medical history and demographics			at -7 to -3																			
Initial histology / centralized review (FFPE tumor block)																						
Previous/concomitant treatments (including corticosteroids)																						
Evaluation of adverse events																						
Radiological evaluation MRI (disease)																						
MRI dosimetry																						
MRI post injection AGuIX (1 h after injection) ^h																						
Examination neurological (MMSE)																						
Clinical examination, weight																						
Vital signs ^a																						
KPS Performance Index																						
Serum pregnancy test ^g																						
Hematology ^b																						
Fasting biochemistry ^c																						
Urine analysis ^d																						
Pharmacokinetics ^f																						
Radiotherapy																						
Temozolomide																						
AGuIX (injection IV) ^j																						
survival assessment																						

Table 2 (continued)

Timepoint	Adjuvant phase														End of treatment visit	Follow-up to progression	Follow-up of survival	
	cycle 3				cycle 4				cycle 5				cycle 6					
	W19	W20	W21	W22	W23	W24	W25	W26	W27	W28	W29	W30	W31	W32				W33
Day	126±4d	133	140	147	154±4d	161	168	175	182±4d	189	196	203	210±4d	217	224	231		
Informed consent																		
Medical history and demographics																		
Initial histology / centralized review (FFPE tumor block)																		
Previous/ concomitant treatments (including corticosteroids)																		
Evaluation of adverse events																		
Radiological evaluation (MRI (disease))				✓													✓	✓
MRI dosimetry																		
MRI post injection AGUIX (1 h after injection) ^h																		
Examination neurological (VIMSE)				✓													✓	✓
Clinical examination, weight	✓			✓				✓									✓	✓
Vital signs ^a	✓			✓				✓									✓	✓
KPS Performance Index	✓			✓				✓									✓	✓

Table 2 (continued)

Timepoint	Adjuvant phase														End of treatment visit	Follow-up to progression	Follow-up of survival	
	cycle 3				cycle 4				cycle 5				cycle 6					
	W19	W20	W21	W22	W23	W24	W25	W26	W27	W28	W29	W30	W31	W32				W33
Week	126 ± 4d	133	140	147	154 ± 4d	161	168	175	182 ± 4d	189	196	203	210 ± 4d	217	224	231		
Day																		
Serum pregnancy test ^a	✓				✓				✓									
Hematology ^b	✓				✓				✓									✓
Fasting biochemistry ^c	✓				✓				✓									✓
Urine analysis ^d	✓				✓				✓									✓
Pharmacokinetics ^e																		
Radiotherapy																		
Temozolomide																		
AGuIX (injection IV) ^f																		5D ^g
Survival assessment																		

^a vital signs include heart rate measurement, systolic and diastolic blood pressure, temperature, pulse

^b hemoglobin, CBC/platelet

^c glucose, sodium, potassium, magnesium, calcium, total protein, creatinine, urea, AST, ALT, alkaline phosphatase, GGT, total bilirubin

^d proteinuria

^e TMZ 150–200 mg/m²/d D1 at D5 every 28 days (the daily dose of 150 mg/m² for the first cycle will be increased to 200 mg/m² for the 2nd cycle in the absence of significant toxicity)

^f pharmacokinetics performed only for phase I patients: blood sampling 7 ml before injection, 15 min, 30 min, 1 h (before MRI), 2 h, 3 h, 4 h (before the beginning of radiotherapy), 6 h and 24 h post injection + urine collection during 6 h

^g for patients of childbearing age

^h only for patients receiving the experimental treatment: Phase I + Phase II experimental arm

ⁱ experimental treatment: for phase I patients (dose of 50 or 75 or 100 mg/kg according to the TITE CRM) and for patients in the experimental arm of phase II (at the dose recommended by phase I) At S1J0, S2J7 and S3J14 the injection will be performed 4 h ± 1 h before the radiotherapy session

^j injection performed 3 to 7 days before the start of radiochemotherapy, and at least 2 days after the MRI dosimetry

Data analysis

Statistical analysis will be realized on modified intention-to-treat (mITT) population, i.e. patients who received at least 1 injection of AGuIX nanoparticles.

The primary endpoint of phase I, the RP2D of AGuIX nanoparticles, will be determined as the highest dose tested for which the percentage of dose-limiting toxicities (DLT) is less than 33%. DLT percentage will be calculated for each tested dose. Progression-free survival, progression-free survival rate at 6 months and 12 months, and overall survival at 6 months and 12 months will be estimated by the Kaplan–Meier method in both arms. The primary endpoint of phase II, 6-month PFS in the experimental arm, will be calculated with its 90% CI, and the result will be interpreted according to the Fleming method.

Missing data will not be replaced. The statistical significance threshold is set at 5%. Statistical analyses will be performed using R software.

Data management and monitoring

The data collected for the study will be recorded in an eCRF (Ennov Clinical software) by each investigating center. The staff with access to the data will be the investigators, the clinical research associates, the project leaders and the biostatisticians. They are authorized professionals and are subject to professional secrecy. The investigator will ensure the accuracy, completeness, and consistency of the pseudonymized patient data recorded and of the provision of answers to data queries.

Regular monitoring reviews will be performed by a clinical research associate mandated by the sponsor. The objectives will be to ensure the proper conduct of the study in each center, the recording of the data generated in writing, their documentation, recording and reporting, in accordance with the legislative and regulatory provisions in force. The monitoring reports will ensure traceability.

Independent data monitoring committee (IDMC)

An independent monitoring will be responsible for reviewing the safety data of treated patients. The committee will meet at the end of phase I and will be able to validate the recommended dose retained, recommend the continuation of the trial in phase II, its interruption, or its modification depending on the adverse events which occurred during the trial.

The committee will meet a second time during phase II, after 20 patients have been treated at the RP2D in the experimental arm (end of the TDL evaluation period) to confirm the choice of the recommended dose.

This committee will consist of 3 members who are not involved in patient recruitment or trial conduct and will include at least 1 radiotherapist and 1 oncologist.

Trial status

The NANO-GBM clinical trial is currently recruiting. Patient enrollment began in March 2022 and is expected to end in March 2024.

Discussion

The NANO-GBM trial is the first study to test the association of intravenous nanoparticles with radio-chemotherapy (STUPP standard protocol) in GBM patients. This trial aims to determine the RP2D of AGuIX nanoparticles and evaluate the efficacy of the combination of AGuIX nanoparticles with radio-chemotherapy and concomitant TMZ in patients with newly diagnosed GBM (with incomplete resection).

The approach to improve the standard treatment of newly diagnosed GBM is based on an optimization of RT, as surgery is always maximal and functional and remains a major prognostic factor, and given that TMZ doses cannot be increased due to associated toxicities.

The accumulation of AGuIX nanoparticles in tumors followed by the interaction between AGuIX nanoparticles and the X-rays, combined with an elimination of nanoparticles in healthy tissues, would allow increasing the effectiveness of RT at the tumor site, without increasing the prescribed X-ray dose. Moreover, it has been demonstrated in preclinical tumor models but also during the First in Human phase I trial that the drug is not active except at the irradiated tumor area: the first clinical data report a good tolerance of a single intravenous AGuIX. The use of AGuIX nanoparticles could therefore allow to overpass the radioresistance to the reference treatment (STUPP protocol) of newly diagnosed unresectable or partially resected GBM while keeping the same level of sparing for healthy tissues. It represents a promising method to improve care of the poorest prognosis patients.

With the use of AGuIX nanoparticles, we expect an improvement of the 6-month progression-free survival rate by 20% for these patients who have the poorest prognosis, without an increase of the delivered total dose of RT and without increase of toxicity in healthy tissues. An improvement in their neurological symptoms linked to the expected response on the residual tumor or in place by increased radiosensitivity is also expected (reduction in signs of intracranial hypertension by reduction in the mass effect or reduction in neurological symptoms).

Abbreviations

DLT	Dose-Limiting Toxicity
EPR	Enhanced Permeability and Retention
GBM	Glioblastoma
MTD	Maximum Tolerated Dose
RANO	Response Assessment in Neuro-Oncology
RP2D	Recommended Phase II dose
TITE-CRM	Time-to-event CRM
TMZ	Temozolomide

Acknowledgements

We are grateful to all the patients and their caregivers. We thank the Cancer Research Patients Committee of the French League Against Cancer for their re-readings of the Patient Information Form. We also thank the members of the Independent Data Monitoring Committee and the investigators.

Authors' contributions

Conception and design: JB, ET, JM, XD, IM, SD, ML; Investigators of the study: JB, JM, XD; Revision of the study design and protocol: SD, KS, ML; Study coordination: JM, ET. Acquisition of data and patient recruitment: JM, JB, XD; Statistical analysis: IM; Obtaining funding and supervision: JM, ET, GLD; Drafting the manuscript: MC, ET; Revision, adaptation and final approval of the manuscript: JB, SD, ML, ODB, GLD. The author(s) read and approved the final manuscript.

Funding

The trial was funded by a public grant, the Programme Hospitalier de Recherche Clinique-Interregional (PHRC-I-19-060) from the French Health Ministry, planned by the Groupement Interrégional de Recherche Clinique et d'Innovation Auvergne Rhône Alpes (GIRCI AURA) and has undergone external peer review as part of funding application. The funding parties are not involved in the design and conduct of the study, nor in the collection, management, analysis, and interpretation of the data. They were not involved in the writing of the manuscript.

Availability of data and materials

Not applicable.

Declarations**Ethics approval and consent to participate**

The study protocol and its amendments obtained approval from the French Ethics Committee (Comité de protection des personnes Ile de France X) (ref 20.12.01.45646) in February 2021 and from the French National Agency for the Safety of Medicines and Health Products (ANSM) (N°Eudra CT 2020-004552-15) in January 2021.

The study is conducted in accordance with the Helsinki Declaration, the Good Clinical Practice (GCP) guidelines of the International Conference on Harmonisation (ICH-E6, 17/07/96), General Data Protection Regulation (GDPR) and local regulatory requirements. Written informed consent will be obtained from each patient by the investigator before any study-related assessment starts.

Consent for publication

Not applicable.

Competing interests

GLD discloses patent No. WO2009/053644 which protects the AGuIX nanoparticles described in this publication. SD, KS, ML, ODB and GLD are employees of NH TherAguix (Meylan, France), which is developing the AGuIX nanoparticles. SD, ML and GLD own shares in this company. The other authors declare that they have no competing interests.

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Received: 31 March 2023 Accepted: 8 April 2023

Published online: 15 April 2023

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III. Conclusion

Bien que le traitement de référence (10,42) ait augmenté la survie des patients, le pronostic à long terme reste médiocre dans le cas du glioblastome. La progression tumorale se produisant en grande majorité à l'intérieur du volume tumoral irradié (164).

L'étude NANO-GBM est la première étude à tester l'association de nanoparticules intraveineuses avec de la radio-chimiothérapie chez des patients atteints de glioblastome. Les doses de radiothérapie ne pouvant être augmentées en raison des toxicités associées, l'utilisation des nanoparticules AGuiX® pourrait permettre de dépasser la radiorésistance des glioblastomes non résécables ou partiellement réséqués. Et cela, tout en conservant le même niveau d'épargne des tissus sains.

Il s'agit d'une méthode prometteuse qui permettrait d'améliorer la prise en charge de ces patients, en améliorant le contrôle local dès la première ligne de traitement. Avec l'utilisation des nanoparticules AGuiX®, nous prévoyons une amélioration du taux de survie sans progression à 6 mois de 20%, sans augmentation de la dose totale de radiations ionisantes délivrée et sans augmentation des toxicités pour les tissus sains.

5. DISCUSSION ET PERSPECTIVES

La prise en charge des gliomes de haut grade par radiothérapie externe reste à ce jour l'un des plus grands défis de l'oncologie. L'équipe de recherche et la division de la recherche clinique du Centre Jean PERRIN a fait de cette problématique l'une de ses priorités depuis de nombreuses années avec notamment l'élaboration de différents protocoles de recherche clinique : L'essai clinique de phase I/II GLYRAD visant à explorer l'efficacité de l'association du LY2228820 à une chimio-radiothérapie (165) et les essais cliniques GMaPIC, NANO-GBM et PROGLIO décrit dans ce manuscrit.

Mon travail de thèse en recherche clinique s'est alors inscrit dans la continuité de ces travaux, en étudiant les relations doses/effets de la radiothérapie externe des gliomes diffus de haut grade. Ainsi, j'ai pu analyser et valoriser les résultats d'une étude évaluant de façon non-invasive la PIC des patients atteints d'une tumeur gliale de haut grade, traités par radiothérapie (GMaPIC). Dans la continuité et de manière complémentaire à cette étude, j'ai élaboré un protocole évaluant les concentrations plasmatiques de l'hPG80 chez une catégorie similaire de patients, également après radiothérapie (PROGLIO). En parallèle de ces projets, pour finir, j'ai participé à l'élaboration d'une étude de phase I/II (NANO-GBM) visant à améliorer les résultats de la radiothérapie des glioblastomes par des nanoparticules. J'ai ainsi pu durant ma thèse, réaliser l'ensemble des étapes nécessaires à l'élaboration d'un protocole de recherche clinique, grâce à ces trois études : Revue de la littérature, conception et rédaction de protocole et autres documents nécessaires à la recherche, budgétisation, gestion réglementaire et administrative, conduite de l'étude, analyses et valorisation.

I. L'étude PROGLIO

L'étude pilote PROGLIO pourrait être critiquée par son absence de comparateur. Elle vise à évaluer les concentrations plasmatiques de l'hPG80 au fil du temps chez des patients atteints de tumeurs cérébrales de haut grade. Il aurait été intéressant de pouvoir comparer les concentrations plasmatiques de l'hPG80 retrouvées dans le sang des patients à des concentrations retrouvées dans un groupe témoin de donneurs de sang sains. Sans cette comparaison, il est impossible d'estimer la spécificité de ce biomarqueur pour les gliomes diffus de l'adulte. En revanche, sans comparateur, il est toujours possible d'étudier les concentrations plasmatiques de l'hPG80 retrouvées dans le sang des patients, d'étudier les

concentrations en fonction de l'évolution tumorale, d'évaluer sa sensibilité à l'irradiation et de comparer les niveaux plasmatiques chez ces patients. La recherche autour de l'hPG80 est très récente. La première publication contenant le mot « hPG80 » date de 2019 et seule 8 publications sont recensées sous Pubmed®, dont l'article du protocole PROGLIO (**Figure 12**). Les études pilotes sont donc primordiales à ce stade afin de démontrer la valeur clinique de ce biomarqueur sanguin notamment en termes de répétabilité, spécificité, sensibilité et robustesse. Les objectifs secondaires de l'étude PROGLIO permettront d'étudier l'implication de ce biomarqueur dans la radiorésistance et d'évaluer si celui-ci peut prédire la réponse aux traitements.



Figure 12 : Résultats de la recherche du mot « hPG80 » sur PubMed®

Source : pubmed.ncbi.nlm.nih.gov

Les résultats de l'essai clinique PROGLIO pourront déterminer la nécessité ou non de réaliser un protocole de recherche clinique à plus grande échelle et multicentrique. Ce second protocole pourrait ainsi comparer les taux de concentration d'hPG80 obtenus entre une population saine et une population atteinte d'un gliome diffus de l'adulte. Il pourrait secondairement évaluer les rôles pronostique et diagnostique de l'hPG80 comme biomarqueur sanguin.

II. L'étude GMaPIC

Les résultats de l'étude GMaPIC dont l'objectif était de comparer la variation de la PIC, détectée à l'aide d'une méthode non-invasive, avec les volumes mesurés à l'IRM sont plutôt équivoques. Les résultats de l'étude ont mis en évidence que la variation relative de la PIC au cours du suivi des patients, par rapport à la baseline, est significativement associée à une augmentation du volume T2/Flair ($p < 0,001$). En revanche, 7,5% des patients inclus dans l'étude GMaPIC n'ont pas été analysés car ils n'ont pas pu réaliser les mesures de la PIC. De plus, sur les 33 patients analysés, 9% des données récoltées n'étaient pas exploitables. Au vu du faible effectif et de la perte de données, la conclusion de l'essai ne peut donc pas être robuste, malgré une significativité statistique. En revanche, cette étude a permis de mettre en évidence l'intérêt de l'utilisation d'une méthode non invasive de la PIC, en continue, chez des patients atteints d'un gliome de haut grade. Presque 94% des patients analysés ont eu une augmentation de la PIC cliniquement significative durant leur suivi. Le taux important d'augmentation de la PIC au sein de cette population et sa corrélation significative avec l'augmentation du volume T2/Flair, malgré un faible effectif, mettent en avant tout l'intérêt de réaliser une nouvelle étude à plus grande échelle et multicentrique. Les dispositifs non invasifs de mesure de la PIC sont au cœur de la recherche clinique en neurologie (166). Les méthodes développées et testées se basent sur l'évolution de la PIC et son estimation en s'appuyant sur les propriétés des structures extra-crâniennes. Elles sont du types ultrasonores, vasculaires, ophtalmologiques ou audiolologiques (167,168). En revanche, aucune de ces techniques, bien que non invasives, ne semble pouvoir remplacer, à elle seule, le gold standard. Ces techniques présentent effectivement des limites. Elles ne sont pas suffisamment précises, sont limitées par différents facteurs dépendant de l'opérateur et ne sont pas toutes applicables en pratique clinique (141). L'étude GMaPIC est la première étude exploratoire à proposer une méthode non-invasive de surveillance de la PIC chez des patients atteints de gliome de haut grade traités par radiothérapie. Elle ouvre donc une nouvelle perspective. La mesure de la PIC de manière non-invasive, pourrait devenir un paramètre clinique prédictif d'efficacité pour les gliomes diffus de l'adulte.

Les résultats de l'essai clinique GMaPIC mettent en avant l'intérêt de réaliser une étude à plus grande échelle et multicentrique. Cette étude pourrait ainsi déterminer avec plus de puissance

si la dernière version du dispositif médical développée par la société ECHODIA pourrait devenir un outil de surveillance non invasif de la PIC.

III. L'étude NANO-GBM

L'évolution tumorale des patients atteints de glioblastome est généralement marquée par des rechutes locales adjacentes à la tumeur primaire, limitant souvent les possibilités thérapeutiques d'une nouvelle résection ou d'une nouvelle radiothérapie. Le type de traitement envisagé dépendra de l'état général du patient et des thérapies précédemment réalisées (34,62).

Ces dernières années plusieurs approches, afin d'améliorer le pronostic des patients atteints de glioblastome, ont été réalisées, notamment *via* l'utilisation de radiosensibilisants. Différentes approches sont possibles comme réduire la radiorésistance dérivant de l'hypoxie tumorale, générer des espèces réactives de l'oxygène ou bien en interférant avec la réparation des lésions de l'ADN induites par les radiations ionisantes. Cependant, les résultats des essais cliniques dans le domaine sont décevants et le pronostic reste toujours aussi insuffisant (169).

L'hypoxie tumorale est un facteur majeur de la radiorésistance, limitant les dommages causés par les radiations ionisantes. Plusieurs médicaments visent à optimiser la perfusion et l'oxygénation des tissus pendant la radiothérapie. Différentes études ont testé l'utilisation de l'oxygène hyperbare afin d'améliorer l'efficacité de la radiothérapie chez des patients atteints de glioblastome. Malheureusement, les résultats de ces études sont plutôt décevants. A ce jour, il manque d'essais cliniques contrôlés randomisés sur de grandes cohortes pour pouvoir statuer de son effet (170–172). Respirer du carbogène pendant les séances de radiothérapie augmente la concentration d'oxygène dans le plasma sanguin, permettant ainsi une meilleure oxygénation des tissus. Ce concept a souvent été associé à la prise orale de nicotinamide permettant de réduire l'obturation des vaisseaux sanguins tumoraux et améliorant ainsi la perfusion tumorale. Cependant, ces approches ont vite été abandonnées car plusieurs essais cliniques ont mis en évidence des effets secondaires importants, sans aucune preuve de bénéfice clinique concernant la survie des patients (173–176). Plus récemment, le bevacizumab a été testé dans cette situation d'hypoxie. Cet anticorps monoclonal anti-VEGF (vascular endothelial growth factor) neutralise l'activité de ce facteur de croissance et inhibe l'angiogenèse tumorale. Il augmente ainsi la perfusion tumorale et réduit l'hypoxie, donc

potentiellement la radiorésistance (177). Plusieurs essais de phase II ont montré des résultats prometteurs (178,179), mais les récents essais de phase III n'ont pas permis d'obtenir un bénéfice en termes de survie (180–182).

Les propriétés cytotoxiques des radiations ionisantes sont diverses. Elles conduisent en majorité à des dommages de l'ADN. Plusieurs radiosensibilisants interfèrent avec les mécanismes de réparation de l'ADN, malheureusement leur utilisation n'est pas concluante dans le cadre des glioblastomes (169). Néanmoins, l'utilisation des inhibiteurs de PARP (poly(ADP-ribose) polymérase) ont suscité beaucoup d'espoir. En inhibant la réparation des cassures simple brin induites par la radiothérapie, les inhibiteurs de PARP provoquent une accumulation de cassures doubles brins, améliorant ainsi potentiellement l'efficacité de la radiothérapie. L'essai clinique randomisé de phase II testant l'ajout du veliparib au traitement standard (STUPP) chez 125 patients atteints d'un glioblastome nouvellement diagnostiqué n'a pu démontrer aucun bénéfice en terme de survie, malgré une bonne tolérance du traitement (183). L'essai clinique OLA-TMZ-RTE-01 de phase I/II testant l'ajout de l'olaparib au traitement standard (STUPP) chez des patients atteints d'un glioblastome nouvellement diagnostiqué est actuellement en cours de recrutement (184,185). Ainsi, à ce jour, aucune preuve de bénéfice n'a été retrouvée.

Aujourd'hui, seul le témozolomide a démontré son efficacité en terme de survie globale faisant de lui le traitement standard des glioblastomes nouvellement diagnostiqués (10,42). Le gemcitabine est un analogue désoxynucléosidique doté de qualités radiosensibilisantes. Il favorise la redistribution des cellules dans le cycle cellulaire, diminue la réparation de l'ADN et favorise l'apoptose (186,187). Sachant qu'il est également capable de passer la barrière hémato-encéphalique, le gemcitabine a été étudié dans plusieurs essais cliniques de phase I et II chez des patients atteints de glioblastome mais n'a pas pu démontrer de bénéfice en terme de survie (188–192). D'autres essais cliniques ont également testé l'utilisation de taxanes (193,194), du 5-Fluorouracil (195,196), ou de platine (197,198), mais malheureusement aucun de ces essais cliniques n'a pu démontrer une efficacité en terme de survie.

L'étude NANO-GBM, s'inscrit également dans ce cadre (voir **ANALYSE BIBLIOGRAPHIQUE** et **TRAVAUX DE RECHERCHES** pour les détails de l'étude). C'est la première étude à tester l'association de nanoparticules intraveineuses avec de la radio-chimiothérapie chez des patients atteints de glioblastome. Les résultats de cette étude pourront déterminer si

l'utilisation des nanoparticules AGuIX® comme radiosensibilisant pourrait permettre de dépasser la radiorésistance des glioblastomes non résécables ou partiellement réséqués. Et cela, tout en conservant le même niveau d'épargne des tissus sains. A ce jour, 8 patients ont été inclus dans la phase I et le comité indépendant de surveillance doit bientôt statuer sur la poursuite de l'étude vers la phase 2 randomisée.

IV. Thèse en recherche clinique

Le caractère récent des thèses en recherche clinique peut engendrer quelques difficultés. Tout d'abord, son format n'est généralement pas conventionnel. Il est nécessaire d'avoir plusieurs projets de recherche afin de pouvoir travailler sur chacune des étapes nécessaires à l'élaboration d'un essai clinique, de l'idée jusqu'à sa valorisation. En effet, il est rare de pouvoir réaliser toutes ces étapes à l'aide d'un seul projet de recherche, car la plupart des essais cliniques, en oncologie, durent plus de 3 ans. De plus, la méconnaissance de ce domaine de recherche peut créer un sentiment de non-reconnaissance auprès de la communauté scientifique très largement issue de la recherche fondamentale. Effectivement, il existe une ignorance de la charge de travail nécessaire à la réalisation d'un essai clinique (contraintes réglementaires, gestion budgétaire et administrative, coordination de projets *ect.*), de l'environnement éthique auquel la recherche impliquant la personne humaine est soumise et des contraintes de la vie-réelle pouvant avoir un impact direct sur les résultats de la recherche (refus de consentement, perdu de vue, décès *ect.*).

Malgré ces quelques difficultés, qui devraient se dissiper dans les années à venir, une thèse en recherche clinique est l'opportunité de maîtriser les compétences suivantes : Veille bibliographique, qualité rédactionnelle, esprit de synthèse et sens de la communication. Ces compétences peuvent également être complétées par l'acquisition des fondements méthodologiques, réglementaires et éthiques de la recherche clinique grâce au DIU-FIEC (formation des investigateurs aux essais cliniques des médicaments). En adéquation avec le marché de l'emploi, la grande majorité des offres concernant des postes de chef de projets en recherche clinique exige une thèse en recherche clinique.

6. ANNEXES

I. Annexe 1 : Travaux collaboratifs en neuro-oncologie

1. L'essai clinique STEP

Durant ces trois années de thèse, j'ai également réalisé des missions supplémentaires d'investigation et de promotion en recherche clinique. Notamment, grâce à l'étude de phase II intitulée STEP évaluant la radiothérapie stéréotaxique hypofractionnée préopératoire des métastases cérébrales (NCT04503772). Cette étude m'a permis d'appréhender le métier d'attaché de recherche clinique (ARC) investigateur, le métier d'ARC moniteur en assurant le contrôle qualité et le métier de chef de projets en réalisant la gestion réglementaire et la coordination de cette étude multicentrique.

Les détails concernant le protocole sont présentés ci-dessous dans l'article du protocole publié dans la revue BMC Cancer.

STUDY PROTOCOL

Open Access

Preoperative stereotactic radiosurgery for brain metastases: the STEP study protocol for a multicentre, prospective, phase-II trial



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Abstract

Background: Surgery is an important therapeutic option for brain metastases. Currently, postoperative stereotactic radiosurgery (SRT) leads to 6-month and 1-year local control estimated at 70 and 62% respectively. However, there is an increased risk of radio-necrosis and leptomeningeal relapse. Preoperative SRT might be an alternative, providing local control remains at least equivalent. It is an innovative concept that could enable the stereotactic benefits to be retained with advantages over post-operative SRT.

Methods: STEP has been designed as a national, multicentre, open-label, prospective, non-randomized, phase-II trial. Seventeen patients are expected to be recruited in the study from 7 sites and they will be followed for 12 months. Patients with more than 4 distinct brain metastases, including one with a surgical indication, and an indication for SRT and surgery, are eligible for enrolment. The primary objective of the trial is to assess 6-month local control after preoperative SRT. The secondary objectives include the assessment of local control, radio-necrosis, overall survival, toxicities, leptomeningeal relapse, distant control, cognitive function, and quality of life. The experimental design is based on a Fleming plan.

Discussion: There is very little data available in the literature on preoperative SRT: there have only been 3 American single or two-centre retrospective studies. STEP is the first prospective trial on preoperative SRT in Europe. Compared to postoperative stereotactic radiotherapy, preoperative stereotactic radiotherapy will enable reduction in the irradiated volume, leptomeningeal relapse and the total duration of the combined treatment (from 4 to 6 weeks to a few days).

Trial registration number: Clinicaltrials.gov: [NCT04503772](https://clinicaltrials.gov/ct2/show/study/NCT04503772), registered on August 07, 2020. Identifier with the French National Agency for the Safety of Medicines and Health Products (ANSM): N°ID RCB 2020-A00403-36, registered in February 2020. Protocol: version 4, 07 December 2020.

Keywords: Brain metastases, Preoperative stereotactic radiotherapy, Local control, Radio-necrosis, Overall survival

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Background

The incidence of brain metastases for patients with cancer varies from 9 to 30% [1–3]. However, it is higher for patients whose primary cancer is lung cancer, breast cancer or melanoma [4, 5]. Brain metastases are associated with poorer prognosis and cause severe side effects, such as the deterioration of cognitive functions, with a negative impact on quality-of-life [6].

Surgery is an important treatment option for brain metastases. However, the two-year local relapse rate is high (46 to 59%) for patients treated only with surgery [7, 8].

Historically, the gold standard postoperative treatment was whole brain radiation therapy (WBRT) leading to a decrease in the number of local relapses, ranging from 10 to 27%, and in distant relapses [7, 8]. However, WBRT has a significant negative impact on cognitive functions and quality-of-life [9, 10]. Postoperative stereotactic radiotherapy (SRT) has thus been developed to limit these side effects without negative impact on overall patient survival, and it has now become the new standard [8, 11].

To date, four prospective studies on postoperative SRT have been published and have shown an estimated local control rate of 70% at 6 months and 62% at one year, with an increased risk of radio-necrosis (nearly 18% at one year) and leptomeningeal relapse (nearly 17% at one year) [9, 12–14]. A phase-III randomized study conducted on 194 patients compared WBRT to postoperative SRT [9]. With a median follow-up of 11 months, the authors showed that the survival time without cognitive deterioration was longer for patients treated with postoperative SRT than for those treated with WBRT (respectively, a median of 3.7 months [CI95 3,45-5,06] vs. 3.0 months [CI95 2,85-3,25]; HR 0,47 [CI95 0,35-0,63]; $p < 0.0001$). Furthermore, six-month cognitive deterioration was significantly less frequent for patients treated with postoperative SRT (-33.6% [CI95 $-45,3-21,8$]; $p < 0.00031$). No differences were found between the groups in terms of overall survival (HR 1,07; [CI95 0,76-1,50]; $p = 0.7$). However, postoperative SRT has several drawbacks: difficulties in delineating the target volume, a high volume of irradiation, a high rate of leptomeningeal recurrence and long overall treatment time (3–6 weeks between surgery and SRT). The alternative to postoperative SRT could be preoperative SRT, providing local control remains at least equivalent. This is an innovative concept that could enable the benefits of SRT to be retained with fewer drawbacks than with postoperative SRT.

To date, three retrospective series of preoperative SRT with a limited number of patients have been published and seem to confirm these advantages [15–17]. The median dose prescribed was 14Gy (1 fraction) on isodose

80% without any margin around the metastasis. Surgery was most often carried out within 24–48 h following SRT.

A retrospective study conducted by Asher et al. on 47 patients treated with preoperative SRT showed 6-month and 12-month survival rates of 77,8 and 60% respectively [15]. The six-month local control rate was 97,8%, falling to 85,6% and 71,8% at 12 and 24 months respectively. With 12 months' hindsight, no radio-necrosis or leptomeningeal relapse was found in the cohort.

A retrospective study compared preoperative and postoperative SRT on a cohort of 180 patients [18]. No difference was found in terms of overall survival ($p = 0,1$), local relapse ($p = 0,24$) or distant relapse ($p = 0,75$). However, postoperative SRT was associated with 2-year leptomeningeal relapse and radio-necrosis rates were higher than those found for preoperative SRT (16,6% vs. 3,2%; $p = 0.01$ and 16,4% vs. 4,9%; $p = 0,01$ respectively). The authors underlined the need for prospective studies assessing preoperative SRT.

To date, no prospective study on preoperative SRT has been published and none is currently being conducted in Europe. Our phase-II trial aims to evaluate preoperative SRT in the treatment of patients with brain metastases with the hypothesis that the local control provided by preoperative SRT will be at least equivalent to that of postoperative SRT, but with a better safety profile.

Methods/design

Study design

STEP has been designed as a national, multicentre, open-label, prospective, non-randomized, phase-II trial to evaluate the efficacy and toxicity of preoperative SRT for patients with brain metastases.

The experimental plan will be run using Fleming's single-stage design without interim evaluation.

This study has been registered on [Clinicaltrials.gov](https://clinicaltrials.gov) (NCT04503772). Seventy patients are expected to be enrolled. The study was started in February 2021 with an 18-month enrolment period and an estimated completion date by July 2023.

Coordination and participating institutions

The Centre Jean PERRIN is the sponsor and is responsible for coordination, trial management, data management and trial monitoring.

This multicentre study is currently being conducted in 7 sites in France. The list of the study sites is available on <https://clinicaltrials.gov/ct2/show/NCT04503772>.

Study objectives and endpoints

Primary objective

The primary objective of the STEP study is to assess 6-month local control after preoperative SRT At the

M6 follow-up visit (i.e. 6 months after preoperative SRT), local control will be evaluated on cerebral MRI. Local relapse is defined as the emergence or progression of nodular contrast within the resection cavity according to the RANO-BM criteria.

Secondary objectives

Assessment of local control at 1 year At the M12 follow-up visit (i.e. 12 months after preoperative SRT), local control will be evaluated on cerebral MRI.

Assessment of the radio-necrosis incidence at 1 year

Radio-necrosis is histologically defined post-operatively, either according to the anatomic-pathological report, or in the absence of salvage surgery, by the appearance of or increase in gadolinium contrast on T1 MRI sequences, associated with an increase in the cerebral blood volume (CBV) ratio (brain blood volume of the tumour / brain blood volume of the non-tumoural white matter) of under 2 on perfusion MRI sequences and/or a standard uptake volume (SUV max) of less than 1.59 on PET scanner at F-DOPA. It will be evaluated at the M12 follow-up visit on cerebral MRI.

Overall survival At each study time-point, patient vital status will be collected. Overall survival is defined as the time between the beginning of preoperative SRT and the date of death from any cause.

Acute and delayed toxicities At each study time-point, except baseline, toxicities will be collected and graded according to NCI CTCAE v5.0. Acute toxicities are those that appear within 3 months after preoperative SRT and delayed toxicities are those that appear more than 3 months after preoperative SRT. In our study, any toxicity ≥ 4 according to NCI CTCAE v5.0 will be considered as a serious adverse event and will be reported immediately by the investigator to the sponsor according to the local regulations.

Assessment of leptomeningeal relapses and assessment of cerebral distant control This will be assessed using MRI and a clinical examination at each study time-point during follow-up (M3, M6, M9, M12).

Assessment of cognitive function and quality of life

At five of the seven study time-points (i.e. baseline, M3, M6, M9 and M12) patients will be asked to complete two questionnaires assessing cognitive functioning and quality of life. These are the Mini Mental State Examination and the EORTC Quality-of-Life questionnaire Core-30.

Determination of local control and predictive factors for complications

This will be conducted on the one hand on the basis of morphological criteria such as the doses received, the volume, etc. and on the other on the basis of medical history, concomitant treatments, etc.

Determination of survival prognosis factors (according to patient and tumour characteristics.)

Biological analysis

A FFPE block of the brain metastasis will be collected for each patient, unless they oppose it on the informed consent form. This block will be stored by the study sponsor and will be used to conduct translational research.

Participant eligibility

The inclusion and non-inclusion criteria are presented in Table 1. Patients will be eligible for the study if they have no more than 4 distinct brain metastases, including one with a surgical indication, an indication for SRT and surgery, and no contraindication for MRI. They will be ineligible if they have metastases from sarcoma or small-cell lung cancer, if they have a documented leptomeningeal disease, or if their survival is estimated under 6 months according to the DS GPA (diagnosis-specific graded prognostic assessment).

Intervention: preoperative stereotactic radiosurgery

Patient positioning and data acquisition

All patients will be irradiated in supine position. Immobilization devices such as stereotactic customized masks will be used to ensure the accuracy and reproducibility of patient positioning during SRT. For all patients, a dosimetric MRI will be required. The dosimetric MRI sequences of interest will be matched with the planning Computed Tomography (Planning-CT). The maximum time lapse between dosimetric MRI and the first fraction of SRT is 7 days.

Volume definition *Delineation of the target volumes*

The gross tumour volume (GTV) is defined as the contrast-enhanced tumour post-gadolinium contrast-enhanced T1-weighted MRI sequences. The GTV will then be extended symmetrically by 2 mm in all dimensions to create the planning target volume (PTV).

Delineation of organs at risk

The following organs at risk will be delineated: healthy brain (corresponding to cranial cavity-GTV), eyeballs, optic chiasm, cochlea, lenses, pituitary gland, hippocampus, optic nerves, brain stem and spinal cord.

Dose prescription and overall treatment duration

Table 1 Selection criteria

Inclusion criteria	Non-inclusion criteria
≤ 4 distinct brain metastases, one with surgical indication	Lymphoma, leukaemia, multiple myeloma, germinal tumours or cerebral primary cancer
Diagnosis of histologically proven breast, digestive, non-small cells lung cancer, kidney or melanoma	Metastases from small-cells lung cancer or sarcoma
≤ 5 cm larger diameter	Mass effect with deflection ≥5 mm from median line or hydrocephaly or compression 4th ventricle, patient neurologically unstable, need for emergency decompressive surgery
Karnofsky performance status ≥70	> 4 brain metastases
No contraindication for MRI	Contraindication to anaesthesia, MRI or gadolinium injection
Possibility for the patient to be treated with both surgery and stereotactic radiotherapy	Proximity of the tumour with organs at risk which do not allow the prescribed dose to be reached in the envelope
≥ 18 years old	Pregnant or breastfeeding woman
Estimated overall survival ≥6 months according to DS GPA	Anti VEGF within 6 weeks before treatment
Written informed consent signed	Documented leptomeningeal injury
Affiliation to the French social security system	History of irradiation of the encephalon in toto
For women of childbearing age including those on LH-RH agonists for ovarian suppression: inclusion negative serum pregnancy test (≤ 7 days prior to the start of preoperative RSH).	History of stereotactic radiotherapy on metastasis to be operated on
	Non-candidate patient for surgery
	Surgical delay > 3 days compared to stereotactic radiotherapy
	Estimated survival < 6 months by DS GPA
	Patient under guardianship or curatorship
	Psychological disorder (cognitive disorders, mental alertness, etc.) or social (deprivation of liberty by judicial or administrative decision) or geographical reasons that may compromise medical monitoring of the trial or compliance with treatment
	Woman of childbearing age without effective contraception
	Patient participating in another intervention study within 4 weeks prior to inclusion

23.1Gy will be prescribed on the 70% isodose line to encompass at least 99% of the PTV, corresponding to a 30Gy dose around the GTV. The total dose will be delivered in 3 fractions, every other day.

The doses delivered to target volumes and organs at risk are presented in Table 2 [19, 20].

Irradiation technique

All treatment will be performed on LINAC, 6 MV X-ray photons, up to 1400 UM/min. The SRT mode is left open to the participating institutions depending on their equipment.

Doses delivered to target volumes (Dmin, 98, 2%, max GTV, Dmin, 98, 2%, max PTV, % PTV coverage by isodose 70%), to organs at risk (Dmax and D2% for at-risk organs and VxGy for healthy brain) and the quality indicators (Paddick's conformity index and gradient index) will be recorded.

Treatment verification and accuracy

An online review of the optimal patient repositioning system will be systematically performed before each fraction according to each centre's policy and

equipment. Any necessary offset correction will be applied.

Treatment interruptions / changes

No changes (major deviations) will be permitted with regard to the target volume selection and delineation,

Table 2 Dosimetry criteria

TARGET VOLUMES	
PTV (GTV + 2 mm)	V23,1Gy ≥ 99% Dmax ≤ 35Gy
GTV	D98% ≥ 29Gy
ORGANS AT RISK	
Brain stem	Dmax < 23,1Gy V18Gy < 0,5 cc
Optic chiasm	Dmax < 17,4Gy
Optic nerves	Dmax < 15Gy
Spinal cord	Dmax < 15Gy
Healthy brain (cranial cavity – GTV)	V23,1Gy < 7 cc V18Gy < 20 cc

the radiation dose prescriptions or the overall treatment duration. Local investigators will carefully follow their patients during treatment and take all adequate measures to avoid any interruption and/or change in the total dose. It is, however, the local investigator's responsibility to interrupt treatment delivery if deemed appropriate in the patient's best interest. Any such interruptions will be recorded in the electronic case report form (eCRF). In case of machine breakdown or a non-working day, all due measures will be taken to avoid prolonging the overall treatment duration.

Time lapse between SRT and surgery

Complete removal of the metastasis will be carried out by the neurosurgeon within ≤ 3 days from the end of the SRT. Postoperative imagery will be performed within 48 h following surgery.

Study procedures and participant timeline

An overview of the study assessments and procedures is presented in Table 3.

Seven consultations are planned for each enrolled patient: inclusion, preoperative SRT, brain metastasis surgery within the 3 following days, and follow-up every three months (M3, M6, M9, M12). The study layout is presented in Fig. 1.

Statistical analysis

Sample size

For sample size calculation, two elements have been taken into account: the primary objective of efficacy at 6

months and the secondary endpoint of 1-year radio-necrosis toxicity.

The efficacy of pre-operative SRT is expected to be at least equivalent to postoperative SRT, estimated at 70%. The experimental plan of the study is Fleming's one-stage design with a sufficient efficacy threshold set at 86% (higher than rates observed in retrospective studies) and an insufficient efficacy threshold set at 70%. In order to control the (one-sided) type I error rate α at 5% and type II error rate β at 10%, the minimum number of patients to be enrolled should be 57.

For the incidence of radio-necrosis at 1 year, to conclude to a decrease compared to that induced in the case of postoperative SRT, a percentage lower than 5% (as observed in the retrospective series) will be accepted and we decided to reject proportions exceeding 15%. To ensure $\alpha < 5\%$ and $\beta < 20\%$, it is sufficient to include 59 patients. The less restrictive hypothesis in terms of power in this second Fleming's design was decided on the basis that it concerns a secondary objective.

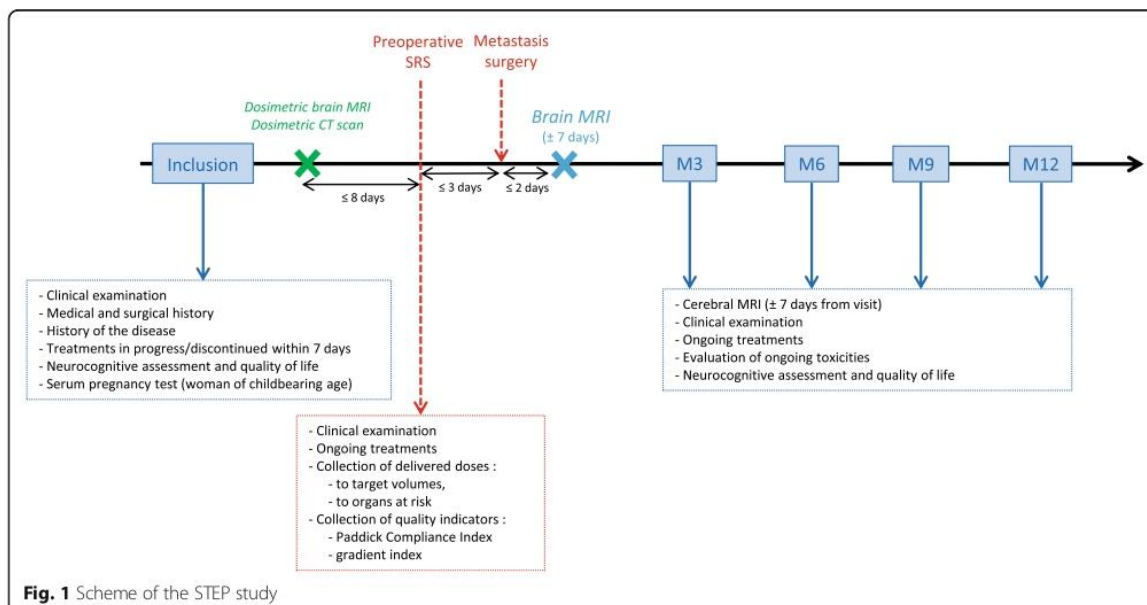
Thus, by including 70 patients, it is possible to compensate for a proportion of more than 15% missing data (for whatever reason) and hence to ensure adequate power of the study in terms of main objective and first secondary objective (the evaluation of the incidence of radio-necrosis).

The treatment will be considered efficacious (and therefore the trial positive) if at least 56/70 of the patients achieve local control. In this case, the estimate of the probability of success will be 80% (90% confidence

Table 3 Data collection schedule

	Inclusion	Preop SRS	Surgery	Follow-up			
				M3	M6	M9	M12
Consent	✓						
Medical and surgical history	✓						
History of the disease	✓						
DS GPA classification	✓						
Serum Pregnancy Test (for woman of childbearing age)	✓						
Clinical examination	✓	✓		✓	✓	✓	✓
Previous and concomitant treatment	✓	✓		✓	✓	✓	✓
Toxicity Evaluation (NCI CTCAE v5.0)		✓	✓	✓	✓	✓	✓
MMSE and EORTC QLQ-C30 questionnaires	✓			✓	✓	✓	✓
Surgery information (date, quality of surgery and post-operative follow-up)			✓				
Dosimetric brain MRI	✓						
Dosimetric CT scan	✓						
Tumor sample (FFPE block)	✓						
Dose delivered to target volumes and organs at risk		✓					
Paddick conformity Index and Gradient Index		✓					
Brain MRI			✓ ^a	✓	✓	✓	✓

^a within 48 h postoperatively (scanner accepted if MRI is not available)



interval of 71.3–86.9%, Jeffreys method), at least. The treatment will be considered to induce an acceptable level of radio-necrosis if no more than 5/70 patients have this toxicity, and, in this case, the estimate of the probability of toxicity will be 7% (90% confidence interval 3.3–13.6%, Jeffreys method), at least.

Data analysis

Primary analysis

The main analysis consists in calculating the local control rate at 6 months, with its 90% confidence interval, and the result will be interpreted according to Fleming's procedure. In the case of non-assessable patients, in order to preserve power and control the alpha risk, the strategy used will be to estimate the local control rate using the Kaplan-Meier method (and to compare it to the rejection rate adjusted on the ratio between the number of assessable patients and the number of patients included).

Secondary analyses

The objectives involving descriptive analyses will call on conventional methods: an evaluation of different toxicities at different times using percentages and confidence intervals. The analysis of survival data (evaluation of local control, distant control, and overall survival) will be carried out using the Kaplan-Meier method (estimation of survival rates at different times with confidence intervals based on cumulative hazard), and Cox proportional hazards model (uni- and multivariate regression, possibly LASSO-penalized) to search for predictive

factors. To investigate predictive factors for complications, a univariate analysis will be performed first, followed by a multivariate logistic regression (penalized if necessary).

Missing data

Missing data will not be replaced (it will be managed by partial elimination). If there is a significant level of missing data, an analysis will be performed to assess its statistical nature and its potential effect on the results. If relevant, an appropriate method of imputation will be envisaged.

Data management and monitoring

The data collected for the study will be entered on an eCRF (Ennov Clinical) by each centre. The people with access to the data will be the investigators, the clinical research associates, the project leaders and the biostatisticians. They are authorized professionals and are subject to professional secrecy. The investigator will ensure the accuracy, completeness, and consistency of the data recorded (pseudonymized patient data) and of the provision of answers to data queries.

Monitoring reviews will be regularly carried out by a clinical research associate mandated by the sponsor. The objectives will be to ensure the correct conduct of the study in each centre, the recording of data generated in writing, its documentation, recording and reporting, in accordance with the legislative and regulatory provisions in force. Monitoring reports will ensure traceability.

Independent data monitoring committee (IDMC)

The IDMC will be composed of three experts: a radiotherapist, a neurosurgeon and a methodologist. The objective of the IDMC will be to review all safety data from the study.

Grade-4 toxicity is expected to remain under 5%. The IDMC will meet on the basis of 2 grade \geq 4 toxicity notifications (NCI CTCAE v5.0).

Trial status

The STEP trial is currently recruiting. Participant recruitment began in February 2021 and recruitment is expected to end in July 2022. The approved protocol is version 4, 07 December 2020.

Discussion

The STEP study puts forward several hypotheses on pre-operative SRT compared to post-operative SRT:

- A reduction in the irradiated volume with better visualization of the contours of the metastases, limiting the risks of radio-necrosis;
- A reduction in post-operative dissemination, limiting leptomeningeal recurrences;
- A reduction in the number of patients (\approx 20%) not treated post-operatively (on account of complications, loss to follow-up, etc.);
- A reduction in the total time of combined treatments: a few days vs. 4 to 6 weeks.

If this study confirms the hypothesis that preoperative SRT reduces the risk of radio-necrosis and local and leptomeningeal recurrence while achieving local control at least equivalent to that of postoperative SRT, the procedure could be a new alternative in the management of patients with brain metastases.

Abbreviations

CBV: Cerebral blood volume; CTV: Clinical target volume; DS-GPA: Diagnosis-specific graded prognostic assessment; eCRF: Electronic case report form; EORTC: European Organisation for Research and Treatment of Cancer; GTV: Gross tumour volume; IDMC: Independent data monitoring committee; MRI: Magnetic resonance imaging; NCI CTCAE: National cancer institute common terminology criteria for adverse events; PET: Positron emission tomography; PTV: Planning target volume; RANO-BM: Response Assessment in Neuro-Oncology Brain Metastases; SRT: Stereotactic radiotherapy; SUV max: Maximum standardized uptake value; WBRT: Whole brain radiotherapy

Acknowledgements

None.

Funding statement

This work was supported by the Inter-Regional Group for Clinical Research and Innovation – Auvergne Rhône Alpes (GIRCI AURA) grant number PHRC-19-070. The funding body reviewed the study protocol. The funding parties are not involved in the design and conduct of the study, nor in the collection, management, analysis, and interpretation of the data. They were not involved in the writing of the present project.

Authors' contributions

Conception and design: JB, GD. Principal investigators of the study: JB, GD, LB. Revision of the study design and protocol: JB, GD, AG, XD, IM. Study coordination: JB, LB, AG, MC. Acquisition of data and patient recruitment: JB, LB, JLL, TK, MC. Radiotherapy quality check (protocol): JB, GD, LB. Statistical analysis: IM. Obtaining funding and supervision: JB, GD, AG. Drafting the manuscript: JB, AG. Revision, adaptation and final approval of the manuscript: All authors. Accountable for all aspects of the work: All authors.

Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

The study protocol and its amendments obtained approval from the French Ethics Committee (Comité de Protection des Personnes Sud-Ouest et Outre-Mer IV) and from the French National Agency for the Safety of Medicines and Health Products (ANSM) (N°ID RCB 2020-A0043–36) in July 2020. The study is conducted in accordance with the Helsinki Declaration, the Good Clinical Practice (GCP) guidelines of the International Conference on Harmonisation (ICH–E6, 17/07/96), General Data Protection Regulation (GDPR) and local regulatory requirements. Written informed consent will be obtained from each patient by the investigator before any study-related assessment starts.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Received: 29 March 2021 Accepted: 15 July 2021

Published online: 28 July 2021

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2. L'étude rétrospective sur les métastases cérébrales du CBNPC

Le travail suivant concerne une étude monocentrique, non-interventionnelle, hors recherche impliquant la personne humaine, approuvée par le comité d'éthique des centres d'investigations clinique Rhône-Alpes-Auvergne. Dans le cadre de ses missions de soin, de recherche et d'enseignement, le Centre Jean PERRIN, participe légitimement à des recherches scientifiques dans un but d'amélioration de la prévention, du dépistage et du soin, dans l'intérêt public dans le domaine de santé. Ainsi, 87 patients atteints d'un cancer bronchique non à petites cellules présentant des métastases cérébrales ont été inclus dans cette étude entre mai 2012 et janvier 2020. Les détails concernant l'étude et les résultats sont présentés ci-dessous, dans l'article publié dans la revue *Clinical and Translational Radiation Oncology*.



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Local control and radionecrosis of brain metastases from non-small-cell lung cancer treated by hypofractionated stereotactic radiotherapy: Evaluation of predictive factors

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ARTICLE INFO

Keywords:

Multifractionated stereotactic radiotherapy
Brain metastases
Non-small-cell lung cancer
Local control
Radionecrosis

ABSTRACT

Background: The objective of our study was to report predictive factors of local control (LC) and radionecrosis (RN) of brain metastases (BM) of non-small cell lung carcinoma (NSCLC) treated by multifractionated stereotactic radiotherapy (MF-SRT) according to French recommendations.

Method: From 2012 to 2020, 87 patients with 101 BM were retrospectively included. The median age was 63 years (37–85). GTV was defined using contrast-enhanced T1w MRI and was isotropically extended by 2 mm to form PTV. Mean maximum BM diameter was 24.5 mm (10–46). Patients were treated with dynamic artherapy from May 2012 to February 2016 and then with VMAT. The total prescribed dose was 23.1 Gy prescribed to the encompassing 70% isodose, in 3 fractions.

Results: LC rates at 6 months, 1 year and 2 years was 95.7%, 90.7% and 87.9% respectively. In multivariate analysis, high GTV Dmin (HR = 0.822, p = 0.012) was in favor of better LC whereas a large maximum diameter was predictive of poor LC (HR = 1.124, p = 0.02). GTV Dmin of 27.4 Gy was identified as a discriminant threshold of LC. In case of GTV Dmin ≥ 27.4 Gy, LC at 1 year was 95.3% versus 75.1% with GTV Dmin < 27.4 Gy. Cumulative incidence of RN at 6 months, 1 year and 2 years was 6.3%, 15.4% and 18.1%, respectively. In multivariate analysis, only dyslipidemia was predictive of RN (HR = 2.69, p = 0.03). No dosimetric predictive factor of RN was found in our study.

Conclusion: MF-SRT (3x7.7 Gy on 70% isodose line, with PTV = GTV + 2 mm; according to French recommendations) of BM from NSCLC gives high LC rates with acceptable RN rate. A GTV Dmin of at least 27.4 Gy could be proposed to optimize dosimetric objectives. No dosimetric predictive factors of RN were found in this study. However, dyslipidemia was identified as a potential predictive factor of RN.

Introduction

Brain metastases are the most common intracranial tumors in adults, accounting for well over half of all brain tumors. They occur in 20–40% of cancer patients during the course of their disease [1,2]. Lung cancer is the leading cause of brain metastases, accounting for 30–50% of cases,

and it is associated with a poor prognosis [3–5]. Brain metastases are generally more frequent in adenocarcinomas than in squamous cell carcinomas and are observed in 26.8% of cases [6]. This rate rises to 38%, especially in NSCLC with ALK rearrangement, and is also high in cases of EGFR mutation [7,8].

Whole brain radiotherapy (WBRT), for a long time the reference for

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<https://doi.org/10.1016/j.ctro.2022.05.008>

Received 30 November 2021; Received in revised form 30 May 2022; Accepted 30 May 2022

Available online 1 June 2022

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local control and symptomatic treatment of brain metastases, has been questioned in recent years because of the lack of survival increase and the risk of cognitive impairment [9,10]. Over time, stereotactic radiotherapy, which includes stereotactic radiosurgery (SRS) and multi-fraction stereotactic radiotherapy (MF-SRT), has become a standard treatment for suitable patients with brain metastases by delivering a lower dose to the healthy brain, with a high rate of local control (LC) and fewer side effects [11].

However, stereotactic radiotherapy can be responsible for inflammatory and necrotic processes resulting in edema with a mass effect that is very difficult to distinguish from a recurrence or a progression of the disease. This can be asymptomatic or affect quality of life. The reported incidence of radionecrosis (RN) varies from 3% to 24% of patients and usually occurs from six weeks up to 15 months after SRT, but can also occur years later [12–15]. Despite the lack of randomized trials, treatment for large brain metastases with MF-SRT regimens (2–5 fractions) may offer a relative reduction of RN compared with SRS, while maintaining high LC [16]. Over the past years, several studies, mainly retrospective, have reported the outcomes of MF-SRT, often including heterogeneous fractionations, and heterogeneous histology [16–20]. French recommendations on the management of brain metastases with stereotactic radiotherapy recommend for MF-SRT a total dose of 23.1 Gy in 3 fractions, on the 70% isodose [5,21]. However, the outcomes of this fractionation have never been clearly published. Thus, the purpose of this study was to assess LC and brain RN rate and identify predictive factors in a retrospective series of 101 unresected brain metastases from NSCLC treated with Linac-based MF-SRT, according to French recommendations [5,21].

Material and methods

Population and metastasis characteristics

Between May 2012 and January 2020, 101 unresected brain metastases from a histologically confirmed NSCLC of 87 patients older than 18 years underwent MF-SRT in our institution and were included in this retrospective study. Previous whole brain radiotherapy (WBRT) was authorized. Patients presenting a tumor other than NSCLC, a tumor size < 1 cm (usually treated with SRS), brainstem metastases, or prior surgery were excluded.

This study was approved by CECIC Rhône-Alpes-Auvergne on 25 September 2020. All characteristics of the 87 patients and the 101 brain metastases from NSCLC are reported in Table 1. Treatment characteristics are reported in Table 2.

The median age was 63.1 (range from 36.5 to 84.8). The population of the study was a majority male (67.8%), most remained in good general state with a performance status ≤ 1 (87.3%), and a majority were treated for a single brain metastasis (62.1%) or two (in 19.5% of cases) with no prior WBRT (75%). Every patient presented a NSCLC, with the most frequent histology adenocarcinoma (82.2%), followed by squamous cell carcinoma (15.8%). Nineteen patients (21.8%) presented a mutation (all patients were tested for), of which the most frequent was KRAS (68.4% of them); other mutations were EGFR (2/19), ALK (2/19), cMET (1/19), and BRCA (1/19).

In 59.8% of patients, systemic therapy was provided at the time of brain MF-SRT. Systemic treatments were mono-chemotherapy in 34.6% of patients (18/52) and chemotherapy in combination with platinum doublet in 29.6% (14/52). One patient received a combination of Paclitaxel and Bevacizumab. In 23% of cases (12/52), systemic treatments were targeted therapies: Erlotinib, a first generation TKI (7/52); Osimertinib, a third-generation TKI (1/52); Alectinib, ALK-inhibitor (1/52), Bevacizumab, anti-VEGF antibody (2/52), which was taken in association with Paclitaxel, and Olaparib, PARP inhibitor (1/52). Seventeen percent of patients (9/52) received immunotherapy such as Pembrolizumab (3/52), Nivolumab (4/52), and Atezolizumab (2/52).

The mean maximum-diameter of brain metastasis was 24.5 mm

Table 1
Patients and brain metastasis characteristics.

Patients characteristics		
Total		87
Gender	Female	28 (32.2%)
	Male	59 (67.8%)
Age – median (min–max)		63.1 (36.5–84.8)
Medical history	HTA	33 (37.9%)
	Diabetes	12 (13.8%)
	Dyslipidemia	23 (26.4%)
Systemic treatment	yes	52 (60%)
	no	35 (40%)
Ps	0	31 (30.7%)
	1	54 (53.5%)
	2	15 (14.9%)
	≥ 3	1 (1%)
Prognostic score - mean (min–max)	SIR	6 (2–9)
	RPA	1.9 (1–3)
	GPA	2.5 (0.5–4)
	DS.GPA	2.4 (0–4)
	lung-molGPA	2 (0.5–3.5)
Number of metastases treated per patient	1	56 (55.4%)
	2	23 (22.8%)
	≥ 3	22 (21.8%)
Brain metastasis characteristics		
Total		101
Tumor volume	Maximum diameter	24.5 (10–46)
	GTV (cc)	5.75 (0.2–26.4)
	PTV (cc)	10.2 (0.7–39)
Prior treatment	WBRT	26 (26%)
	SRT	1 (1%)
NSCLC histology	Adenocarcinoma	83 (82%)
	Epidermoid carcinoma	16 (16%)
	other	2 (2%)
Mutation	yes	19 (19%)
	no	82 (81%)
Location	cerebellar	21 (21%)
	frontal	32 (32%)
	occipital	15 (15%)
	parietal	17 (17%)
	temporal	14 (14%)
	other	2 (2%)

Abbreviations. PS = performance status; SIR = score index for radiosurgery; RPA = recursive partitioning analysis; DS-GPA; DS.GPA = diagnosis-specific graded prognostic assessment; lung-molGPA = lung-molecular graded prognostic assessment; GTV = gross tumor volume; PTV = planning target volume; WBRT = whole-brain radiotherapy; SRT = stereotactic radiotherapy.

(min–max: 10–46). Twenty-two percent were smaller than 20 mm, and 13% were larger than 30 mm.

Treatment specifications

Planning-CT images were acquired with a 1.25 mm slice thickness and fused with the dosimetric magnetic resonance imaging (MRI) sequences of interest using Iplan®, version 4.1 (Brainlab). The MRI included 3 sequences: a FLAIR 3D MRI sequence, a T1 3D MPRAGE MRI sequence with contrast agent, and a T1 GE. The maximum delay between dosimetric MRI and first fraction was 7 days.

During the planning-CT and radiotherapy session, patients were

Table 2
Multifractionated stereotactic radiotherapy (MF-SRT; 3x7.7 Gy on the 70% isodose line) characteristics.

Technique		
OTT (days) mean (mean – max)	Dynarc	59 (58%)
	Vmat	42 (42%)
GTV doses (Gy)		5.8 (4–10)
	Dmin	27.7 (7.3–31.6)
	D98%	29.2 (9–32)
	Dmean	31.5 (26.6–33.5)
	Dmax	33.2 (27.7–35.9)
PTV doses (Gy)	Dmin	21.8 (6.1–27.3)
	D98%	24.9 (7.4–29.5)
	Dmean	29.9 (25–32.1)
	Dmax	33.1 (27.7–35.9)
	V70%	99.3 (73.2–100)
	(Brain – GTV) doses (Gy)	V23.1
V21		8 (1.6–31.1)
V18		10.8 (2.1–39.7)
V14		16.6 (3.1–61)
V10		28.3 (5.2–99.4)
V5		76.9 (15.6–249.4)

immobilized with a noninvasive thermoplastic mask (Brainlab, Munich, Germany) and repositioned daily with an integrated ExacTrac X-ray 6D system (Brainlab, Munich, Germany) which has the ability for pre-treatment positioning.

The target volume was identified on the fused planning-CT and MRI, and the gross tumor volume (GTV) was generated on the post-gadolinium contrast-enhanced T1-weighted MRI sequence. Clinical Target Volume (CTV) definition was identical to the GTV and planning target volume (PTV) was defined as a 2 mm three-dimensional expansion around the GTV. GTV and PTV mean volume were 5.75 cc (min – max: 0.16 – 26.4) and 10.18 cc (min – max: 0.73 – 39.33) respectively. Organs at risk (OARs) delineated were the cranial cavity, healthy brain (entire cranial cavity - GTV), brainstem, optic nerves, chiasma, eyeballs, lenses, and cochlea. For all patients, irradiation was performed with a 6 MV photon beam from a linear accelerator (NovalisTx®), equipped with a high definition MultiLeaf Collimator (HD MLC 120) (Varian Medical Systems, Palo Alto, CA, USA and Brainlab, Munich, Germany).

Two radiotherapy methods were used over two periods. From May 2012 to February 2016, dynamic arc therapy (with 4–5 non coplanar arcs) was used. The dose prescribed was 33 Gy to the isocentre and 23.1 Gy (70%) at the envelope covering the PTV, delivered in 3 fractions. From March 2016 to January 2020, VMAT (volumetric modulated arc therapy), using one full coplanar arc and three partial non-coplanar arcs spaced by 45°, was used. The prescribed dose was 33 Gy for the GTV and 23.1 Gy (70% isodose) for the PTV delivered in 3 fractions, corresponding in practice to a prescribed dose to the 70% isodose line to achieve 99% target coverage of the PTV. Delineation and dose prescription corresponded to French national recommendations [5,21]. MF-SRT was delivered every other day. In case of proximity with OARs, the prescribed dose could be adjusted to meet dose constraints [22]. Concerning Treatment Planning Systems (TPS) for DynArc, final calculations were performed using Iplan® TPS, version 4.1 (Brainlab), using a pencil-beam algorithm with a spatial resolution of 2.5 mm. For VMAT, final calculations were performed using the AAA algorithm on Eclipse® TPS version 13.5 (Varian Medical Systems). The arc optimization algorithm, the Progressive Resolution Optimizer used in Rapidarc®, optimized leaf position, dose rate, and gantry speed.

Before the start of the treatment, patients received oral corticosteroid at an initial dose of 1 mg/kg decreasing on 4 weeks, to prevent brain edema.

Follow-up

Follow-up included MRI (including T1 Gadolinium sequences with dynamic susceptibility-weighted contrast-enhanced [DSC] perfusion), coupled with a clinical examination every 3 months. Treatment related-toxicities such as RN, edema and hemorrhages were recorded using the National Cancer Institute's Common Terminology Criteria for Adverse Events (NCI-CTCAE) Version 4.0.

Local control failure was defined by a recurrence in the previously irradiated volume using RANO-BM criteria [23], characterized as an increase of at least 20% in sum longest distance relative to nadir, associated with either a relative cerebral blood volume (CBV) > 2.0 at dynamic susceptibility-weighted contrast-enhanced perfusion images (calculated for each lesion by the ratio of the tumor CBV on the mean CBV value of normal white matter) or a maximum lesion to maximum background uptake ratio (SUVLmax/Bkgmax) > 1.59 at F-DOPA PET-CT (for 2 patients). RN was defined by stable or shrinking lesions over a 6-month period associated with a rCBV < 2.0 in perfusion images, or a SUVLmax/Bkgmax < 1.59, or on the basis of histologic findings after brain metastasectomy [18,24].

Last follow-up was defined as the date of death or the date of the last consultation with a cerebral IRM, during the period of the study that ended November 2020. Median follow-up was 12 months (range = 1 – 90). Overall survival (OS) was defined as the time between the last session of MF-SRT and the patient's death.

Statistical analysis

RN and LC rates, progression-free survival (PFS), and overall survival (OS) were estimated from the end of MF-SRT using the Kaplan-Meier calculation method. Then the log-rank test was performed to compare survival curves. Predictive factors for LC, OS, and time to RN are investigated using univariate and multivariate Cox regression models. For LC and RN, a competing risk analysis with death as a concurrent risk was performed, as a sensitivity analysis.

Concerning LC, the following factors were assessed in the univariate analysis: histological type, presence or absence of a mutation, presence or absence of a systemic treatment at the time of brain SRS, prior WBRT, GTV volume, PTV volume, largest diameter, location, laterality (right or left), depth (distance from the cranial vault to the surface of the lesion), overall treatment time, doses delivered to GTV and PTV (Dmin, D98%, Dmean, D2%, Dmax), V70% PTV (V70% is the volume of the structure receiving a dose ≥ 70% prescribed dose, i.e. prescription isodose), and radiotherapy technique (non-coplanar dynamic arc therapies or VMAT).

For OS, the same factors were analyzed in the univariate analysis, to which were added age, gender, comorbidities (diabetes, dyslipidemia, high blood pressure), performance status, number of brain metastases, and prognostic scoring systems for BM patients including the Score Index For Radiosurgery (SIR), the Recursive Partitioning Analysis (RPA), the Graded Prognostic Assessment (GPA), the disease specific Graded Prognostic Assessment (DS-GPA) and the lung-molGPA which incorporates recently reported gene alteration data, predicting the outcomes of NSCLC.

Concerning RN, the following factors were included: age, gender, comorbidities (diabetes, dyslipidemia, high blood pressure), presence or absence of a systemic treatment at the time of brain SRS, prior WBRT, GTV volume, PTV volume, largest diameter, location, depth, overall treatment time, radiotherapy technique (non-coplanar dynamic arcs therapy or VMAT) and doses delivered to healthy brain parenchyma (brain - GTV): V23.1Gy, V21Gy, V18Gy, V14Gy, V10Gy, V5Gy (definition: V_xGy (cc) is the volume of the structure receiving a dose ≥ x Gy).

All variables with a p-value < 0.1 in the univariate analysis were used to build a multivariate regression model, using the LASSO algorithm in order to perform variable selection.

For identifying the optimal GTV Dmin threshold dose, a time-dependent ROC (Receiver Operating Characteristics) curve was

performed to identify the optimal threshold value. Then, a Kaplan-Meier curve of the two populations was estimated and compared using a log-rank test. As a complementary analysis obtaining a p-value adjusted for multiple testing, we performed maximally selected rank statistics threshold analysis using the *r*-package *maxstat* with p-value approximation via conditional Monte-Carlo. Intergroup differences were compared using Fisher's exact test for categorical variables and the Wilcoxon-Mann-Whitney for continuous variables. A p-value < 0.05 was considered indicative of a statistically significant difference. Statistical analyses were performed using the R software, version 4.1.0 (R-Project, GNU GPL, <https://cran.r-project.org/>).

Results

Local control

Considering death as competing risk factor, the LC rates were 95.7%, 90.7% and 87.9% at 6, 12, and 24 months respectively (Fig. 1A). No difference in LC was found between DynArc and VMAT techniques. In univariate analysis, predictive factors of better LC were other locations than the cerebellar (HR = 0.282, CI 95% = 0.086 – 0.926, *p* = 0.047), higher GTV D_{min} (HR = 0.774, CI 95% = 0.676 – 0.887, *p* = 0.002), higher GTV $D_{98\%}$ (HR = 0.781, CI 95% = 0.684 – 0.892, *p* = 0.003), higher GTV D_{mean} (HR = 0.725, CI 95% = 0.540 – 0.973, *p* = 0.049), higher $V_{70\%}$ PTV (HR = 0.835, CI 95% = 0.750 – 0.929, *p* = 0.01), no prior WBRT (HR = 3.625, CI 95% = 1.098 – 11.970, *p* = 0.037), lower PTV volume (HR = 1.073, CI 95% = 1.005 – 1.145, *p* = 0.045), and lower maximum diameter (HR = 1.100, CI 95% = 1.031 – 1.174, *p* = 0.006).

In multivariate analysis, higher GTV D_{min} (HR = 0.822, CI 95% = 0.706 – 0.957, *p* = 0.012) and lower maximum diameter (HR = 1.124, CI 95% = 1.044 – 1.210, *p* = 0.002) remained significant factors of predictive LC. In the competing risk model considering death as competing event, only higher GTV D_{min} (HR = 0.826, CI 95% = 0.757 – 0.902, *p* < 0.001), and lower maximum diameter (HR = 1.152, CI 95% = 1.055 – 1.259, *p* = 0.002) remained significant.

Histology type, the presence of a mutation, and systemic treatment were not predictive factors of LC, whether in univariate analysis or multivariate analysis.

Concerning GTV D_{min} , we identified a discriminant threshold-value of 27.4 Gy (AUC = 0.69, CI 95% = 0.49–0.87), using a time dependent ROC curve. The 1-year LC was 97.1% versus 73.1% for GTV $D_{min} \geq 27.4$ Gy and GTV $D_{min} < 27.4$ Gy respectively (*p* = 0.002 by logrank test). A complementary analysis, using maximally selected rank statistics with p-value adjustment for multiple testing, provided the same threshold of 27.4 Gy with an adjusted p-value of 0.02 (Fig. 2A and 2B).

Radionecrosis and other toxicities

Considering death as a competing risk factor, the 6-month, 1-year and 2-year actual risks of RN were 6.3%, 15.4%, and 18.1% respectively. Symptomatic RN was described in 5.9% of cases. Predictive factors of RN found in univariate analysis were high blood pressure (HR = 3.126, CI 95% = 1.210 – 8.077, *p* = 0.017) and dyslipidemia (HR = 3.614, CI 95% = 1.429 – 9.137, *p* = 0.009). Dyslipidemia was the only significant predictive factor of RN in multivariate analysis (HR = 3.436, CI 95% = 1.140 – 10.355, *p* = 0.028) and in the competing risk model (HR = 2.69, CI 95% = 1.076 – 6.72, *p* = 0.03).

We reported the following mean volumes of healthy brain (brain – GTV): $V_{23.1Gy}$, V_{21Gy} , V_{18Gy} , V_{14Gy} , V_{10Gy} and V_{5Gy} , which were 6.43 cc, 8.02 cc, 10.76 cc, 16.64 cc, 28.38 cc, and 76.86 cc respectively. There were no dosimetric predictive factors of RN, whether in univariate analysis or multivariate analysis.

Other symptoms observed during and after MF-SRT were hemorrhage, neurological deficit, intra-cranial hypertension and epilepsy, in 7%, 8%, 4% and 2% of cases, respectively.

Survival

PFS at 6 months, 1 year, and 2 years was 67.9%, 40.9%, and 17.4% respectively. Median PFS was 10 months (Fig. 1B).

OS at 6 months, 1 year, and 2 years was 80.8%, 56.7%, and 34.1% respectively (Fig. 1C). Median OS was 14 months. In univariate analysis, significant prognostic factors of lower OS were higher age (HR = 1.035, CI 95% = 1.004 – 1.066, *p* = 0.024) and poorer PS status (1.423, CI 95% = 0.805 – 2.515, *p* = 0.215), whereas higher SIR score (HR = 0.661, CI 95% = 0.537 – 0.813, *p* < 0.001), higher GPA score (HR = 0.493, CI 95% = 0.333 – 0.731, *p* = 0.001), higher DS-GPA score (HR = 0.669, CI 95% = 0.507 – 0.883, *p* = 0.008), higher lung-molGPA (HR = 0.483, CI 95% = 0.315 – 0.743, *p* = 0.001) and presence of mutation (HR = 0.500, CI 95% = 0.244 – 1.022, *p* = 0.041) were predictive factors of higher OS. In multivariate analysis, a higher SIR, GPA, and lung-molGPA score remained significant prognostic factors of OS.

Discussion

In our retrospective series, we reported the outcomes of 101 unresected brain metastasis in 87 patients from NSCLC treated with MF-SRT. To our knowledge, this is the first published study to assess the outcomes of MF-SRT, and study predictive factors of LC and RN, in a homogeneous population of patients with intact brain metastases of NSCLC, treated by MF-SRT in 3 fractions according to French recommendations (3x7.7 Gy on the 70% isodose line; PTV = GTV + 2 mm) [21]. In particular, we found that a $D_{min} < 27.4$ Gy to the GTV could be a predictive factor of worse LC; and that dyslipidemia could be a predictive factor of RN.

NSCLC is the main etiology of brain metastasis but in most studies assessing brain RN or LC, the underlying cancers were heterogeneous with various fractionations [16–20]. Only one recent study conducted by Minniti et al. compared the effectiveness of MF-SRT for large brain metastases from NSCLC; however this study included both resected and intact brain metastases [25]. In the group treated for intact brain metastases, 6-month LC was 96% and 12-month LC was 92%, which is quite similar to our results with 6-month and 12-month LC of 95% and 87% respectively [25]. In the study by Minniti et al., patients were treated with 3x9Gy on the 80% isodose line, with PTV = GTV + 1 mm; which is quite similar to our fractionation according to French recommendations with 3x7.7 Gy on the 70% isodose line, with PTV = GTV + 2 mm. Garsa et al reported, in 2014, predictive factors of individual tumor LC after SRS for NSCLC brain metastases in which the estimated local control at 12 months was 74%. However, in this trial all patients were treated by Gamma Knife single-fraction SRS and the median prescription dose was 20 Gy (range 14–24 Gy). Cerebellar tumor location, larger tumor volume, and lower conformity index were significant independent predictors of local failure. The adjusted 1-year local control rate for cerebellar lesions was 60% compared with 77% for supratentorial lesions (controlling for tumor volume and conformality index), which support our results since we found in our study that cerebellar location was a predictive factor of lower rates of local control but only in univariate analysis [26]. However, Vogelbaum et al, in an analysis of 202 patients with multiple types of metastatic malignancies treated with SRS, did not find any difference between infratentorial and supratentorial metastasis [27]. We also reported that maximum diameter (HR = 1.124, CI 95% = 1.044 – 1.210, *p* = 0.002) was associated with a worse prognosis for LC.

Furthermore, one of the interests of our study was to report dosimetric predictive factors of LC and characterize a minimum dose delivered to the GTV of 27.4 Gy. In our study, the 1-year LC was significantly improved when GTV $D_{min} \geq 27.4$ Gy (97.1% versus 73.1%, *p* = 0.013). Even if a dose–effect relation is well known in single fraction SRS [28], it is much less studied and reported in MF-SRT. Furthermore, D_{min} to the GTV is rarely specified and studied in published MF-SRT series, even if it might be a dosimetric factor of interest since PTV margins vary from 0 to 2 mm in MF-SRT leading to a varying dose in the

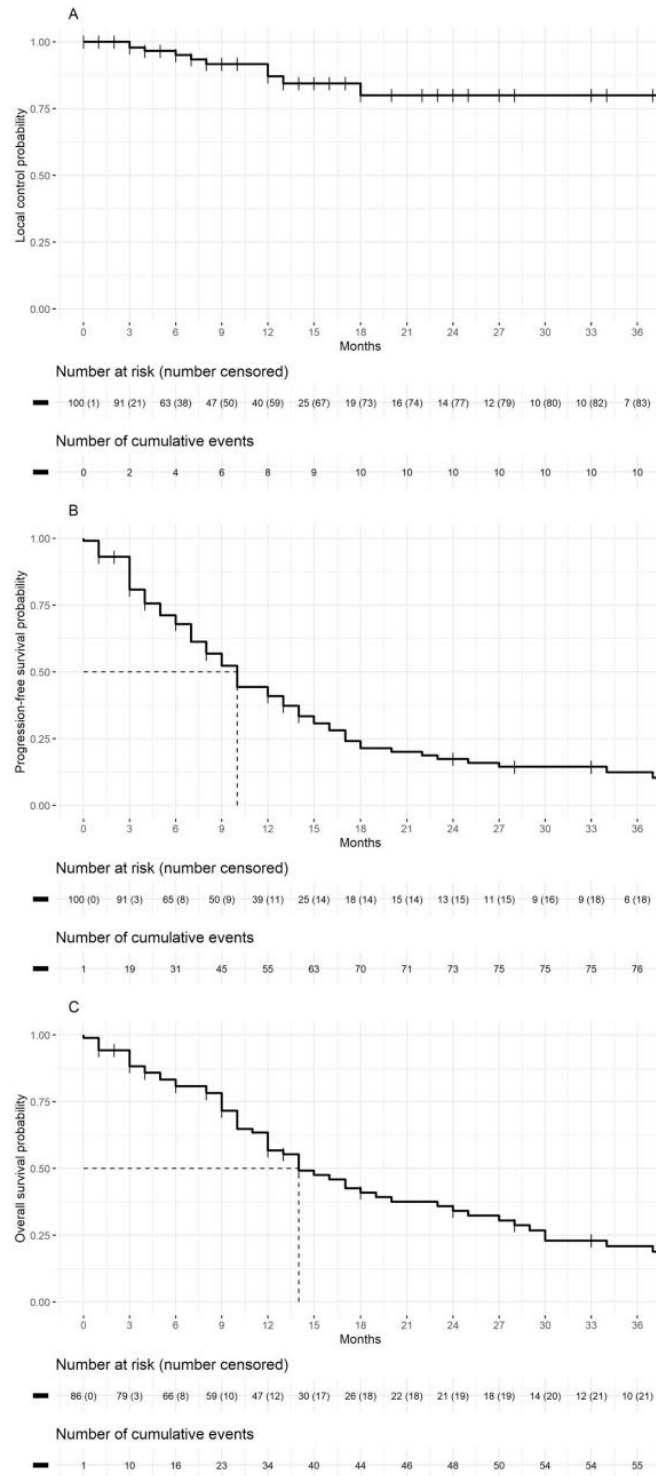


Fig. 1. Probability of local control (1A), progression-free survival (1B) and overall survival (1C) for the 87 patients receiving MF-SRT for 101 brain metastases from NSLC. Abbreviations: MF-SRT, multifractionated stereotactic radiotherapy; NSLC, non-small-cell lung cancer.

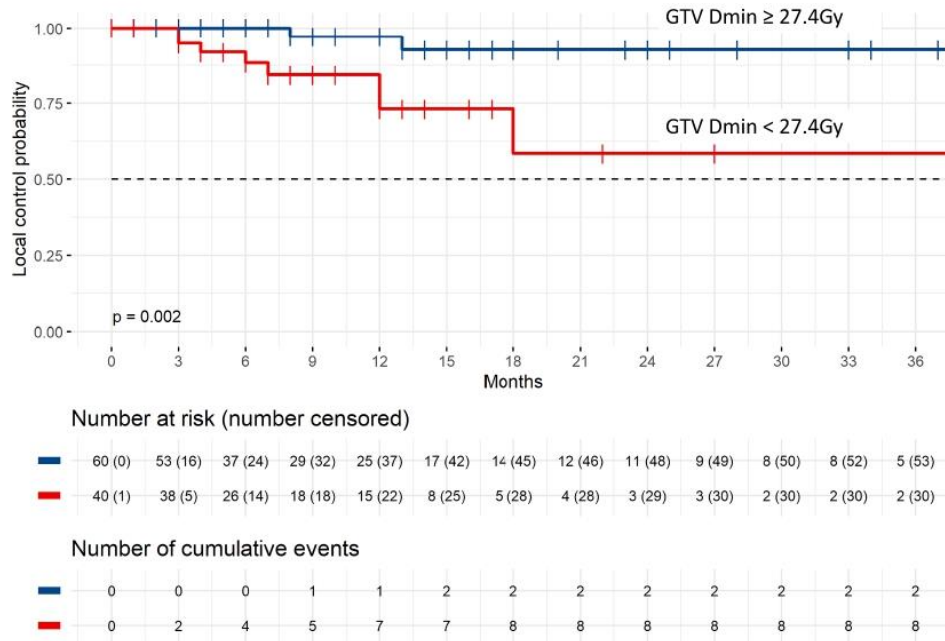


Fig. 2. Comparison of local control curves of all 101 treated brain metastases between those receiving a GTV Dmin < 27.4 Gy in three fractions vs ≥27.4 Gy. Abbreviations: GTV = gross tumor volume; Dmin = minimum dose.

GTV, whereas GTV is always delineated in the same manner. To overcome this limitation, in 2020 Dupic et al published a study which focused on the minimum or near-minimum (D98%) dose delivered to the GTV and not the marginal dose prescribed to the PTV influenced by the use of margins. Therefore, this study demonstrated that a GTV D98% higher than 29 Gy in 3 fractions was a significant predictive factor of local control in MF-SRT for brain metastases from various histology [19].

Dosimetric data collected for each brain metastasis from NSCLC is not sufficient on its own to explain local control. Another important factor for LC is the choice of systemic treatment. Indeed, the chosen agent must be effective against the primary cancer, but also be able to cross the blood–brain barrier. It is systematically discussed before local treatment. Even if most systemic therapies do not easily pass the blood–brain barrier, the anarchic neo-angiogenesis of brain metastases, responsible for an alteration of the blood–brain barrier, makes the diffusion of some cytotoxic agents possible. For example, platinum-based chemotherapies induce equivalent cerebral and extra-cerebral response rates, usually between 30 and 50% [29]. Tumors with high PDL1 expression (≥50%) are accessible to Pembrolizumab in first-line treatment [30]. Other immunotherapies such as Nivolumab or Atezolizumab may be used with satisfactory safety of use based on brain response rates. Mutations are often present in adenocarcinoma and enable the development of innovative targeted therapies, specifically TKI (Tyrosine Kinase Inhibitor) for EGFR mutations or ALK gene rearrangement. Osimertinib is the preferred drug in the case of EGFR mutations due to a better penetration in the central nervous system compared to 2nd generation TKI, and a better control of brain metastases [31]. A clear decrease in brain progression with Alectinib versus Crizotinib, 12% versus 45% (cause-specific hazard ratio 0.16, p < 0.001) associated with an intrabrain response rate of 88% for Alectinib versus 50% for Crizotinib, was observed in ALEX study [7]. In our study, systemic treatment concerned 60% of patients: chemotherapy (63%), Avastin (5.7%), targeted therapies (19.2%): Erlotinib (13.5%), Alectinib (1.9%), Olaparib (1.9%), and immunotherapy (15.3%). Diverse treatments were administered, and do not lead to any conclusions about their

association with LC.

RN is the most common adverse event of MF-SRT, but its pathophysiology is poorly understood. Several risk factors have been described, including the total dose of radiotherapy delivered, tumor volume, fractionation, healthy brain parenchyma irradiation and previous irradiation. Other factors mentioned include overall treatment time, infratentorial location, and combination with chemotherapy [17,18,20,32–36]. In our study, 1-year actual RN risk rate was 15.4%. Among RN cases, symptoms were observed in 5.9%. In the series by Minniti et al. [18], retrospectively comparing SRS vs MF-SRT (using more or less the same MF-SRT as in our study) for multiple histology brain metastases, the cumulative 1-year risks of RN were 18% vs 9% (p = 0.01), in favor of MF-SRT. The cumulative 2-year risk of RN for MF-SRT was about 18% at two years, as in our study. For lesions > 3 cm, the cumulative 1-year risk was 14% for MF-SRT vs 33% for SRS (p = 0.01). Symptomatic RN was 4%. More recently, Minniti et al. [25], using the same MF-SRT schedule, published a series of 241 postoperative or intact NSCLC brain metastases. The 1-year cumulative incidence rates of RN were 15% and 7% after postoperative SRT and SRT alone, respectively. 5.7% of the patients treated with MF-SRT. In a meta-analysis conducted in 2018 by Lehrer et al. lower rates of RN were found with 18.2% (95% CI, 9.3%–32.5%) for SRS group and 7.1% (95% CI, 4.4%–11.3%) for MF-SRT group [16]. The RN rates reported in our study might appear slightly higher than in the above cited studies, however symptomatic RN are in the exact same range. Furthermore, the exact diagnosis of RN is difficult as discussed in the RANO-BM working group report [23] and in the recent review from Milano et al. (*American Association of Physicists in Medicine Working Group on Stereotactic Body Radiotherapy*) [37]. In this review, several studies (n = 15) have reported RN risks after MF-SRT (2–5 fractions). Patients selected for MF-SRT often had bulkier disease and/or tumors in critical locations. Toxicity after MF-SRT appears to be relatively lower vs. single-fraction SRS). More recently, a large series of 334 multiple histology intact brain metastases treated in 5 daily fractions of MF-SRT (median dose of 30 Gy in 5 fractions) was published [38]. Fifty-two metastases (15.6%) had an adverse radiation effect, of which 32 (9.5%) were symptomatic RN. In

our study, other symptoms observed during and after MF-SRT were hemorrhage, neurological deficit, intra-cranial hypertension and epilepsy, in 7%, 8%, 4% and 2% of cases, respectively. However, these results are difficult to interpret since these symptoms are not necessarily toxicities related to the irradiated lesion, in patients who may have other intracerebral lesions. In MF-SRT (3 fractions), the most significant prognostic factor reported to date for RN is the brain volume receiving high doses: V23.1 Gy \geq 5 cc [39], V21Gy \geq 20.9 cc [40] and V18Gy $>$ 30.2 cc [18]. In our study, no dosimetric factors were independent predictive factors of RN in univariate or multivariate analysis, probably because of the limitation of healthy brain parenchyma irradiation (mean V23.1 Gy, V21Gy and V18Gy of 6.4 cc, 8.0 cc and 10.8 cc respectively, which are globally lower than the previous published thresholds). Dyslipidemia was the only significant predictive factor of RN in multivariate analysis (HR = 3.436, CI 95% = 1.140 – 10.355, $p = 0.028$) in our study. To date, no data in the literature have been reported showing an association between dyslipidemia and RN. Although the pathophysiological mechanisms of RN are not yet completely understood, vascular damage is observed a few months to years after the end of SRT, followed by glial and neuronal lesions. Once present, these lesions are usually irreversible and progressive. The vascular phase is characterized by vasogenic edema, followed by hyalinization responsible for a thickening of the vascular wall with parietal thrombi until a fibrinoid necrosis [41,42]. This imbalance could contribute to the secondary cytotoxic edema that leads to tissue necrosis. Oligodendrocytes are very radio-sensitive and their destruction is responsible for demyelination. The lesions then mainly affect the white matter, while the cortex is partly spared. Atherosclerosis is an inflammatory response to injury to the arterial wall and lead to necrosis. Actually, the accumulation of LDL cholesterol in the intima will oxidize and be captured by macrophages involving a chronic inflammatory reaction. This endothelial remodeling leads to an increasing endothelial parietal thickness that may be responsible for ischemic necrosis [37]. Disorders of microvascularization caused by dyslipidemia may partially explain our result; nevertheless, high blood pressure and diabetes are also responsible for microangiopathy but did not appear as predictive factors of RN. However, this result remains difficult to interpret, indeed we do not have sufficient data regarding the lipid metabolism disorder which may concern triglycerides or cholesterol. We also have no data on statin intake and the number of patients included seems low to conclude. More investigation is necessary for stronger conclusions about the observed correlation between dyslipidemia and increased risk for radiation necrosis.

Conclusion

In conclusion, MF-SRT delivered according to French recommendations (i.e 3x7.7 Gy on the 70% isodose line, with PTV = GTV + 2 mm) for brain metastasis from NSCLC results in high LC rates with acceptable RN rate. The LC, RN and OS rates we reported in this retrospective series of brain metastasis from NSCLC are consistent with results observed in series with heterogeneous primary tumors. GTV D_{min} remained a predictive factor of better LC in NSCLC brain metastasis with a threshold of GTV D_{min} \geq 27.4 Gy. No dosimetric predictive factors of RN were found in this study. However, dyslipidemia was identified as a potential predictive factor of RN, which may be explained by microvascular disorders. Further studies are needed to explore this hypothesis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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II. Annexe 2 : Travaux collaboratifs en cancérologie des voies aéro-digestives supérieures

1. L'étude rétrospective TOCIT

Le travail suivant concerne une étude monocentrique, non-interventionnelle, hors recherche impliquant la personne humaine, approuvée par le comité d'éthique des centres d'investigations clinique Rhône-Alpes-Auvergne. Dans le cadre de ses missions de soin, de recherche et d'enseignement, le Centre Jean PERRIN, participe légitimement à des recherches scientifiques dans un but d'amélioration de la prévention, du dépistage et du soin, dans l'intérêt public dans le domaine de santé. Ainsi, 113 patients atteints d'un cancer des VADS localement avancé et traités par chimiothérapie d'induction de type TPF ont été inclus dans l'étude TOCIT entre juin 2009 et décembre 2017. Les détails concernant l'étude et les résultats sont présentés ci-dessous, dans l'article publié dans la revue BMC Cancer.

RESEARCH ARTICLE

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Predictive factors of toxicity of TPF induction chemotherapy for locally advanced head and neck cancers



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Abstract

Background: The rate of toxic deaths related to induction chemotherapy in the treatment of locally advanced head and neck cancers is unacceptable and calls into question this therapeutic strategy, which is however highly effective in terms of rate and speed of response. The purpose of the study was to investigate predictive factors of toxicity of induction chemotherapy with docetaxel, cisplatin, and 5-fluorouracil (TPF) in locally advanced head and neck cancers (LAHNC).

Methods: Between June 2009 and December 2017, 113 patients treated consecutively with TPF were included retrospectively. Patients were receiving induction chemotherapy for either an inoperable cancer or laryngeal preservation. For inoperable cancer, induction chemotherapy was proposed to patients presenting either a large tumor with strong symptoms (dyspnea, dysphagia, pain) or a tumor with rapid progression. Risk factors were chosen among the initial patient and tumour characteristics and chemotherapy modalities.

Results: Eighty-nine patients (79%) were male; the median age was 58 years [32–71]. Sixty-nine (61%) patients were treated for inoperable cancer and 44 (39%) for laryngeal preservation. 45% had stage IVa cancer, 28% stage III and 25% stage IVb. Sixty percent of patients had a partial response after TPF, 22% had a complete response, 12% were stable, 5% were progressing, and 1% had a discordant response. Thirty-four patients (30%) received enteral feeding during induction chemotherapy with TPF. The possibility of oral feeding without a tube was predictive of a better response ($p = 0.003$). Seven (6%) patients died during TPF. There was an increased risk of death with preexisting liver dysfunction (liver dysmorphia on imaging or decrease prothrombin rate) ($p = 0.032$). There was an increased risk of grade ≥ 3 infection if an enteral feeding occurred during the period of induction chemotherapy ($p = 0.03$).

Conclusions: TPF induction chemotherapy had an 82% objective response rate with 6% toxic deaths. Nutritional status and the presence of hepatic dysfunction are significant risk factors to be taken into account in therapeutic decisions.

Keywords: Head and neck cancer, Induction chemotherapy, TPF, Toxicity, Nutritional status, Hepatic dysfunction

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Background

Sixty percent of head and neck squamous cell carcinomas (HNSCC) are diagnosed at locally advanced stages [1]. Until the early 1990s, local treatments (surgery and/or radiotherapy [RT]) were the key components of treatment for locally advanced head and neck cancers (LAHNC) [2, 3] with a high rates of relapse and morbidity [4, 5]. To improve cure rates and functional outcomes, chemotherapy has been integrated into various strategies (concurrent radiochemotherapy, induction chemotherapy and a combination of both) [6–13]. These strategies have been applied in patients with non-operable cancers [6–11, 14] and in patients with resectable disease who are candidates for organ preservation [9, 12, 13, 15–17].

Induction chemotherapy combining cisplatin-5-fluorouracil (PF) followed by RT was initially approved for organ preservation in locally advanced, operable laryngeal squamous cell carcinomas (SCC) requiring total laryngectomy [17, 18]. In 2006, the GORTEC 2000–01 [19] trial demonstrated the superiority of the docetaxel-cisplatin-5-fluorouracil (TPF) combination to the PF induction regimen in the management of these laryngeal SCC eligible for an organ preservation strategy. For inoperable LAHNSCC, a large meta-analysis [11] compared TPF to PF induction, also showing TPF's superiority. Thus, TPF induction chemotherapy, already used in laryngeal preservation strategies, has been extended to all inoperable LAHNSCC with large tumour (T3 - T4) or lymph node extension (N2c - N3) with high risk of micro-metastases, without any formal proof of its superiority or non-inferiority compared to the standard treatment of concomitant radiochemotherapy.

The exact place of TPF induction chemotherapy for LAHNSCC over standard concomitant radiochemotherapy [10] has been questioned by several studies [20–25]. The National Comprehensive Cancer Network (NCCN) guidelines include induction chemotherapy with TPF followed by RT alone or by radiochemotherapy [26] as a validated treatment option. Indeed, TPF induction chemotherapy continues to be used due to a high response rate and an unequalled rapidity of response, often allowing a rapid regression of severe symptomatology (dyspnea, dysphagia, pain...).

However, TPF induction chemotherapy introduces a high risk of severe toxicities. These toxicities, in patients with many co-morbidities, can be fatal, with death rates ranging from 0 to 6.7% [8, 20–23, 27]. In addition, 20–30% of patients starting induction chemotherapy do not receive all of the radiotherapy +/- concomitant chemotherapy [8, 28, 29]. Thus, this treatment strategy may show better outcomes in better-selected patients with a lower risk of toxicity and better chances of receiving the full course of treatment [21–23, 25, 30, 31]. For now,

there are no consensus criteria for selecting these patients. The main aim of this study was to identify predictive factors of toxicity of docetaxel, cisplatin, and 5-fluorouracil (TPF) in locally advanced head and neck cancers (LAHNC), in order to better select patients likely to benefit from this treatment.

Methods

Inclusion population and study endpoints

Between June 2009 and December 2017, 113 patients treated consecutively in our institution with TPF induction chemotherapy were analyzed retrospectively. Patients were receiving induction chemotherapy for either an inoperable cancer or laryngeal preservation. For inoperable cancer, induction chemotherapy was proposed to patients presenting either a large tumor with strong symptoms (dyspnea, dysphagia, pain) or a tumor with rapid progression.

According the French legislation, the database has been declared to the French National Commission on Informatics and Liberty. All patients have been informed about the research by a non-opposition letter. They were free to oppose to the used of their personal data for this study. Study ethics approval was obtained on 18 November 2020 (CECIC Rhône-Alpes-Auvergne, Grenoble, IRB 5921).

The primary endpoint of this study was to identify predictive factor of grade ≥ 3 toxicities of TPF induction chemotherapy. Secondary endpoints included the search of predictive factors of treatment-related deaths, predictive factors of overall response rates and overall survival.

TPF induction chemotherapy

The induction chemotherapy regimen combined docetaxel 75 mg/m² on D1, cisplatin 75 mg/m² on D1 or cisplatin 20 mg/m² from D1 to D4, and 5-fluorouracil 750 mg/m² as a continuous infusion from D1 to D5 every 21 days. Intravenous hydration of 2 to 3 L was administered depending on whether patients were receiving single-dose or fractionated cisplatin. The antiemetic protocol combined aprepitant, corticosteroids, ondansetron and anti-D2. All patients received systematic GCSF as primary prophylaxis in accordance with international recommendations [32]. Premedication with methylprednisolone 50 mg twice daily was given the day before, the day of and the day after docetaxel in order to prevent hypersensitivity reactions, cutaneous adverse reactions and retention syndromes. Systematic dihydropyrimidine dehydrogenase (DPD) deficiency screening was not realized in the study period. According to local recommendations, systematic ciprofloxacin as primary prophylaxis was not realized.

Treatment following TPF induction chemotherapy

Radiotherapy delivered curative doses on the initial tumor/tumor bed of 60 and 66 Gy for operated patients and 70 Gy for non-operated patients, in fractions of 2 Gy to 2.12 Gy through conformal technique or volumetric modulated arc therapy (VMAT). RT was administered alone or concurrently with chemotherapy, which consisted of carboplatin AUC 1.5/week for 7 weeks or cisplatin 40 mg/m²/week for 7 weeks or cisplatin 100 mg/m² W1, W4, W7 or cetuximab 400 mg/m² at D-8 and 250 mg/m²/week for 7 weeks.

Patient follow-up

Acute hematologic and non-hematologic adverse events occurring during induction chemotherapy or radiotherapy were evaluated according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

Responses obtained after TPF and two to three months after the end of radiation therapy were also reported. Tumour response was evaluated clinically by nasofibroscope and/or CT imaging according to the Response Evaluation Criteria in Solid Tumours (RECIST) 1.1. Patients' responses included complete and partial response, and treatment failure included patients with progression or no response (stable disease) in order to increase the power of the analyses.

Statistical analysis

Statistical analyses were performed using R software (v. 3.5.1, R-Project, GNU GPL [33]). The normality of the continuous variables was tested using a Shapiro-Wilk test. For the statistical hypothesis testing, the α risk was fixed to 5%. Initial patient and tumour characteristics and chemotherapy modalities were analyzed in search of predictive factors of toxicity, death, progression-free survival (PFS) and overall survival (OS). Risk factors of toxicity studied were age, gender, alcohol consumption, presence of hepatic dysmorphia, prothrombin level, diabetes, high blood pressure, history of ischemic heart disease, chronic obstructive bronchitis, obliterative arterial disease of the lower limbs, performance status, weight loss before and during induction chemotherapy and receiving enteral feeding during induction chemotherapy indication for laryngeal preservation or inoperable disease and stage of disease.

The methods used were Fisher's exact test (for the relationship between pairs of categorical variables), the Wilcoxon-Mann-Whitney test (in case of a continuous and a categorical variable), and a Cox model (for time-to-event data). Statistical significance in the Cox model was assessed using the Wald statistic. Multivariate Cox models were fit using a variable selection method based on a LASSO-type penalized regression using all factors with a p -value < 0.1 in the univariate analysis.

Survival was defined as the time between diagnosis and death, regardless of cause, and living patients had their conditions at the date of the last follow-up statistically censored. Event-free survival was calculated as the time from the date of diagnosis to the date of progression (local, regional, or distant) or death, with patients' conditions at the last follow-up being censored if no event had occurred. Kaplan-Meier estimates were used for survival rate evaluation, and comparison between survival curves was based on the logrank test.

Results

Characteristics of the population

Sixty-nine (61%) had inoperable LAHNC and 44 (39%) were treated in a laryngeal preservation strategy. The patient and tumor characteristics are summarized in Table 1 and Table 2. The median age was 58 years [32–71]. 96% of patients had a World Health Organization (WHO) performance status of 0 or 1. 70% of patients had a history of alcohol and tobacco intoxication. The most frequent tumor sites were the larynx (27%). 92% of cancers were SCC, 7% undifferentiated carcinoma and 1% adenocarcinoma. All tumors were locally advanced with 45% of them classified as stage IVa, 28% stage III and 25% stage IVb. In inoperable tumors, there were one stage II (1%), 9 stage III (13%), 33 stage IVa (48%) and 26 stage IVb (38%). In laryngeal preservation, there were one stage II (2%), 23 stage III (52%), 18 stage IVa (41%) and 2 stage IVb (5%).

The median weight loss at diagnosis was 4.1 kg. 50% of patients experienced stable weight, 23% weight loss between 5 and 10% from baseline, 19% between 10 and 20 and 8% \geq 20%. The median serum albumin level was 39.6 g/L (95% CI [37.6–39.8]). Thirty-four patients (30%) had a feeding tube due to initial weight loss.

TPF induction chemotherapy and treatment following TPF

88% of patients received TPF induction chemotherapy with single-dose cisplatin on D1 and 12% received fractionated cisplatin 20 mg/m² from D1 to D4 from the first treatment. The data concerning TPF induction chemotherapy are presented in Table 3. Sixty-four patients (57%) received the full course of TPF induction chemotherapy as originally planned (without cancellation or dose reduction).

The modalities of treatment following TPF induction chemotherapy are presented in Table 3. The median time from the last chemotherapy treatment to the start of radiotherapy was 36 days [24–107] for inoperable LAHNC and for laryngeal preservation. Thirteen percent of patients received conformational technique and 87% received volumetric modulated arc therapy (VMAT). Radiotherapy delivered curative doses on the initial tumor/tumor bed of 60 and 66 Gy for operated patients and 70 Gy for non-operated patients, in fractions of 2 Gy to 2.12 Gy.

Table 1 Characteristics of 113 patients treated with TPF^a induction chemotherapy for locally advanced head and neck cancer

Male sex – no. (%)	89 (79)
Average age [min-max] (years)	58 [32–71]
WHO Performance Index – no. (%)	
0	56 (50)
1	52 (46)
2	5 (4)
Alcohol and tobacco use – no. (%)	
Alcohol and tobacco use	79 (70)
Tobacco	19 (17)
Alcohol	4 (3)
No alcohol and tobacco use	11 (10)
Comorbidities – no. (%)	
Hepatic dysfunction	6 (5)
Hepatic dysmorphia	10 (13)
Diabetes	9 (8)
High blood pressure	17 (15)
Ischemic heart disease	2 (2)
Obliterative arteriopathy of the lower limbs	5 (4)
Obstructive pulmonary disease	8 (7)
Stroke	2 (2)
Weight loss – median [min-max] (Kg)	–4,1[–28–7,81]
Serum Albumin – median [IC-95%] (g/l)	39,6 [37,6–39,8]
Oral feeding possible at diagnosis – no. (%)	
Yes	88 (78)
No	25 (22)
Enteral nutrition during TPF – no. (%)	
Yes	34 (30)
No	79 (70)

^aTPF docetaxel, cisplatin, 5-fluorouracil

Of the 113 patients included, a total of 39 patients (35%) received the full treatment initially planned (induction chemotherapy followed by radiotherapy or radiochemotherapy) without cancellation or dose reduction. Causes for discontinuation of induction chemotherapy or dose reduction were varied: febrile aplasia, acute renal failure, grade ≥ 3 hand-foot syndrome, mucositis, vomiting, diarrhea, radiodermatitis, radiomucitis and death. No patients did not receive concomitant chemotherapy due to induction chemotherapy toxicity.

TPF induction chemotherapy toxicities and predictive factors of toxicity

TPF induction chemotherapy toxicities are described in Table 4. Predictive factors of these toxicities are described in Table 5.

Table 2 Tumor characteristics of 113 patients treated with TPF^a induction chemotherapy for locally advanced head and neck cancer

Squamous cell carcinoma – no. (%)	102 (90)
Primary disease site – no. (%)	
Oral cavity	7 (6)
Oropharynx	26 (23)
Nasosinus	11 (10)
Nasopharynx	10 (9)
Hypopharynx	27 (24)
Larynx	31 (27)
Adenopathy without primitif	1 (1)
Laryngeal preservation – no. (%)	44 (39)
Stage – no. (%)	
II	2 (2)
III	32 (28)
IVA	51 (45)
IVB	28 (25)
Tumor size – no. (%)	
T1	3 (3)
T2	15 (13)
T3	52 (46)
T4	42 (37)
Tx	1 (1)
Nodal status – no. (%)	
N0	26 (23)
N1	13 (11)
N2	53 (47)
N3	21 (19)

^aTPF docetaxel, cisplatin, 5-fluorouracil

Indication for laryngeal preservation vs. inoperable disease was not significantly correlated with the different TPF induction chemotherapy toxicities studied (see supplementary data Table S1).

Hematotoxicity

14% grade ≥ 3 anemia, 6% grade ≥ 3 thrombocytopenia, 35% grade ≥ 3 neutropenia and 27% grade ≥ 3 febrile neutropenia were observed. In univariate analysis, female gender ($p = 0.042$), alcohol consumption ($p = 0.02$), pre-existing liver disease ($p = 0.037$) and a lowered prothrombin rate ($< 80\%$) ($p = 0.045$) were correlated with an increased risk of ≥ 3 grade anemia. In univariate analysis, age ($p = 0.039$), pre-existing liver disease ($p = 0.003$), and liver dysmorphia on initial imaging ($p = 0.027$) were correlated with an increased risk of grade ≥ 3 thrombocytopenia. No predictive factors for ≥ 3 grade neutropenia or febrile neutropenia were found. In multivariate

Table 3 Therapeutic sequence for the 113 patients treated with TPF induction chemotherapy for locally advanced head and neck cancer

Induction chemotherapy with TPF* regimen	
Number of cycles – no. (%)	
4	6 (5)
3	78 (69)
2	19 (17)
1	10 (9)
Fractionated cisplatin to C1	
Yes	14 (12)
No	99 (88)
Patients who received full induction chemotherapy – no. (%)	70 (62)
Induction chemotherapy protocol modifications – no. (%)	
Interruption	18 (16)
Report	7 (7)
Dose reduction	30 (31)
Radiotherapy	
Delay between the end of induction and the start of radiotherapy – median [min-max]	36 days [24–107]
Overall treatment time – median [min-max]	49 days [27–72]
Technique – no. (%)	
IMRT*	91 (87)
Conformational	13 (13)
Interruption – no. (%)	1 (1)
Concomitant radiochemotherapy (excluding laryngeal preservation and post-operative irradiation)	
Concomitant chemotherapy – no. (%)	49 (43)
Carboplatin AUC 1.5/week for 7 weeks	25 (51)
Cisplatin 40 mg/m ² /week for 7 weeks or cisplatin 100 mg/m ² S1, S4, S7	18 (37)
Cetuximab 400 mg/m ² at D-8 and 250 mg/m ² /week for 7 weeks	6 (12)
Interruptions in concomitant chemotherapy – no. (%)	26 (53)
Carboplatin	12 (46)
Cisplatin	10 (39)
Cetuximab	4 (15)
Patients who received the full course of treatment – no. (%)	39 (35%)

*TPF docetaxel, cisplatin, 5-fluorouracil

*IMRT intensity-modulated radiotherapy

analysis, there was an increased risk of ≥ 3 grade anemia ($p = 0.049$) in patients with a lowered prothrombin rate.

Other toxicities

In univariate analysis, female gender was correlated with an increased risk of grade ≥ 3 nausea ($p = 0.011$) and

Table 4 Toxicities of induction chemotherapy with TPF in patients treated for locally advanced head and neck cancer. Toxicity was assessed according to CTCAE classification V4.0

	Grade ≥ 3	Grade 4	Grade 5
Anemia	16 (14%)	0 (0%)	0 (0%)
Neutropenia	39 (35%)	32 (34%)	2 (2%)
Febrile neutropenia	30 (27%)	1 (1%)	5 (6%)
Thrombocytopenia	7 (6%)	1 (1%)	1 (1%)
Nausea/Vomiting	24 (22%)	2 (3%)	0 (0%)
Mucositis	20 (18%)	1 (2%)	0 (0%)
Diarrhea	19 (17%)	0 (0%)	0 (0%)
Nephrotoxicity	0 (0%)	0 (0%)	0 (0%)
Digestive hemorrhage	6 (5%)	1 (1%)	1 (1%)

grade ≥ 3 mucositis ($p < 0.001$) and a lowered prothrombin rate at diagnosis ($< 80\%$) was correlated with an increased risk of gastrointestinal bleeding ($p = 0.03$).

In multivariate analysis, there was an increased risk of infection ($p = 0.047$) in patients receiving enteral nutrition during induction chemotherapy. Thirty-six patients (32%) developed an infection during induction chemotherapy.

Deaths related to induction chemotherapy

There were 6% deaths ($n = 7$) during TPF induction chemotherapy (3/44 for laryngeal preservation patients, and 4/69 for inoperable patients). Five occurred after the first cycle. Four of them developed febrile neutropenia, one of which was complicated by colitis with gastrointestinal perforation. One patient presented a pneumopathy without neutropenia. One death occurred after the second cycle (sudden cardiopulmonary arrest at home) and another one after the third course (pneumonia in the context of febrile neutropenia). In univariate analysis, an increased risk of death related to induction chemotherapy was found in cases of liver disease ($p = 0.032$), a lowered prothrombin rate at diagnosis ($< 80\%$) ($p = 0.021$) or hepatic dysmorphism on initial imaging ($p = 0.044$). Neither sex, age, tumor stage, location, general condition, weight loss before or during TPF, nor enteral nutrition were significantly predictive of induction-chemotherapy-related death. In multivariate analysis, no factor was significantly correlated with the risk of death.

TPF induction chemotherapy response rates

Twenty-two percent of patients had a complete response after induction chemotherapy, 60% had a partial response, 12% had a stable disease, 5% had continued progression, and 1% had a discordant response in the primary tumour along with lymphadenopathy.

In univariate analysis, predictive factors of a better response (complete or partial response vs. stable or

Table 5 Predictive factors of toxicities of induction chemotherapy with TPF in patients treated for locally advanced head and neck cancer (multivariate analysis). Toxicity was assessed according to CTCAE classification V4.0

	Anemia Grade ≥ 3	Neutropenia Grade ≥ 3	Febrile neutropenia Grade ≥ 3	Infection	Death
Age	NS	NS	NS	NS	NS
Female sex	NS	NS	NS	NS	NS
Alcohol use	NS	NS	NS	NS	NS
Liver dysfunction	NS	NS	NS	NS	NS
Hepatic dysmorphia	NS	NS	NS	NS	NS
Prothrombin rate	0.049	NS	NS	NS	NS
Diabetes	NS	NS	NS	NS	NS
HBP	NS	NS	NS	NS	NS
Ischemic heart disease	NS	NS	NS	NS	NS
Obstructive pulmonary disease	NS	NS	NS	NS	NS
Obliterative arteriopathy of the lower limbs	NS	NS	NS	NS	NS
Performance status	NS	NS	NS	NS	NS
Weight loss before TPF	NS	NS	NS	NS	NS
Weight loss during TPF	NS	NS	NS	NS	NS
Enteral feeding during TPF	NS	NS	NS	0.047	NS

progressive) were the possibility of oral feeding without a feeding tube ($p = 0.03$), higher albuminemia ($p = 0.006$), and weight loss $< 5\%$ at diagnosis ($p = 0.043$). In multivariate analysis, indication for inoperable disease was correlated with better response vs. laryngeal preservation ($p = 0.04$).

Overall treatment response rates and survival data

The median follow-up was 48 months [0–103], in which 20% of patients had a recurrence (Table 3). Forty-two percent of patients ($n = 47$) were dead at last follow-up. Nine deaths were treatment-related (19%), 7 occurred during induction chemotherapy and 2 during radiotherapy. The

median overall survival was 74 months and the median PFS was 20 months.

In multivariate analysis, the necessity of enteral feeding at diagnosis and a poor response (stable disease or progression) after induction chemotherapy were predictive of worse PFS ($p = 0.003$ and $p < 0.001$ respectively) and of worse OS ($p = 0.001$ and $p < 0.001$, respectively) (Fig. 1).

Discussion

TPF induction chemotherapy used in the management of LAHNC has anti-tumor efficacy, with an overall response rate of 82% in our study and from 44 to 81% in

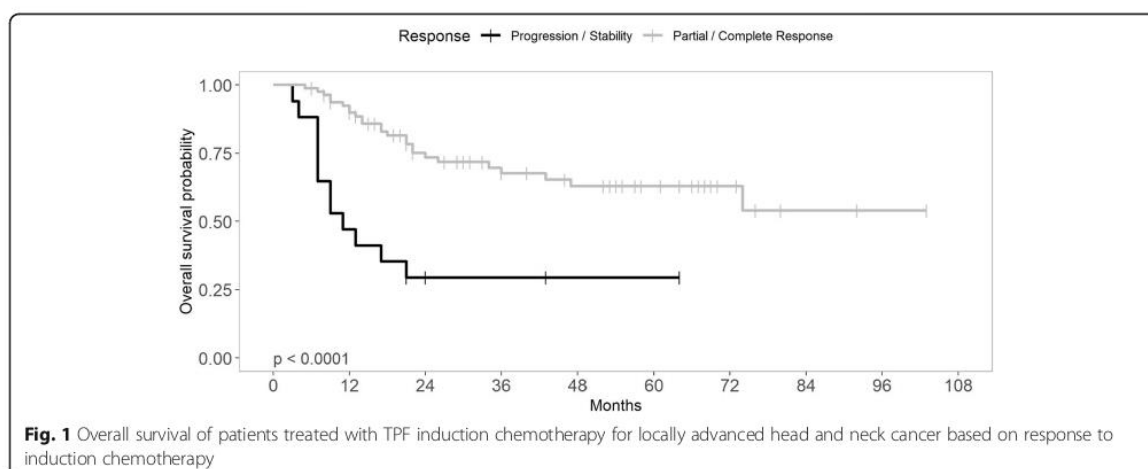


Fig. 1 Overall survival of patients treated with TPF induction chemotherapy for locally advanced head and neck cancer based on response to induction chemotherapy

the literature for inoperable tumour [8, 20, 22, 23, 30] and about 80% for laryngeal preservation [18]. However, this induction protocol resulted in high rates of severe toxicities as well as a non-negligible rate of death.

The number of patients in our study remains relatively small (113 patients) but represents, to our knowledge, the largest “real life” trial published to date. This study is also retrospective, and thus represents a bias for the interpretation of the results. We included patients receiving TPF induction chemotherapy, either in a laryngeal preservation strategy or for inoperable LAHNC or locally advanced undifferentiated nasopharyngeal carcinoma. This population was therefore heterogeneous, but since the main endpoint of this study was TPF toxicity, this was not a major bias in the interpretation of the results, especially since we have not demonstrated significant differences in toxicities between patients receiving TPF as part of a laryngeal preservation protocol and those receiving TPF for an inoperable tumour. However, efficacy and survival data (secondary endpoints) in our study are to interpret with caution due to the heterogeneity of the population.

We showed that the introduction of an enteral feeding during induction chemotherapy increased the risk of infection ($p = 0.047$ in multivariate analysis). In addition, the prognosis of these patients appears to be generally worse. Indeed, the objective response, progression-free survival and overall survival rates were correlated with the possibility or not of oral feeding at diagnosis. Patients with head and neck cancer are at high risk of undernutrition due to previous alcohol and tobacco intoxication, but also due to impaired swallowing or limited mouth opening that may be caused by the tumor itself [34–36]. This risk may be increased by the mucosal and digestive toxicities specific to TPF. The infectious risks associated with enteral feeding can be of two types: firstly, there is a risk of pneumopathy, either by massive inhalation of nutrient solution or by repeated occult inhalation. Secondly, there is a risk of infection directly related to the placement of the gastrostomy. Our study suggests that patients whose nutritional status is such that they require enteral feeding are not good candidates for TPF induction therapy. Especially for patients with a laryngeal preservation strategy, radiochemotherapy may be more appropriate. However, there was no correlation between the markers of nutritional status used (albuminemia, weight loss) and toxicities, suggesting that these routinely used clinical and biological markers are insufficient. However, concerning albuminemia, our results are to be interpreted with caution since 25% of the data were missing, and in the absence of a systematic C-reactive protein (CRP) dosage. The search for other markers of nutritional status, particularly sarcopenia, would be interesting.

We found 6% iatrogenic deaths in patients receiving TPF induction chemotherapy for LAHNC, a rate similar to the ones found in the literature [8, 20–23, 30]. In our institution, for inoperable LAHNSCC, we reserve TPF induction chemotherapy for patients with rapidly evolving and/or highly symptomatic tumors with a large tumor burden. This is a population similar to that of the GORTEC 2007–02 trial with a death rate of 6.7% [30]. We found that the risk of death was increased in cases of pre-existing liver disease (alcoholic cirrhosis). Liver biology may be normal even in cases of proven cirrhosis and therefore does not by itself allow optimal patient selection. Patients diagnosed with hepatic cirrhosis are usually not treated with TPF, but retrospective analysis of the data from this patient cohort has allowed us to find, a posteriori, signs of cirrhosis prior to chemotherapy in some patients. The extension work-up for these head and neck cancers usually includes a cervico-thoracic CT scan more or less a PET scan for inoperable locally advanced tumors. Therefore, we do not always have at our disposal morphological liver imaging. The systematic performance of an abdominal CT scan or liver ultrasound could be discussed in order not to administer TPF chemotherapy to patients with hepatic dysmorphia.

An option for optimizing induction chemotherapy would be to lighten the chemotherapy regimen to decrease its toxicity, especially for inoperable LAHNC. The use of the modified TPF scheme [37] or the combination of docetaxel, cisplatin and cetuximab, which has achieved response rates of more than 50% [38] with an acceptable safety profile in relapsed or metastatic situations, could be an alternative to TPF. A combination of docetaxel, cisplatin, anti-PD-1 or anti-PD-L1 would also be worth comparing to TPF in terms of the efficacy and safety results of the combination of chemotherapy and anti-PD1 in relapsed or metastatic situations [39].

Conclusions

The role of TPF induction chemotherapy in the management of locally advanced head and neck cancers remains to be defined. As found in the literature, our study shows that TPF induction chemotherapy has a high objective response rate, but significant morbidity with 6% toxic deaths. Nutritional status and the presence of hepatic dysfunction (liver dysmorphia on imaging or decrease prothrombin rate) seem to be the major elements to be taken into account in therapeutic decisions. Indeed, the need for enteral feeding at the beginning of treatment is associated with an increased risk of toxicities and poorer survival data. The presence of hepatic dysfunction is correlated with an increased risk of hematotoxicity grade ≥ 3 and an increased risk of death.

Abbreviations

CTCAE: Common Terminology Criteria for Adverse Events; HNSCC: Head and neck squamous cell carcinomas; LAHNC: Locally advanced head and neck cancers; OS: Overall survival; PF: Cisplatin-5-fluorouracil; PFS: Progression-free survival; RECIST: Response evaluation criteria in solid tumours; RT: Radiotherapy; TPF: Docetaxel-cisplatin-5-fluorouracil; VMAT: Volumetric modulated arc therapy

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12885-021-08128-5>.

Additional file 1: Table S1. Statistical analysis of toxicity profile differences between the laryngeal preservation group and inoperable disease group.

Acknowledgements

None.

Authors' contributions

Guarantor of integrity of the entire study: MB, JB. Study concepts and design: MB, AFD, ML, JB. Literature research: MB, ML, MC, JB. Clinical studies: MB, ML, MC, JB. Experimental studies / data analysis: MB, AFD, JMi, JMo, NPD, NS, XD, JB, IM. Statistical analysis: IM. Manuscript preparation: MB, MC, AG, JB. Manuscript editing: ML, AG, XD, JMi, JMo, NPD, NS. The author(s) read and approved the final manuscript.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Availability of data and materials

Inquiries about datasets analyzed for this study can be directed to the corresponding author.

Declarations**Ethics approval and consent to participate**

All methods were performed in accordance with the relevant guidelines and regulations. According to the French legislation, the database has been declared to the French National Commission on Informatics and Liberty. Study ethics approval was obtained on 18 November 2020 from ethics committee "Comité d'Ethique des Centres d'Investigation Clinique CECIC Rhône-Alpes-Auvergne, Grenoble, IRB 5921". According to the French legislation, and the ethics committee recommendations, all patients received a written informed form about this study. They were free to oppose to the use of their personal data for this study by a non-opposition letter. According to the French legislation, and the ethics committee recommendations, and due to the retrospective nature of this study, a written consent from the patients was not necessary (only a non-opposition letter).

Consent for publication

Not applicable.

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Received: 5 January 2021 Accepted: 23 March 2021

Published online: 07 April 2021

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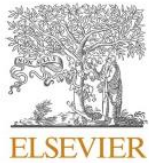
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2. L'étude ancillaire de l'essai clinique STEREO POSTOP

Le travail suivant concerne une étude ancillaire de l'essai clinique phase II, multicentrique STEREO POSTOP GORTEC 2017–03 (NCT03401840), évaluant la radiothérapie stéréotaxique hypo fractionnée post-opératoire des cancers de l'oropharynx et de la cavité buccale avec des marges à risque. L'étude ancillaire visait à étudier l'impact dosimétrique de l'ajout d'arcs non coplanaires à la technique VMAT et d'évaluer les toxicités aiguës sur les 10 premiers patients inclus dans cette étude. Les détails concernant l'étude et les résultats sont présentés ci-dessous, dans l'article publié dans la revue *Clinical and Translational Radiation Oncology*



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Postoperative SBRT in the treatment of early-stage oropharyngeal and oral cavity cancers with high-risk margins: A dosimetric comparison of volumetric modulated arc therapy with or without non-coplanar arcs and acute toxicity outcomes from the STEREOPOSTOP GORTEC 2017–03 phase 2 trial

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ARTICLE INFO

Keywords:

Stereotactic body radiotherapy
Postoperative
Early stage
Head and neck cancers

ABSTRACT

Background and purpose: The STEREO POSTOP GORTEC 2017–03 phase 2 trial (NCT03401840) evaluates postoperative stereotactic body radiotherapy (SBRT) in case of high-risk margins for pT1–T2/N0 oropharyngeal and oral cavity tumors. The present ancillary study aimed to compare the dosimetric impact of adding non-coplanar arcs to the volumetric modulated arc therapy (VMAT) technique and to evaluate acute toxicities on the first patients included in this trial.

Materials and methods: Ten patients were included. Patients were treated with Novalis TX®. The total dose was 36 Gy (100% isodose line) in 6 fractions, treated every other day. Two treatment plans were created for each patient: one plan using 2 coplanar arcs only (VMATc) and one plan using coplanar and 3 non-coplanar arcs (VMATc + nc). Acute toxicity was evaluated according to NCI CTCAE criteria V4.03.

Results: Median age was 62 years. Localization of tumor was the mobile tongue for 6 patients, floor of mouth for 2, cheek for 1, and gingiva for 1. Six patients had pT2N0 tumors (AJCC 7th edition) and 4 had pT1N0. Mean CTV and PTV volumes were 36.4 and 56.1 cc respectively. Mean PTV coverage by the 36 Gy isodose was 98.2% for both techniques ($p = ns$), with comparable conformity indexes (1.1 for VMATc vs 1.07 for VMATc + nc; $p = 0.23$). VMATc + nc had a significantly better gradient index (3.45 vs 2.97; $p = 0.01$), resulting in a significantly better sparing of most organs at risk. For example, mean Dmean to the oral cavity, lips, and homolateral parotid were respectively of 16.8 Gy, 11.1 Gy, and 10.4 Gy for VMATc vs 14.8 Gy ($p = 0.005$), 8.1 Gy ($p = 0.001$), 6.5 Gy ($p = 0.04$) for VMATc + nc. No grade ≥ 4 or higher acute toxicity was reported. The most common acute toxicity was grade ≥ 2 mucositis.

Conclusion: VMATc + nc had better dosimetric outcomes than VMATc and has become the standard technique for patients treated in the STEREO POSTOP GORTEC 2017–03 trial (NCT03401840) in our institution. Acute toxicity appears acceptable.

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<https://doi.org/10.1016/j.ctro.2022.11.007>

Received 19 July 2022; Received in revised form 8 November 2022; Accepted 10 November 2022

Available online 14 November 2022

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Background

Early-stage oropharyngeal and oral cavity cancers are mainly squamous cell carcinomas. Their incidence is rising [1]. Multidisciplinary management is usually needed. Primary surgery is one of the mainstay treatments [2]. Negative tumor margins are recommended (>5mm) [3,4]. If feasible, a re-resection of any positive margin is preferred. Otherwise, postoperative radiotherapy is indicated [5–8]. Limited adjuvant postoperative radiotherapy to the primary site for patients with pT1-T2 tumors and negative neck dissection, is a therapeutic option [8,9]. Both fractionated external beam radiotherapy and brachytherapy can have a role in this setting. Brachytherapy is a highly conformal radiotherapy technique that allows high-dose delivery to small volumes within a short overall treatment time [10–12]. However, implantation is not always technically possible and brachytherapy necessitates a highly experienced team and appropriate infrastructures. Post-operative external beam radiotherapy can also be used but the overall treatment time is longer (6–7 weeks) [13–17]. Another possible alternative could be postoperative hypofractionated Stereotactic Body Radiotherapy (SBRT), which is investigated in the STEREO POSTOP GORTEC 2017–03 multicentric phase 2 trial (NCT03401840) [18]. It is an attractive option because it delivers a highly conformal dose of radiation in a limited number of fractions, with steep dose gradients resulting in reduced normal tissue irradiation [19]. To our knowledge, STEREO POSTOP GORTEC 2017–03 (NCT03401840) is the first-in-human trial to deliver postoperative SBRT in this specific indication.

This manuscript presents the outcomes of an ancillary study issued from the STEREO POSTOP GORTEC 2017–03 trial (NCT03401840). The purpose of this ancillary study was to compare the dosimetric impact of adding non-coplanar arcs to the volumetric modulated arc therapy (VMAT) technique on a Novalis-type accelerator and to report the acute toxicity profile of the first ten patients from the STEREO POSTOP GORTEC 2017–03 trial (NCT03401840) [18].

Material and methods

Patients

This ancillary study included the ten first patients included in the STEREO POSTOP GORTEC 2017–03 (NCT03401840) phase 2 trial in our institution. The first patient was included in January 2018. A total of 90 patients was included. The entire detailed protocol has been published previously [18]. Main inclusion criteria included: squamous cell carcinoma of the oral cavity (except lips) or oropharynx; pT1 or pT2 (AJCC 7th edition) with an indication of postoperative tumoral bed irradiation (positive margin R1, close margin <5 mm or margin estimated at risk); N0 after surgical treatment (neck dissection or sentinel lymph node biopsy), or pN1 without extracapsular extension; and no prior radiotherapy. Main exclusion criteria included: pT3 or pT4 (AJCC 7th edition); pT2 >3 cm and R1 with concurrent chemoradiotherapy decided in multidisciplinary tumor board; lymphovascular invasion; distant metastasis; and lack of at least one of the following elements: pre-operative medical imaging, endoscopy report, surgery report, and pathological report. The primary endpoint of the STEREO POSTOP GORTEC 2017–03 (NCT03401840) phase 2 trial was 2-year late toxicity.

All patients in this ancillary study were treated with Novalis TX® (Varian Medical Systems, Palo Alto, CA, USA and Brainlab, Munich, Germany). This ancillary study was foreseen in the study protocol [18].

Treatment preparation

All patients had a dental examination, including clinical and radiological examination. When indicated, extraction of dental elements was carried out. Adequate dental care (including daily fluoride application if necessary) was realized, at least during follow-up.

A planning CT of 1.25-mm thickness was acquired in supine position,

including the whole skull to the lower border of the clavicle. Patients were immobilized using a noninvasive stereotactic thermoplastic mask. In the protocol [18], the use of devices for the immobilization of the tongue was left to the discretion of the investigators. In the present ancillary study, no specific device was used for the immobilization of the oral tongue for the 10 patients.

According to the study protocol [18], the CTV was defined as the initial tumor bed including the positive or close margins with a margin of 5 to 10 mm according to the anatomical barriers and extension pathways. In the case of flap reconstruction, CTV also included the junction normal tissue/flap +5 mm proximity flap. A 2-mm set-up margin was implemented around the CTV to create the PTV. Delineation of the organs at risk (OARs) was realized according to Brouwer et al. [20]. When necessary, a 2-mm margin was applied to the OARs to create the planning OARs volumes (PRVs).

According to the study protocol [18], the total dose was 36 Gy in 6 fractions, treated every other day; corresponding to biological effective dose (BED) BED_{10} of 64.2 Gy for the tumor (equivalent to BED_{10} of 60 Gy in 30 fractions), a BED_{10} of 54.4 Gy for early effects (equivalent to BED_{10} of 74 Gy in 37 fractions), and a BED_3 of 108 Gy for late effects (equivalent to BED_3 of 66 Gy in 33 fractions) [21,22].

Patients were treated with a volumetric modulated arc therapy (VMAT) technique with arcs of 6-MV photons. Treatment specifications were as follows: the prescription isodose line was 100 % of the prescribed dose (36 Gy), to encompass at least 95 % of the PTV, with no >5 % of the PTV receiving > 110 % of the prescribed dose i.e. 39.6 Gy. The prescription isodose line was chosen as 100 % due to the postoperative situation. Final calculations were performed using the AAA algorithm on Eclipse® TPS version 15.6 (Varian Medical Systems). The arc optimization algorithm, the *Photon Optimizer* used in Rapidarc®, optimized leaf position, dose rate, and gantry speed. Optimization parameters with *Normal Tissue Objectives* (NTO) were used to spare healthy tissues. The maximum dose rate was set at 600 MU/min.

For the treatment, daily pre-positioning was performed using an ExacTrac® stereoscopic X-ray system (Brainlab, Munich, Germany) and a robotic couch with six of freedom, and final positioning was performed using cone-beam CT.

Dosimetric comparison

Two treatment plans were created for each patient: one plan using coplanar arcs only (VMATc) and one plan using coplanar and non-coplanar arcs (VMATc + nc). The ten patients were ultimately treated with VMATc + nc.

VMATc plans were created with two full coplanar arcs (Fig. 1). The first arc was planned in a clockwise direction and the second in a counter-clockwise direction. For all the plans, the collimator was rotated to 30° for the first arc and to 330° for the second arc to reduce the tongue-and-groove effect.

VMATc + nc plans were created with one full coplanar arc and 3 partial non-coplanar arcs spaced by about 45° (Fig. 1). The maximum arc rotation amplitude was 160°. The rotation of the collimator was 10°, 350°, 350°, 350° and 10° for the first, second, third, and fourth arcs respectively.

Acute toxicity assessment

Acute toxicity was defined as any ≤ 3-month toxicity related to SBRT according to NCI CTCAE criteria V4.03. To evaluate early toxicity, 3 visits with a physical evaluation were planned during SBRT: at the first fraction (day1), the fourth (expected date: day8), and the last fraction (day11 to day13). After SBRT treatment, a visit was planned 1 week after the last fraction, at 1 month, and at 3 months. The 10 patients used for the dosimetric analysis were the same as the ones included in the acute toxicity assessment.

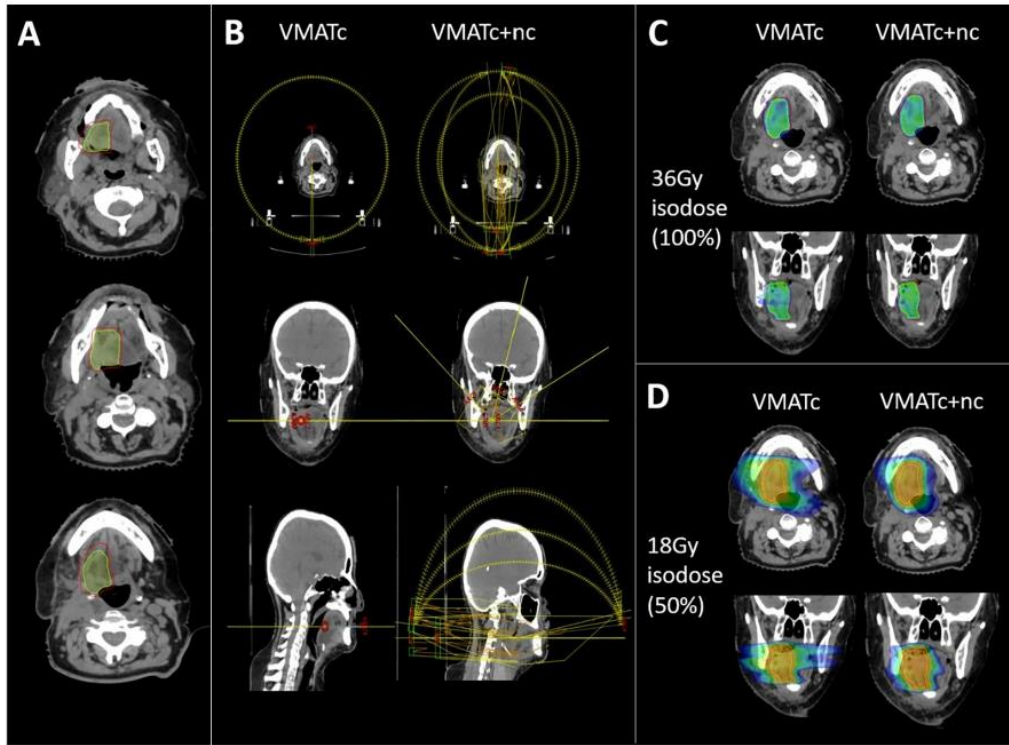


Fig. 1. Treatment planning of a patient with a pT1-R1 tumor of the posterior right mobile tongue. A/ Axial views of the planning CT with the CTV (yellow) and PTV (red). B/ Axial, coronal and sagittal views of VMAT technique with coplanar arcs only (VMATc), and of VMAT technique with coplanar and non-coplanar arcs (VMATc + nc). C/ Axial and coronal views representing the 36 Gy isodose (100 %) for VMATc and VMATc + nc. D/ Axial and coronal views representing the 18 Gy isodose (50 %) for VMATc and VMATc + nc. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Statistical analysis

The plan analyses were based on dose-volume histogram (DVH) data. For target volume coverage, V100% (36 Gy), and the maximum dose (Dmax) to the PTV were noted. We also calculated three indexes for the PTV: the inverse Paddick conformity index (CI), the gradient index (GI), and the homogeneity index (HI).

The inverse Paddick CI is defined as follows:

$$CI = \frac{(Total\ volume\ receiving \ge Dcoverage) + (PTV\ volume)}{(PTV\ volume\ receiving \ge Dcoverage) (PTV\ volume\ receiving \ge Dcoverage)}$$

A value of 1 is the ideal case. The larger the value, the less conformal the treatment.

The GI is defined as follows:

$$GI = \frac{(Total\ volume\ receiving \ge 50\%Dcoverage)}{(Total\ volume\ receiving \ge 100\%Dcoverage)}$$

The GI describes the steepness of the dose fall-off from the 36 Gy isodose (Dcoverage in our case) to the 18 Gy isodose (50 % of Dcoverage). The larger the value, the shallower the gradient.

The HI is defined as follows:

$$HI = \frac{D2\% - D98\%}{Dmean}$$

where D2 % was the dose delivered to 2 % of the PTV volume, D98 % was the dose delivered to 98 % of the PTV volume, and Dmean was the mean dose to the PTV. Small values of HI indicated more homogeneous irradiation of the PTV.

For organs at risk, Dmean and/or D2% were noted.

Statistical analyses were performed using R v2.15.1 (<https://www.cr>

[an.r-project.org](https://www.cr)). To compare the dosimetric indices for the different modalities, non-parametric Wilcoxon tests for paired samples were used. If the associated p-value was less than the significance level ($\alpha = 0.05$), it was assumed that there was a statistically significant difference between the compared data sets. Due to the low number of patients, data concerning acute toxicities were only descriptive.

Results

Patient characteristics

All patients' characteristics are detailed in Table 1. The median age was 62 years (min–max: 36–81). Six of the 10 patients had mobile tongue tumors, 2 had floor of mouth tumors, 1 had a cheek tumor, and 1 had a gingiva tumor. Six of the 10 patients had T2 tumors (AJCC 7th edition) and 4 had T1 tumors. Five of the 10 patients had a flap reconstruction surgery. The indications of postoperative SBRT for the 10 patients were as follows: 1 positive R1 margin, 8 close margin <5 mm, and 1 extensive microscopic perineural invasion. Median follow-up was 12 months (min–max: 3 – 33).

The mean CTV volume was 36.4 cc (min–max: 22.3–65.9) and the mean PTV volume was 56.1 cc (min–max: 37.6–92.3).

Dosimetric comparison

Table 2 summarizes dosimetric parameters for both VMATc and VMATc + nc techniques. Mean PTV coverage (prescription isodose 36 Gy) was 98.2 % for both techniques (p = ns), with a comparable CI (mean CI of 1.1 for VMATc vs 1.07 for VMATc + nc; p = 0.23).

Table 1
Patients characteristics.

	Age	Localization	T stage	N stage	Indication	Flap	CTV (cc)	PTV (cc)
Patient 1	41	Floor of mouth	T2	N0	Close margin	Yes	49.5	75.0
Patient 2	36	Mobile tongue	T2	N0	Close margin	No	41.6	64.1
Patient 3	71	Gingiva	T2	N0	Close margin	No	28.2	44.9
Patient 4	68	Mobile tongue	T1	N0	Close margin	No	27.6	43.3
Patient 5	34	Mobile tongue	T1	N0	Perineural invasion	Yes	27.7	45.6
Patient 6	51	Cheek	T1	N0	Close margin	Yes	65.9	92.3
Patient 7	63	Mobile tongue	T2	N0	Close margin	Yes	35.4	54.2
Patient 8	80	Mobile tongue	T2	N0	Close margin	No	22.3	37.6
Patient 9	61	Mobile tongue	T1	N0	Close margin	No	29.0	46.7
Patient 10	69	Floor of mouth	T2	N0	R1 margin	Yes	36.9	56.8

Table 2
Summary of dosimetric results comparing volumetric modulated arc therapy with coplanar arcs only (VMATc) or with coplanar and non-coplanar arcs (VMATc + nc).

	Indices	VMATc	VMATc + nc	p-value
PTV	V36Gy (%)	98.2	98.2	ns
	CI	1.1	1.07	0.23
	HI	0.1	0.07	0.004
	GI	3.45	2.97	0.01
	Dmax (Gy)	35.7	33.8	0.005
Jaw	D2% (Gy)	30.3	27.8	0.01
	Dmean (Gy)	12.6	10.6	0.009
HL Cheek	D2% (Gy)	34.2	33.3	0.02
	Dmean (Gy)	19.2	15.2	0.007
CL Cheek	D2% (Gy)	16.0	11.4	0.006
	Dmean (Gy)	10.6	7.4	0.01
Lips	D2% (Gy)	26.7	23.1	0.003
	Dmean (Gy)	11.1	8.1	0.001
Spinal cord	D2% (Gy)	10.0	6.4	0.001
Brainstem	D2% (Gy)	5.0	4.8	0.78
HL Parotid	Dmean (Gy)	10.4	6.5	0.04
CL Parotid	Dmean (Gy)	6.2	3.07	0.02
Oral Cavity	D2% (Gy)	29.8	28.6	0.36
	Dmean (Gy)	16.8	14.8	0.005

Data are presented as mean doses of all patients ± standard deviation. VxGy = volume receiving at least xGy; and Dx% is the minimum dose received by x% of the structure volume.

CI = Conformity Index; HI = Homogeneity Index; GI = Gradient Index; Dmax = maximum dose; Dmean = mean dose; HL = homolateral; CL = contralateral; Gy = Gray.

Treatment plans were significantly more homogeneous with VMATc + nc (mean HI of 0.1 for VMATc vs 0.07 for VMATc + nc; p = 0.004) with a significantly better gradient index (mean GI of 3.45 for VMATc vs 2.97 for VMATc + nc; p = 0.01).

Most of the organs at risk were significantly better spared with VMATc + nc (Table 2). For example, the mean Dmean to the oral cavity was 16.8 Gy for VMATc vs 14.8 Gy for VMATc + nc (p = 0.005), mean Dmean to the lips was 11.1 Gy for VMATc vs 8.1 Gy for VMATc + nc (p = 0.001) and mean Dmean to the homolateral parotid was 10.4 Gy for VMATc vs 6.5 Gy for VMATc + nc (p = 0.04).

Acute toxicity

Patients' acute toxicities are summarized in Table 3. There was no grade ≥ 4 acute toxicity. The ten patients experienced grade ≥ 2 mucositis (3 grade 2 and 7 grade 3). For all patients, the maximum grade of mucositis was reached 1 week after the end of the treatment; and progressively decreased to disappear at 1 month for 40 % of patients, and at 3 months for 100 % of patients. Xerostomia was noticed for 5 of the 10 patients, all grade 1. There were no grade ≥ 3 dysphagia (4 grade 2 and 5 grade 1). At 3 months, dysphagia was improved in all the patients, with only 4 patients with persistent grade 1 dysphagia. Epidermitis was noticed in 3 of the 10 patients (2 grade 2 and 1 grade 1) and

Table 3
Acute toxicities related to SBRT treatment.

	Mucositis	Xerostomia	Dysphagia	Epidermitis	Others
Patient 1	Grade 2	None	Grade 2	Grade 2	Mycosis (Grade 2) Tongue edema (Grade 2)
Patient 2	Grade 3	Grade 1	Grade 1	None	Pain (Grade 2)
Patient 3	Grade 3	None	Grade 1	None	None
Patient 4	Grade 3	None	Grade 1	None	None
Patient 5	Grade 3	Grade 1	Grade 2	None	Mycosis (Grade 2) Pain (Grade 1)
Patient 6	Grade 3	Grade 1	None	Grade 2	Trismus (Grade 2)
Patient 7	Grade 3	Grade 1	Grade 2	None	Cheilitis (Grade 2)
Patient 8	Grade 2	None	Grade 1	None	None
Patient 9	Grade 2	Grade 1	Grade 1	Grade 1	Mycosis (Grade 2)
Patient 10	Grade 3	None	Grade 2	None	None

was also totally resolved at 1 month. Two of the 10 patients experienced pain (1 grade 2 and 1 grade 1). One patient experienced grade 2 tongue edema, one had grade 2 trismus, and one had grade 2 cheilitis.

Discussion

This study is the first ancillary study from the STEREO POSTOP GORTEC 2017–03 trial (NCT03401840) [18]. This phase 2 trial evaluates postoperative SBRT in the treatment of early-stage oropharyngeal and oral cavity cancers with high risk margins. In this trial, SBRT is limited to the primary site for patients with pT1-T2 tumors and negative neck dissection [8,9]. Omitting neck irradiation for pN0 patients is a controversial topic. The main series reporting this strategy for localized tumors come from post-operative brachytherapy with favorable outcomes [23,24]. A total of 90 patients are planned to be included in the STEREO POSTOP GORTEC 2017–03 trial (NCT03401840). The primary endpoint of this trial is 2-year late toxicity. Here, we report the results of a dosimetric study of the 10 first patients treated with a Novalis-type accelerator as well as acute toxicity results. We compared the dosimetric impact of adding non-coplanar arcs using a VMAT irradiation technique. We found that both VMATc and VMATc + nc were highly conformal techniques (CI of 1.1 and 1.07 respectively, p = 0.23), but that VMATnc resulted in a steeper dose gradient (GI of 2.97 vs 3.45 for VMATc, p = 0.01). This steeper dose gradient resulted in better organs at risk sparing (Table 2). For example, the mean Dmean to the homolateral parotid gland was reduced by 3.9 Gy (10.4 Gy for VMATc vs 6.5 Gy for

VMATnc, $p = 0.05$), mean Dmean to the lips was reduced by 3 Gy (11.1 Gy for VMATc vs 8.1 Gy for VMATnc, $p = 0.0004$), and mean Dmean to the oral cavity was reduced by 2 Gy (16.8 Gy for VMATc vs 14.8 Gy for VMATnc, $p = 0.005$). We found that this dosimetric impact was sufficiently meaningful to use VMATc + nc as the reference technique for all the patients included in this trial in our institution. To date, SBRT in head and neck cancers has been mainly validated in the case of reirradiation. Recently, the International Stereotactic Body Radiotherapy Consortium (ISBRTC) has published a survey of current practices in SBRT for head and neck cancer reirradiation [25]. Of the 15 international institutions included in this survey, a majority of the institutions (11 out of the 15) used linear accelerators with cone-beam CT to treat patients, and 9 institutions used a VMAT technique. However, there were no precisions regarding whether non-coplanar arcs were used or not. The department of radiation oncology from the University of Pittsburgh Cancer Institute is probably the most important team that had published in the field of head and neck cancers SBRT [26–31]. They describe that in their experience, they initially favored Cyberknife almost exclusively. However, with advances in treatment delivery and image guidance, they transitioned to almost exclusively linear accelerators with cone-beam CT (Trilogy and Truebeam). They use both static IMRT and VMAT plans and only coplanar beams or arcs are used (except for skull base lesions for which non-coplanar arcs are commonly incorporated) [27]. To date, this dosimetric study is the first published to demonstrate that non-coplanar arcs might be useful in head and neck SBRT (other than skull base).

The acute toxicity profile that we report here appears favorable. However, this report only concerned 10 patients. The most common acute toxicity that we report was grade 2 to 3 acute mucositis (Fig. 2). This toxicity profile seemed comparable with the one reported in the series of post-operative brachytherapy. Goineau et al. [23] published a series of 112 patients treated with post-operative interstitial low dose rate (LDR) ^{192}Ir brachytherapy for mobile tongue squamous cell carcinoma. The main acute toxicity, present in all patients, was grade ≥ 2 mucositis. Ferenczi et al. [24] published a series of 44 patients treated with high dose rate tumor bed brachytherapy for floor of mouth tumors. They reported 75 % of grade ≥ 2 acute mucositis. Even if the acute toxicity profile that we reported here seemed favorable, it is rather late toxicity that should be looked at closely in this situation. Indeed, the STEREO POSTOP GORTEC 2017–03 trial (NCT03401840) includes pT1/pT2 N0 oral cavity or oropharyngeal squamous cell carcinomas with high risk margins, which have a potential long survival. The reports of late toxicity and oncological long-term outcomes are expected for 2023.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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3. L'étude rétrospective RAPIDARCO

L'étude RAPIDARCO est une étude monocentrique, non-interventionnelle, hors recherche impliquant la personne humaine, approuvée par le comité éthique IRB (Institutional Review Board) du Centre Jean PERRIN. Dans le cadre de ses missions de soin, de recherche et d'enseignement, le Centre Jean PERRIN, participe légitimement à des recherches scientifiques dans un but d'amélioration de la prévention, du dépistage et du soin, dans l'intérêt public dans le domaine de santé. L'étude RAPIDARCO a ainsi été proposée à chaque patient ayant un cancer des VADS et traités par VMAT au Centre Jean PERRIN. L'objectif étant de recueillir des données, tout au long de leur parcours de soin, avec notamment des données démographiques (âge, sexe, poids, *etc.*), des données anatomopathologiques, ainsi que des données relatives à leur cancer (historique, traitements, évolution, données dosimétriques, recueil des toxicités, *etc.*). Ainsi, 809 patients ont été inclus dans l'étude entre mars 2011 et décembre 2019. Les données ont été enregistrées rétrospectivement de mars 2011 à juin 2022 par des radiothérapeutes, une interne en radiothérapie et moi-même.

Cette étude est à l'origine, d'un premier projet porté par le Dr Emmanuel CHAUTARD. Ce projet qui est en cours vise à caractériser l'infiltrat immun des cancers des VADS. Les prélèvements anatomopathologiques de 90 patients inclus dans l'étude RAPIDARCO ont été récupérés afin d'explorer l'expression d'un panel de gènes impliqués dans l'immunité tumorale grâce à une approche innovante (Nanostring) et de réaliser une analyse quantitative et qualitative de l'infiltrat immun par immunohistochimie. L'enjeu à plus long terme est de pouvoir sélectionner les patients pouvant tirer un bénéfice d'une combinaison d'immunomodulateurs avec la radiothérapie.

Le deuxième projet, porté par le Dr Cécile MIONE, dans le cadre de sa thèse de médecine et moi-même a pour objectif d'évaluer les résultats oncologiques de patients opérés d'un carcinome épidermoïde de l'oropharynx et de la cavité buccale, traités par VMAT post-opératoire au Centre Jean PERRIN, en étudiant tout particulièrement les profils d'échec loco-régional. La chirurgie est le traitement de référence pour les cancers des VADS localisés ou localement avancés. Après une chirurgie, la radiothérapie peut également être nécessaire afin de diminuer le risque de rechute locorégionale. Elle est recommandée pour les patients présentant des caractéristiques défavorables, notamment avec un stade avancé et des marges insuffisantes (199,200). Une chimiothérapie concomitante est également recommandée, en

particulier pour les patients présentant des caractéristiques pathologiques à haut risque, c'est-à-dire avec une infiltration ganglionnaire extra-capsulaire et/ou une marge chirurgicale positive (199,200). La VMAT est aujourd'hui la technique de radiothérapie recommandée pour le traitement des cancers des VADS (201). Mais c'est une technique complexe, dont la délimitation du volume cible va conditionner les résultats du traitement notamment en termes de contrôle loco-régional (202,203). Force est de constater que peu d'études ont été publiées sur de la radiothérapie post-opératoire par VMAT chez des patients atteints de carcinome épidermoïde de l'oropharynx et de la cavité buccale, une analyse rétrospective a été réalisée grâce aux données des patients inclus dans l'étude RAPIDARCO. L'objectif été d'évaluer les résultats oncologiques de ces patients en étudiant tout particulièrement les profils d'échec loco-régional. Les détails concernant cette étude et les résultats sont présentés ci-dessous, dans l'article soumis dans la revue Radiation Oncology pour lequel je suis co-1^{ère} auteure.

Outcomes among oropharyngeal and oral cavity cancer patients treated with postoperative volumetric modulated arctotherapy

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ABSTRACT

Background

Presently, there are few published reports on postoperative radiation therapy for oropharyngeal and oral cavity cancers treated with IMRT/VMAT technique. This study aimed to assess the oncological outcomes of this population treated with postoperative VMAT in our institution, with a focus on loco-regional patterns of failure.

Material and Methods

Between 2011 and 2019, 167 patients were included (40% of oropharyngeal cancers, and 60% of oral cavity cancers). The median age was 60 years. There was 64.2% of stage IV cancers. All patients had both T and N surgery. 34% had a R1 margin, 42% had perineural invasion. 72% had a positive neck dissection and 42% extranodal extension (ENE). All patients were treated with VMAT with simultaneous integrated boost with three dose levels: 66Gy in case of R1 margin and/or ENE, 59.4-60Gy on the tumor bed, and 54Gy on the prophylactic areas. Concomittant cisplatin was administrated concomitantly when feasible in case of R1 and/or ENE.

Results

The 1- and 2-year loco-regional control rates were 88.6% and 85.6% respectively. Higher tumor stage (T3/T4), the presence of PNI, and time from surgery >45 days were significant predictive factors of worse loco-regional control in multivariate analysis ($p=0.02$, $p=0.04$, and $p=0.02$). There were 17 local recurrences: 11 (64%) were considered as infield, 4 (24%) as marginal, and 2 (12%) as outfield. There were 9 regional recurrences only, 8 (89%) were considered as infield, and 1 (11%) as outfield. The 1- and 2-year disease-free survival (DFS) rates were 78.9% and 71.8% respectively. The 1- and 2-year overall survival (OS) rates were 88.6% and 80% respectively. Higher tumor stage (T3/T4) and the presence of ENE were the two prognostic factors significantly associated with worse DFS and OS in multivariate analysis.

Conclusion

Our outcomes for postoperative VMAT for oral cavity and oropharyngeal cancers are encouraging, with high rates of loco-regional control. However, the management of ENE still seems challenging.

Introduction

Surgery is one of the cornerstone treatments for oropharyngeal and oral cavity cancers [1]. Adjuvant postoperative radiation therapy is recommended for patients with adverse features, including advanced disease and inadequate margins. The addition of concomitant chemotherapy is recommended, particularly for patients who have 'high risk' pathological features including extranodal extension (ENE) and/or a positive surgical margin [1,2].

Intensity Modulated Radiation Therapy (IMRT) or Volumetric Modulated Arcttherapy (VMAT) is today the recommended radiation technique for the treatment of head and neck cancers [3]. IMRT/VMAT for head and neck cancers is a complex technique both for target volume delineation and treatment planning [4,5]. The delineation of the target volumes is an essential step conditioning the results of the treatment, particularly in terms of loco-regional control [6–9]. Presently, there are few published reports on postoperative radiation therapy for oropharyngeal and oral cavity cancers treated with IMRT/VMAT technique. This study aimed to assess the oncological outcomes of operated patients with oropharyngeal and oral cavity squamous cell carcinomas (SCC), treated with postoperative VMAT in our institution, with a focus on loco-regional patterns of failure.

Material and Methods

Patients

The database maintained by the Department of Radiation Oncology at our institution was used to identify patients treated with postoperative VMAT for oropharyngeal or oral cavity SCC from May 2011 to December 2019. Patients with distant metastases or concomitant malignancies at the time of diagnosis, histology other than SCC, R2 margins, and/or a previously irradiated cancer of the head and neck were excluded.

One hundred and sixty-seven patients were retrospectively reviewed. Patient characteristics are described in Table 1.

The initial location of the tumors was the oropharynx in 40% of cases (n=67) and the oral cavity in 60% (n=100) of cases. The median age of the patients was 60 years (20 - 94 years). One hundred and twenty-five patients were smokers (75%). The WHO performance status was assessed as 0 for 78 patients (46,7%), 1 for 73 patients (43,7%), 2 for 15 patients (8,9%), and 3 for 1 patient (0,7%). The TNM stages (UICC 2009) were as follows: there were 23 stage II (13.7%), 37 stage III (22.1%), 94 stage IVa (56.4%), and 13 stage IVb (7.8%).

Treatment

The overall treatment strategies were individualized for each patient following recommendation by a multidisciplinary tumor board.

- Surgery:

All patients included underwent surgery on the primary tumor accompanied by lymph node dissection (unilateral for 46.1% and bilateral for 53.9%). The operative technique depended on the location and initial extension of the disease. Sixty-seven patients (40%) had a free flap inserted during surgical reconstruction.

- Anatomic pathology:

Fifty-six patients (34%) had a positive R1 margin, and sixty-three (37%) had a close margin (<5mm). Seventy patients (42%) had peri-neural invasion (PNI), and 48 (29%) had lympho-vascular invasion. One hundred and twenty one patients (72%) had positive neck dissections, and 68 patients (42%) had ENE (Table 1).

- Radiotherapy:

Patients underwent radiotherapy in case of advanced tumors (T3-T4), close or positive margins, lymph node involvement with or without ENE, the presence of lymphatic-vascular space invasion, and/or PNI [10,11]. The median interval from surgery to initiation of radiotherapy was 46 days (24 - 100). All patients were irradiated with VMAT (Rapidarc®, Varian Medical Systems, Palo Alto, CA, USA) with simultaneous integrated boost (SIB). A planning CT-scan, supine, in the treatment position, was performed, with 2.5-mm-thick slices, and a personalized thermoformed mask with 5 attachment points. Preoperative imaging was merged with the planning CT scan to guide the contouring of this surgically reshaped area. Target volumes and organs at risk were delineated according to the different expert recommendations [12–17]. Three clinical target volumes (CTV) were typically defined. CTV1

(59.4 to 60 Gy) was defined as the preoperative tumor bed with a margin (1 to 2 cm), and manually adjusted according to anatomical barriers. CTV2 (54 Gy) was defined as subclinical tumor sites at risk, according to the risk of tumor involvement [14–16], and as prophylactic nodal irradiation, following guidelines [12,13,15]. CTV3 (66 Gy) was occasionally identified, in cases of ENE and/or positive margin. Planning target volumes (PTV) related to positioning errors and movements were obtained by adding a 4 mm margin around the CTVs. Treatment was delivered in 30-33 fractions.

The treatment planning system was Eclipse® (Varian Medical Systems). Treatment plans followed the recommendations of the International Commission of Radiation Units report n°83 [18] . The treatment was delivered using a Clinac IX® or Novalis TX® (Varian Medical Systems) linear accelerators delivering 6-MV photons, with daily position control by KV/KV beams or CBCT.

- Chemotherapy:

Seventy-two patients (43%) underwent concomitant chemotherapy. The main indications were R1 positive margins and/or ENE among patients under 70 years of age and in the absence of contraindications [10,11,19]. Concomitant chemotherapy protocols included either three-weekly high dose Cisplatin, or weekly Cisplatin (Table 1). Five patients (3%) received neoadjuvant chemotherapy with Taxotere-Cisplatin-5Fluoro-Uracile.

Follow-up

Weekly evaluations were performed by the radiation oncologist for all patients during radiation treatment. The first post-treatment follow-up was at 3 months after radiation completion. Then, every 3 months for the first and the second year, alternating surgeon and radiation oncologist, and at least twice a year for up to 5 years, and thereafter yearly. A follow-up imaging was performed at 3 months and then annually.

Recurrences

Local and regional recurrences were confirmed via radiologic imaging (i.e. progression in subsequent images or high SUV on PET imaging) or via pathology specimens (i.e. from surgical biopsy). Diagnostic contrast-enhanced CT and/or PET/CT or MRI documenting the initial evidence of local recurrence were investigated. Radiologically evident recurrence volumes were manually segmented and reviewed by four experienced radiation oncologists (CM, JMO,

JMI and JB). The corresponding original planning CTs were identified and the original plans were restored. Recurrence CT was co-registered with initial planning CT using a deformable image registration (Aria with MIRS application, version 2.1, Varian Medical Systems) [20,21]. The recurrence volume was transferred to the initial planning CT and was subsequently deformed according to the deformable co-registration. A clinical validation was carried out by the radiation oncologists. The most likely point of origin of the recurrence was defined clinically by the radiation oncologists, based on their knowledge of anatomy and cancer spread pathways according to Due et al. [22,23]. If the point of origin of the recurrence was outside the initial target volume, recurrence was considered to be “outfield”; if the point of origin of the recurrence was inside the initial target volume, recurrence was considered to be “infield”; and if the point of origin of the recurrence was on the boundary of the initial target volume, recurrence was considered to be “marginal”.

Statistical analysis

Groups defined by initial tumor localization were compared using Fisher’s exact test and the Wilcoxon-Mann-Whitney test. The Kaplan-Meier method was used to calculate survival curves. The last day of radiation therapy was used as time zero. Comparisons between survival curves were made using the log-rank test. Median follow-up was estimated using the reverse Kaplan-Meier method. Factors associated with survival were analyzed using univariate Cox regression models followed by penalized multivariate models, obtained by including all variables with a p-value < .05 in the univariate analysis and model selection with the LASSO method. All analyses were performed using R statistical software version 4.1.0 (R-Project, GNU GPL). P-values under 0.05 were considered significant.

The factors associated with survival analyzed were: gender (male/female), tobacco use (never/ current or stopped), WHO stage (0/1-3), tumor status (T1-T2/T3-T4), number of pathological lymph nodes (≥ 3 / < 3), tumor differentiation (good/moderate, poor), lymphovascular invasion, PNI, ENE, margin status (R0/close, R1), radiotherapy-treatment time and surgery-radiotherapy time.

Results

Local, regional and loco-regional control

The median follow-up was 33 months. During follow-up, 26 patients (16%) developed loco-regional recurrences: 12 local, 9 regional, and 5 both local and regional (Table 2).

The 1 and 2-year local control rates were 93% and 90% respectively for the overall population. For oral cavity cancers, the 2-year local control rate was 85.1% vs 96.7% for oropharyngeal cancers ($p=0.031$). Higher tumor stages (T1-2 vs T3-4; $p<0.001$) and presence of PNI ($p<0.001$) were predictive factors for poorer local control in univariate and multivariate analysis ($p=0.03$ and $p=0.01$ respectively) (Table 3).

The 1 and 2-year regional control rates were 93.1%. A number of lymph node over 3 was the only predictive factor for a poorer regional control in univariate and multivariate analysis ($p=0.009$ and $p=0.006$ respectively) (Table 3).

The 1- and 2-year loco-regional control rates were 88.6% and 85.6% respectively (Figure 1), with 85.3% and 80.1% among oral cavity cancer and 93.6% and 93.6% among oropharynx cancer ($p=0.022$). Higher tumor stages (T1-2 vs T3-4; $p=0.002$), presence of PNI ($p=0.008$) and time from surgery to initiation of VMAT ($p=0.03$) were predictive factors for poorer loco-regional control in univariate and multivariate analysis respectively ($p=0.02$, $p=0.04$, and $p=0.02$ respectively; Table 3; Figure 1).

Local and regional patterns of recurrence

Seventeen patients (10%) developed a local recurrence and 13 patients (8%) a regional recurrence, of whom 5 patients had both a local and regional recurrence. The initial characteristics and patterns of recurrence are described in Table 2.

Concerning the 17 local recurrences, 11 (64%) were considered as infield, 4 (24%) as marginal, and 2 (12%) as outfield. Concerning the 9 regional recurrences only, 8 (89%) were considered as infield, and 1 (11%) as outfield.

Metastasis

During follow-up, there were 26 metastatic events (15.6%) with a 2-year metastasis-free survival probability of 84.5% (Figure 2). Ten patients treated for an oropharyngeal cancer (2-year probability of metastasis-free survival = 85.9%) and 16 patients treated for an oral cavity

tumor (2-year probability of metastasis-free survival = 83.3%) developed secondary localizations (bone, lung, cutaneous and liver) with no difference according to the initial tumor location ($p=0.74$). In univariate analysis, PNI and ENE were associated with poorer metastasis free survival ($p=0.049$, $p=0.002$). In multivariate analysis, only ENE remained significant ($p=0.004$; Table 3, Figure 2).

Survival

The 1- and 2-year disease-free survival (DFS) rates were 78.9% and 71.8% respectively (Figure 3). Higher tumor stages (T1-2 vs T3-4; $p= 0.001$) and presence of ENE ($p=0.03$) were unfavorable prognostic factors for DFS in univariate and multivariate analysis ($p=0.002$ and $p=0.02$ respectively; Table 3; Figure 3).

Forty-two patients (25%) died during follow-up. The 1- and 2-year overall survival (OS) rates were 88.6% and 80% respectively (Figure 4). In univariate analysis, WHO stage ≥ 1 ($p = 0.03$), presence of ENE ($p = 0.01$) and T3-T4 tumors ($p=0.004$) were unfavorable prognostic factors for OS. In multivariate analysis, the presence of ENE and T3-T4 tumors were independent unfavorable prognostic factors for OS ($p=0.01$ and $p=0.01$ respectively; Table 3; Figure 4).

Discussion

IMRT/VMAT has been increasingly used over the last two decades for the treatment of head and neck cancers, and is today the gold standard technique in radiation therapy for these cancers. However, the data regarding the outcomes associated with IMRT/VMAT in the post-operative setting for oral cavity and oropharyngeal cancers is very limited [24–31]. Thus our series, despite its relatively small number of patients ($n=167$), is one of the largest reported to date. The outcomes reported in our series are in line with those reported in the literature [25,26,29,31–34] (Table 4). We found that postoperative VMAT reached high rates of local and loco-regional control. We found that the presence of ENE, despite present-day radiochemotherapy techniques, remains a major issue.

There are a few limitations to our study that need to be highlighted. Potential biases, inherent in any retrospective analysis, could have affected the results of this study. Because of the retrospective nature of the study, certain data missing in the medical files could not be assessed for all patients. For example, the depth of invasion for oral cavity cancers was often missing, not allowing to use the latest UICC classification. We also found that the collection of

all toxicities were not robust enough to allow good quality interpretation of the data, so we only focused on the oncological outcomes. This series was also single-center. However, all patients were treated in the same institution with surgery and post-operative radiotherapy in fairly homogeneous manner for both dose prescription and delineation.

Concerning local recurrences, during follow-up only 17 patients (10%) developed a local recurrence with actuarial 1 and 2-year local control rates of 93% and 90% respectively. The vast majority of these local recurrences were found to be *infield*. Higher tumor stages and presence of PNI were the only predictive factors in multivariate analysis for poorer local control. R1 margins were not found to be predictive factors for local control. This is in line with various recent reports that have also found that R1 margins were no longer a factor of poorer local control since the emergence of radiochemotherapy [24,27,29,35]. It seems that the negative impact of R1 margins has been fully negated by using higher radiotherapy doses (usually 66Gy) and radiochemotherapy. However, this does not seem to be the case for ENE. Indeed, in our series, the presence of ENE was found to be a prognostic factor for poorer DFS, metastasis-free survival, and OS. A recent series of 439 patients with head and neck SCC (all localizations) treated with postoperative radiotherapy also reported the negative prognostic value of ENE despite the use of radiochemotherapy [35]. This finding highlights the need to find new strategies for patients with ENE treated with postoperative radiochemotherapy, as in the ongoing NIVOPOSTOP trial testing the addition of Nivolumab to standard cisplatin-based chemo-radiation (NCT03576417).

We also found that oral cavity cancers generally had poorer outcomes than oropharyngeal cancers, with 2-year LC rates of 85.1% vs 96.7% ($p=0.031$), and 2-year OS rates of 77.3% vs 83.8% (non-significant, probably due to a lack of statistical power). This is in line with previously reported series [25,29,36].

Regarding chemotherapy, 72 patients (43%) were treated with radiotherapy combined with chemotherapy. The local control and OS rates for these high-risk patients were similar to those for patients with a lower risk of recurrence, treated without concomitant chemotherapy. It is possible that without concomitant chemotherapy the outcome among high-risk patients would have been worse [29]. The total number of failures in this series is however too small to enable any robust calculations between subgroups.

Conclusion

Our outcomes for postoperative VMAT for oral cavity and oropharyngeal cancers are very encouraging, with high rates of loco-regional control (85.6% at 2 years). However, the management of ENE still seems challenging, as these events were identified as highly unfavorable prognostic factors.

List of abbreviations

CTV: clinical target volume
DFS: disease-free survival
ENE: extranodal extension
IMRT: intensity modulated radiation therapy
OS: overall survival
PNI: peri-neural invasion
PTV: planning target volume
SCC: squamous cell carcinoma
SIB: simultaneous integrated boost
VMAT: volumetric modulated Arctherapy

Declarations

Ethics approval and consent to participate: This retrospective study was approved by our local ethics committee. All patients were informed and were free to oppose their participation in this study.

Consent for publication: not applicable

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request

Competing interests: The authors declare that they have no competing interests

Funding: no specific funding for this study

Authors' contributions: CM, MC, ML and JB designed the study. CM, MC, JMo, JMI, IM, EC, MB, MK, NS, FM, NFD collected the data and performed the analysis. CM, MC, EC, ML and JB interpreted the data, and JD wrote the paper. All authors read and approved the final manuscript.

Acknowledgements: not applicable

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Table 1: Patient and disease characteristics

Characteristics	N (%)
Gender	
Male	122 (73)
Female	45 (27)
Median Age (range)	60 (20-94)
Site	
Oral Cavity	100 (60)
Oropharynx	67 (40)
T-stage	
1-2	72 (43)
3-4	95 (57)
N-stage	
0-1	72 (43)
2-3	95 (57)
UICC Stage 2009	
II	23 (13,7)
III	37 (22,1)
IVa	94 (56,4)
IVb	13 (7,8)
p16	
Positive	24 (14)
Negative	38 (23)
Unspecified	105 (63)
Smokers	125 (75)
WHO status	
0-1	151 (90)
2-3	16 (10)
Treatment	N (%)
Chemotherapy	
Concomitant	72 (43)
Neoadjuvant	5 (3)
No	90 (54)
Nodal surgery	
Bilateral	90 (53.9)
Unilateral	77 (46.1)
Tumor characteristics	N (%)
Extranodal extension+	68 (42)
Tumor differentiation	
Well	74 (44)
Moderate	56 (34)
Poor	19 (11)
Unspecified	18 (11)
Lymphovascular invasion	
Yes	48 (29)
No	117 (70)
Unspecified	2 (1)
Perineural invasion	
Yes	70 (42)
No	91 (54)
Unspecified	6 (4)
Margin	
R1	56 (34)
R0 close < 5mm	63 (37)
R0	41 (25)
Unspecified	7 (4)

Table 2: Prognostic and predictive factors*PNI +: presence of perineural invasion, RT: radiation therapy, ENE +: presence of extranodal extension*

	Prognostics factors	Univariate Analysis HR (CI 95%)	Multivariate Analysis HR (CI 95%)
Local control	T3-T4	14.135 (1.873 – 106.702) p<0.001	9.88 (1.296 – 75.315) p=0,03
	PNI +	6.633 (1.887 – 23.323) p<0.001	5.127 (1.453 – 18.089) p=0,01
Regional control	Lymph node >3	4.906 (1.581 – 15.225) p=0.009	4.906 (1.581 – 15.225) p=0.006
Loco-regional control	T3-T4	3.979 (1.504 – 10.524) p=0.002	3.172 (1.167 – 8.624) p=0.02
	Time from surgery to RT	1.035 (1.008 – 1.062) p=0.03	1.041 (1.003 – 1.082) p=0.04
	PNI +	2.911 (1.294 – 6.549) p=0.008	2.714 (1.17 – 6.296) p=0.02
Metastasis-free survival	PNI+	2.213 (0.992 -4.936) p=0.049	1.939 (0.853 – 4.407) p=0.11
	ENE +	3.549 (1.543-8.165) p=0.002	3.498 (1.506 – 8.122) P=0.0044
Disease-free survival	T3-T4	2.551 (1.409 – 4.617) p=0.001	2.544 (1.399 – 4.625) p=0.002
	ENE +	1.785 (1.055 – 3.02) p=0.03	1.921 (1.127 – 3.273) p=0.02
Overall survival	WHO stage 1-3	1.997 (1.067 – 3.74) p=0.03	1.651 (0.868 – 3.14) p=0.13
	T3-T4	2.606 (1.308 – 5.192) p=0.004	2.529 (1.252 – 5.111) p=0.01
	ENE +	2.167 (1.17 – 4.013) p=0.01	2.295 (1.213 – 4.345) p=0.01

Table 3: Initial characteristics and analysis of the patients who developed a local and/or regional recurrence

R1= positive margin, R<5mm=close margin <5mm, R0=negative margin, ENE +: extranodal extension, ENE -: positive node without extranodal extension, N - : no positive node, Surgery – RT interval: interval between surgery and the first day of radiotherapy

	Patients	TNM	Age at diagnosis	Location	Nodal Extension	Margin	PN I	Flap	Chemotherapy	Surgery-RT interval (days)	Prescribed Dose (Gy)	Treatment Time (days)	Time to recurrence (months)	Recurrence anatomical description	Recurrence analysis
Local T recurrence only	1	T4aN2aM0	52	Mobile tongue	ENE +	R1	Yes	Yes	Yes	39	66	46	9.2	Tongue/tongue flap junction	Infield.
	2	T3N2cM0	78	Base of tongue	ENE +	R1	Yes	Yes	No	41	66	50	7.1	Base of Tongue	Infield.
	3	T4aN0M0	50	Mandible	N0	R1	Yes	No	No	53	66	50	20.7	Homolateral mandible	Infield.
	4	T4aN2cM0	54	Floor of mouth	ENE +	R1	Yes	Yes	Yes	49	66	49	15.7	Fibula flap region	Infield
	5	T3N1M0	77	Mobile tongue	ENE -	R0	No	No	No	47	66	45	16.4	Mobile tongue	Infield.
	6	T3N1M0	59	Floor of mouth	ENE -	R1	Yes	No	Yes	48	66	46	4.2	Mobile tongue/floor of mouth junction	Infield
	7	T4N1M0	72	Retromolar area	ENE +	R<5 mm	Yes	Yes	No	51	66	48	12.3	Digastric muscle	Outfield
	8	T4aN0M0	62	Floor of mouth	N0	R<5 mm	Yes	Yes	No	48	60	38	2.7	Fibula flap region	Marginal
	9	T3N1M0	60	Upper lip	ENE +	R0	Yes	Yes	Yes	83	60	48	3.1	Upper jaw	Infield.
	10	T3N0M0	56	Base of tongue	N0	R1	Yes	Yes	Yes	38	66	47	29.1	Macroscopic PNI along the lingual nerve	Infield
	11	T1N0M0	65	Tonsil	N0	R<5 mm	Yes	No	No	39	66	45	4.8	Mobile Tongue	Marginal
	12	T3N0M0	79	Hard Palate	N0	R1	Yes	No	No	41	66	44	6.6	Hard Palate	Marginal
Both local and regional recurrence	13	T4aN2cM0	42	Floor of mouth	ENE +	R<5 mm	Yes	Yes	Yes	48	60	38	4.7	T: Tongue / flap junction	T: Infield.
	14	T3N0M0	77	Cheek	N0	R1	No	Yes	No	35	66	45	27.8	T: Mandible	T: Infield
	15	T4aN2bM0	65	Retromolar area	ENE -	R1	No	Yes	Yes	41	66	43	11	T: Infratemporal fossa	T: Outfield
	16	T4aN2cM0	26	Mobile tongue	ENE +	R1	Yes	Yes	Yes	35	66	45	1.4	T: Base of tongue	T: Infield.
	17	T4N2cM0	20	Floor of mouth	ENE +	R<5 mm	Yes	Yes	Yes	41	60	45	3	T: Oropharynx Hypopharynx	T: Marginal
Regional N recurrence only	18	T4aN2cM0	53	Mobile Tongue	ENE + IIB controlateral	R1	Yes	Yes	Yes	42	66	48	4	IVa controlateral	Infield (54Gy)
	19	T3N0M0	51	Retromolar area	N0	R0		Yes	No	49	60	50	3.8	Ib homolateral	Infield (54Gy)
	20	T2N2cM0	54	Oropharynx	ENE + IIB homolateral	R1		No	Yes	42	66	51	39	VIIA homolateral	Infield (54Gy)

21	T3N2bM0	80	Mobile tongue	ENE + IIA homolateral	R<5 mm	No	No	No	44	66	47	4	IV homolateral	Infield (54Gy)
22	T1N2aM0	74	Left Tonsil	ENE + IIB homolateral	R0	No	No	No	43	66	44	12	VIIA homolateral	Infield (60Gy)
23	T4aN3bM0	79	Hard Palate	ENE + IIA homolateral	R1	Yes	No	No	48	60	39	8.5	II A IIB III contralateral (unilateral irradiation)	Outfield
24	T1N2cM0	49	Mobile tongue	ENE + IIA bilateral	R1	No	Yes	Yes	44	66	48	4	IB homolateral	Infield (60Gy)
25	T2N2bM0	56	Floor of mouth	ENE + IIA homolateral	R<5 mm	Yes	Yes	Yes	43	66	50	9	IIB-IV-V homolateral	Infield (60Gy)
26	T4N1M0	80	Floor of mouth	ENE - IIB homolateral	R1	Yes	Yes	No	43	60	41	4	IIB homolateral	Infield (60Gy)

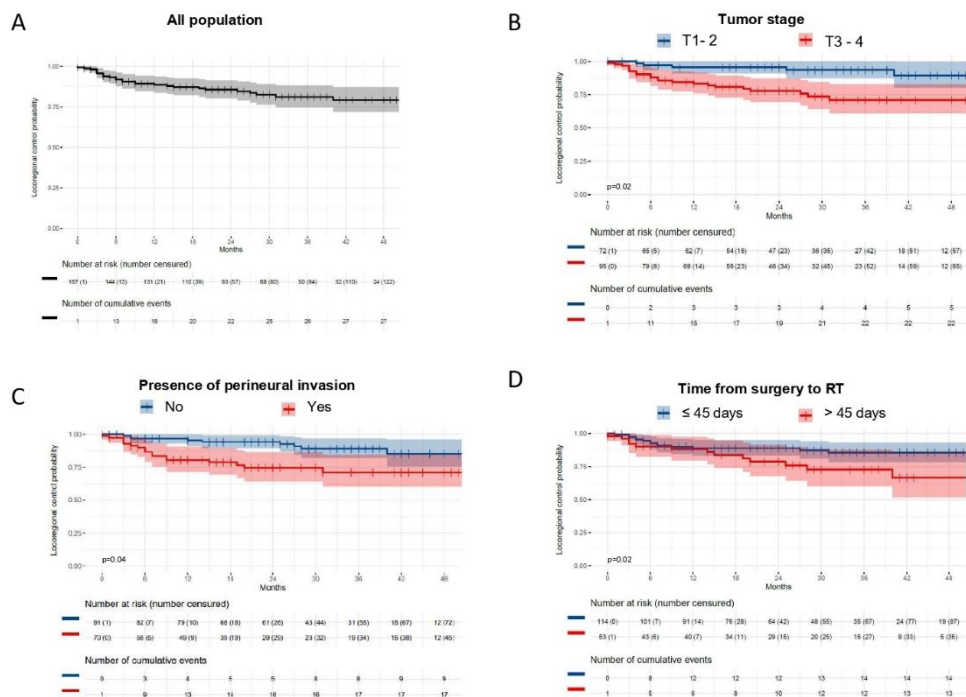


Figure 1 : Loco-regional control among A) the entire population, and B) according to tumor staging, and C) the presence of extranodal extension

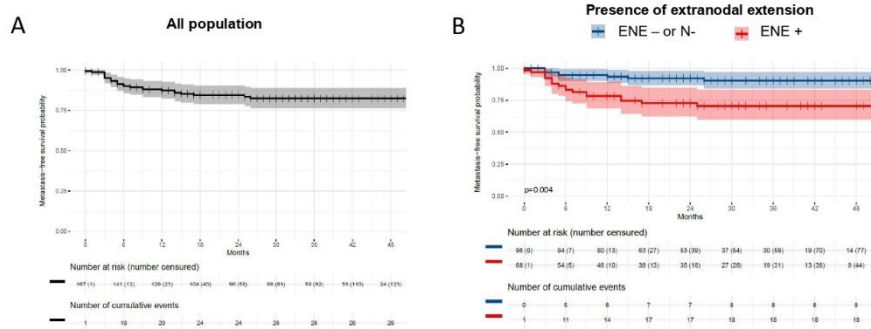


Figure 2 : Metastasis-free survival among A) the entire population, and B) according to the presence of extranodal extension

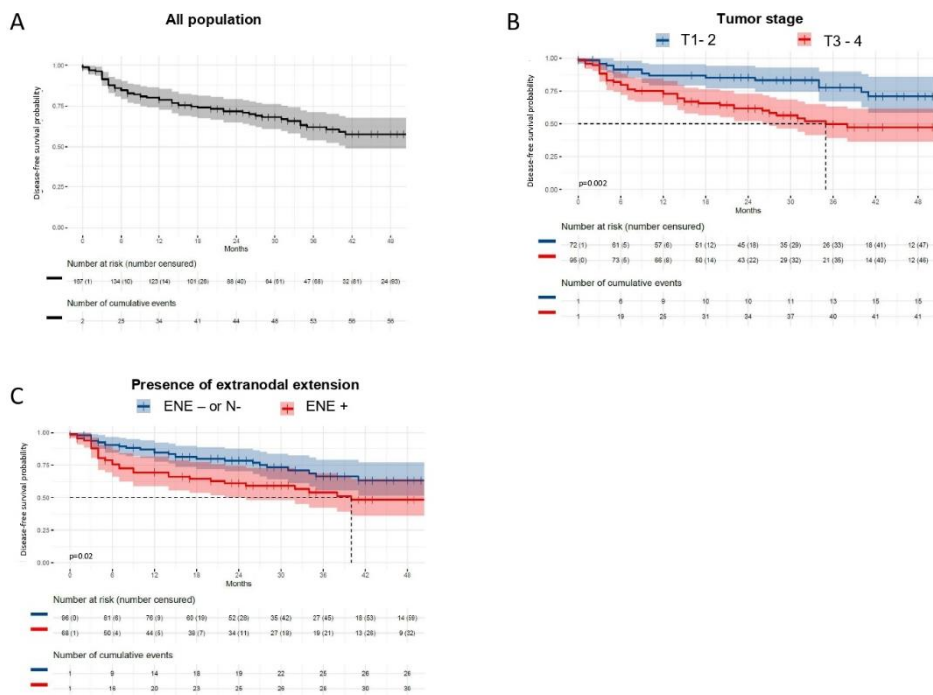


Figure 3 : Disease-free survival among A) the entire population, and B) according to tumor staging, and C) the presence of extranodal extension.

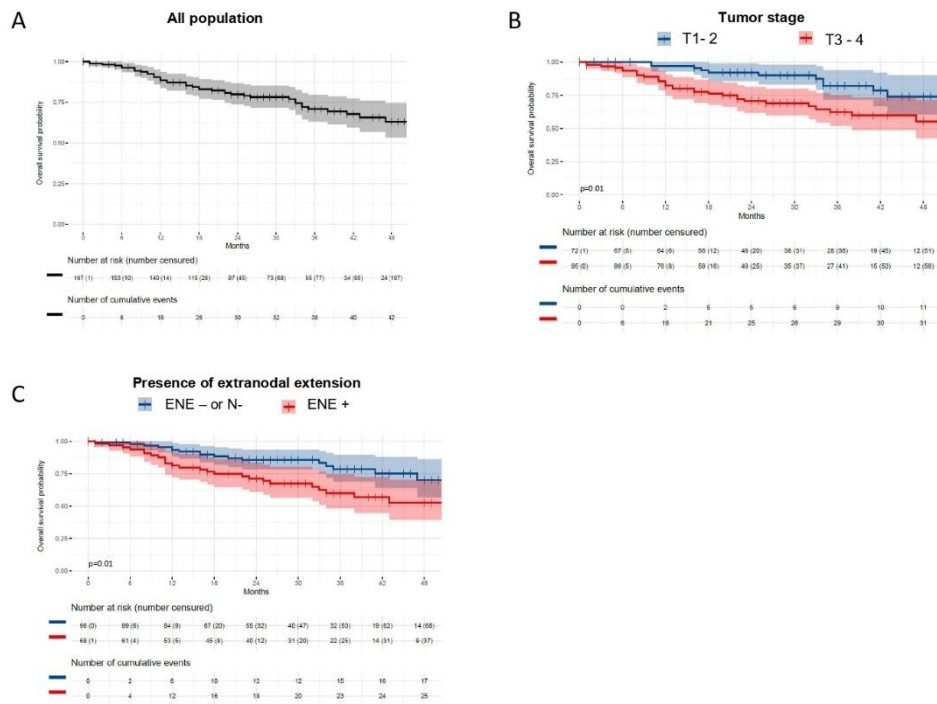


Figure 4 : Overall survival among A) the entire population, and B) according to tumor staging, and C) the presence of extranodal extension.

III. Annexe 3 : Participation au concours « Ma thèse en 180 secondes » à Clermont-Ferrand

Le concours « Ma thèse en 180 secondes » est un concours international de vulgarisation scientifique organisé par le CNRS et France Universités. Il permet aux doctorants francophones de présenter son sujet de recherche à un auditoire profane et diversifié. Cette présentation doit être réalisée en trois minutes, avec une seule diapositive. Le but étant de présenter ses recherches de façon claire et concise tout en étant convaincant. Les présentations font souvent référence à la culture populaire ou à des métaphores. L'humour est également un outil très utilisé car il permet de décomplexifier la science.

Dans le cadre de ma thèse, j'ai participé à la finale régionale du concours Ma thèse en 180 secondes à Clermont-Ferrand le lundi 06 mars 2023. Ma participation à ce concours est visionnable en ligne via le lien suivant : <https://youtu.be/InkiwhdiYag>

Ma présentation s'est focalisée sur la présentation du protocole de recherche clinique GMaPIC et de ses résultats, illustrés par la série littéraire Harry Potter. Le script étant le suivant : *« En mangeant des dragées de chez Bertie Crochue, les oreilles d'Harry se mettent à souffler et faire du bruit. Bon, je sais ce n'est pas la meilleure référence scientifique pour parler d'une thèse qui porte sur la mesure de la pression intracrânienne chez des patients ayant un cancer du cerveau. Mais ce n'est pas parce que c'est magique, que ce n'est pas réel ?*

Effectivement ! Savez-vous que l'on peut mesurer la pression à l'intérieur de notre crâne par nos oreilles ?

Des cellules situées au fond de notre oreille sont dotées de petits cils qui vont bouger à la réception d'un son. Ce mouvement va être transformé en un signal électrique pour notre cerveau et va également renvoyer une partie des ondes vers l'extérieur. Nos oreilles font donc du bruit ! Oui, oui je vous l'assure ! Si vous ne l'aviez jamais remarqué, c'est tout à fait normal, ce son n'est pas perceptible à l'oreille du moldu.

Un dispositif médical clermontois permet d'envoyer des ondes acoustiques dans l'oreille et d'enregistrer à l'aide d'un micro les ondes qui seront renvoyées vers l'extérieur. Le but étant de mesurer le décalage entre le son envoyé et le son reçu.

Donc dans le cas d'une augmentation de la pression intracrânienne, ces mesures seront impactées, car il y aura un changement de pression dans l'oreille, ce qui augmentera ou diminuera le décalage entre le son envoyé et reçu.

Dans le cadre de ma thèse, vous l'aurez compris, afin de faciliter ces mesures, j'ai donné des dragées de chez Bertie crochue à mes patients. Plus sérieusement, j'ai utilisé ce dispositif médical chez des patients ayant un cancer du cerveau.

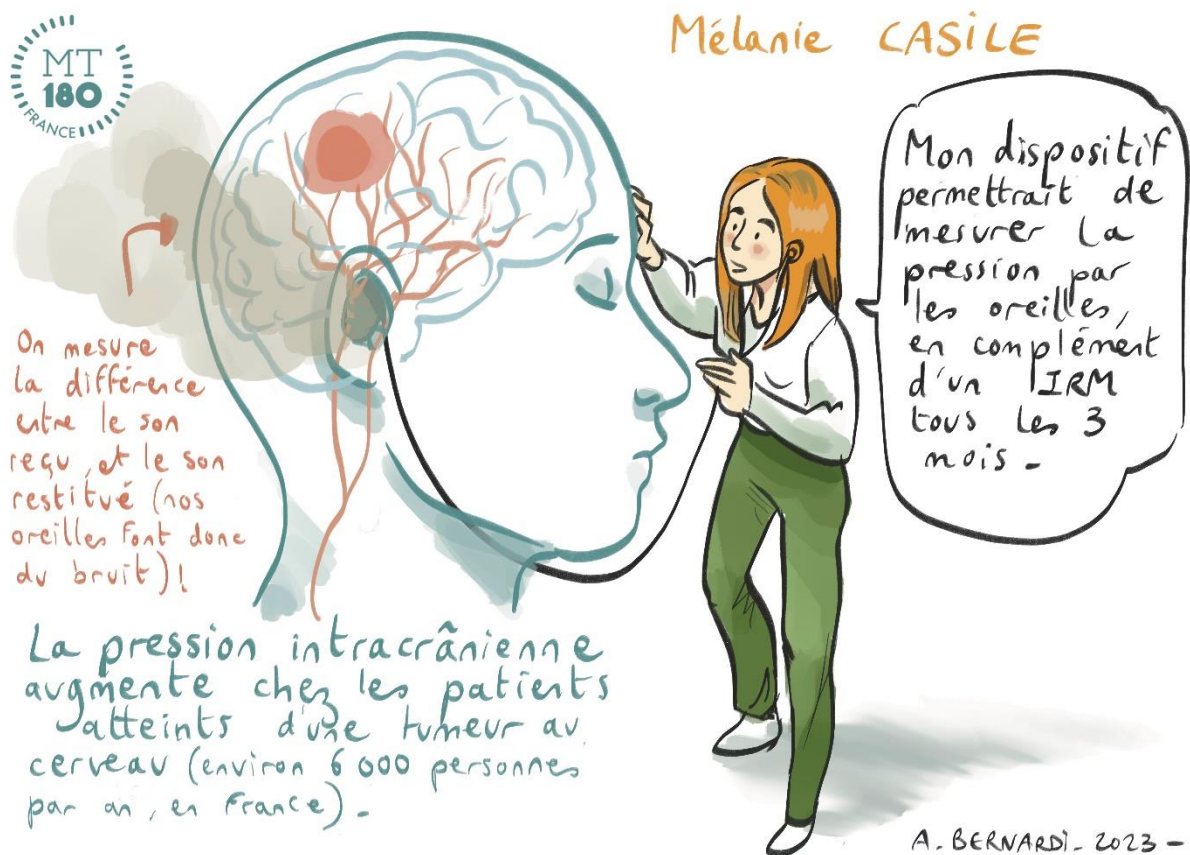
Les résultats de mon étude mettent en avant que la majorité ont eu une augmentation de la pression intracrânienne durant leur suivi. Ceci pourrait s'expliquer par le développement du cancer qui va prendre de plus en plus de place dans un espace clos et inextensible qui est notre crane. De plus, j'ai déterminé que cette augmentation de pression pourrait être assimilée à une progression de la maladie.

En routine, une imagerie est réalisée tous les 3 mois afin de déterminer si le cancer progresse ou reste stable. La survie de ces patients est d'environ 1 an, donc attendre 3 mois peut être trop long.

Mais ce dispositif médical, qui n'est pas sorcier à utiliser, pourrait devenir complémentaire à ces imageries afin d'alerter en cas d'une suspicion de progression. Ce qui permettrait de modifier les traitements le plus rapidement possible et ainsi améliorer la qualité de vie de ces patients. Ce qui est primordial pour ce type de cancer.

Le cancer du cerveau touche environ 6000 personnes par an en France mais malheureusement peu d'avancées ont été réalisées depuis 20 ans. Les résultats de cette étude sont donc pleins d'espoirs qui ne sont pas magiques mais bien scientifiques ! »

De plus, dans le cadre cet événement, une facilitatrice graphique, Anne Bernardi, a réalisé une synthèse visuelle de ma présentation en une planche de bande-dessinée. Cette technique est également appelée « scribing ». Le résultat est le suivant :



IV. Annexe 4 : Poster présenté au congrès du Cancéropôle Lyon Auvergne Rhône-Alpes 2023

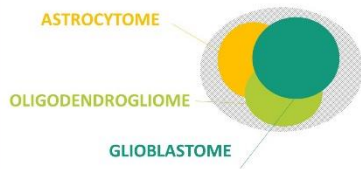
Etude GMaPIC Mesure non invasive de la pression intracrânienne pour les patients atteints de gliomes de haut grade traités par radiothérapie et/ou chimiothérapie

Mélanie CASILE^{1,2,3}, Emilie THIVAT^{1,2,3}, Fabrice GIRAUDET⁴, Angeline GINZAC^{1,2,3}, Ioana MOLNAR^{1,2,3}, Julian BIAU⁵, Blandine LOURENCO⁴, Paul AVAN⁴, Xavier DURANDO^{1,2,3,6}

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LES GLIOMES DIFFUS DE L'ADULTE DE HAUT GRADE

Les tumeurs cérébrales primitives les plus fréquentes chez l'adulte se développent aux dépens des cellules gliales permettant le soutien fonctionnel et structural du système nerveux.



SURVIE MEDIANE
(Glioblastome)
14 à 16 MOIS^{1,2}

SURVIE SANS PROGRESSION
(Glioblastome)
< 7 MOIS^{1,2}

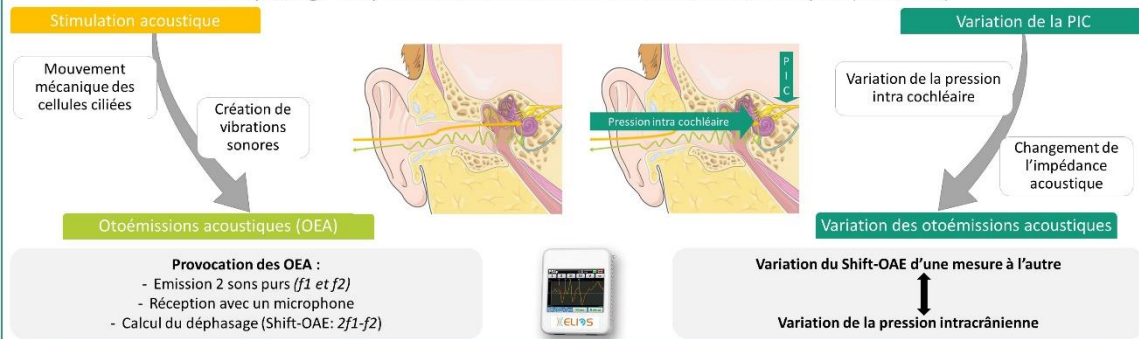
SURVEILLANCE tous les 2 à 4 mois :
IRM basée sur les critères RANO
« *response assesment in neuro-oncology* »



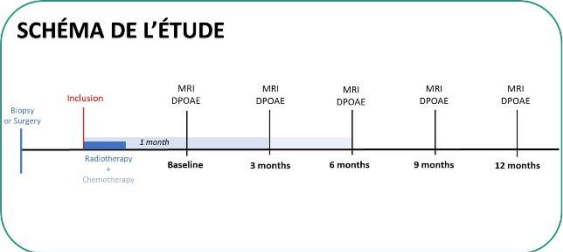
La présence d'une tumeur cérébrale, son évolution ou l'apparition d'un œdème peut entraîner des variations de la pression intracrânienne (PIC) pouvant être à l'origine d'une hypertension intracrânienne (>20 mmHg).

ÉVALUATION NON INVASIVE DE LA PIC :

Le déphasage des produits de distorsion des otoémissions acoustiques (Shift-OAE)



Objectif de l'étude : Comparer la variation de la pression intracrânienne, détectée par une méthode non invasive basée sur la surveillance du shift-OAE, avec les résultats des IRM, chez des patients atteints de gliome.



RESULTATS DE L'ÉTUDE

Le changement de pression intracrânienne par rapport à la baseline au cours du suivi des patients est significativement associé à une augmentation du volume T2/Flair ($p < 0,001$)

L'essai GMaPIC a démontré une association entre les variations de la pression intracrânienne et une augmentation du volume T2/Flair.

Ce dispositif pourrait devenir un outil de surveillance de la pression intracrânienne en complément de l'imagerie. Il permettrait de prévenir une progression tumorale dans le but d'adapter les traitements le plus rapidement possible afin d'améliorer la qualité de vie de ces patients.

V. Annexe 5 : Prix communication Oral – JED 2023

26^{ème} édition des Journées de l'Ecole Doctorale
Sciences de la Vie, Santé, Agronomie et Environnement



Prix

CENTRE JEAN-PERRIN

CASILE Makani

ayant présenté ses travaux de recherche lors des 26^{èmes} journées de l'Ecole Doctorale,

a reçu le **prix Centre Jean-Perrin** d'une valeur de **250 €**

Clermont-Ferrand, 5 au 6 Juin 2023

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