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Option : Epidémiologie / *Epidemiology*

Par / *By* Edouard LHOMME

**ANALYSE DES DETERMINANTS ET MODELISATION DE LA REPONSE IMMUNITAIRE
POST-VACCINATION DANS LES STRATEGIES VACCINALES EXPERIMENTALES**

***ANALYSIS OF THE DETERMINANTS AND MODELISATION OF THE POST-
VACCINATION IMMUNE RESPONSE IN EXPERIMENTAL VACCINE STRATEGIES***

Sous la direction de / *Under the supervision of:* Dr Laura RICHERT

Soutenue le 25 novembre 2019 / *Defended on 25 November 2019*

Membres du Jury / *Members of the PhD committee*

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Résumé

Les essais cliniques de vaccins posent des défis méthodologiques particuliers, principalement liés à la spécificité du développement des vaccins, les schémas des essais cliniques, de l'absence d'un corrélat de protection validé et de la complexité des techniques immunologiques évaluant l'immunogénicité des candidats vaccins. Celles-ci nécessitent des recherches méthodologiques pour définir les méthodes les plus appropriées. Cette thèse porte sur la recherche méthodologique visant à optimiser les méthodes utilisées dans le développement clinique des vaccins, notamment pour proposer et développer des méthodes statistiques de modélisation de l'immunogénicité, en prenant comme exemple les essais cliniques des vaccins contre le VIH et le virus Ebola.

Nous avons d'abord étudié la dynamique des réponses immunitaires après la vaccination et montré qu'il faudrait tenir compte des temps de mesure précoce dans les futurs essais cliniques pour mieux comprendre le rôle des lymphocytes T CD4 auxiliaires et évaluer leur rôle prédictif dans la réponse immunitaire aux vaccins. Ensuite, nous avons développé une nouvelle approche de modélisation bivariée pour l'analyse de la réponse cellulaire mesurée par la technique de cytométrie en flux de marquage des cytokines intracellulaires (ICS). Cette nouvelle méthode a montré de très bonnes performances statistiques et devrait devenir la nouvelle méthode statistique standard pour les analyses ICS dans les essais vaccinaux. Ce travail aura un impact direct sur l'évaluation de la réponse du ICS dans les essais cliniques de vaccins.

En ce qui concerne la réponse humorale, nous avons montré qu'il subsiste des incertitudes importantes sur les déterminants de la réponse anticorps après une vaccination préventive contre le virus Ebola. Cela met l'accent sur l'intérêt d'harmoniser les méthodes de mesure et les schémas d'étude. De plus, il indique le besoin de mettre en place des essais cliniques randomisés à bras multiples sur le vaccin contre le virus Ebola pour une comparaison précise de l'immunogénicité entre les différentes stratégies vaccinales.

Nous avons présenté enfin la méthodologie d'un essai randomisé de phase 2 contre le virus Ebola évaluant trois stratégies vaccinales dans quatre pays d'Afrique de l'Ouest, et plus particulièrement pour finir une réflexion méthodologique et éthique sur la question de l'inclusion des personnels de l'essai dans un essai clinique vaccinale contre le virus Ebola en période non-épidémique.

Les méthodes développées dans le cadre de cette thèse contribueront à améliorer la conception et l'analyse des futurs essais vaccinaux, et pourraient également être transposées plus largement à d'autres domaines de recherche.

Mots clés : vaccin ; immunogénicité ; essai clinique ; modélisation ; VIH ; Ebola

Summary

Specific methodological challenges exist in vaccine clinical trials, due principally to specificities of vaccine development, clinical trial design, absence of validate correlate of protection, and complexities of new immunological assays for evaluating immunogenicity of vaccine candidates. These require methodological research to define the most appropriate methods. This thesis focuses on methodological research to optimize methods used in the clinical development of vaccines, especially to propose and develop statistical methods to model immunogenicity, using HIV and Ebola vaccine clinical trials as an example.

We first investigated the dynamics of the immune responses post-vaccination and showed that early sampling time points should be considered in future clinical trials to better understand the role of the early CD4 helper T cells and to evaluate their predictive role in the immune response to vaccines. Then, we developed a new bivariate modelling approach for the analysis of the cellular immune response (assessed by intracellular cytokine staining, ICS) that showed good statistical performances and should become the new statistical standard method for ICS analyses in vaccine trials. This work will have a direct impact on the assessment on the ICS response in vaccine clinical trials.

Regarding the humoral response, we showed that there are still significant uncertainties in the determinants of the antibody response after preventive vaccination against Ebola virus disease. This emphasizes the interest of harmonizing measurement methods and study designs. Furthermore, it indicates the need of randomized multi arm Ebola vaccine trials for accurate comparison of immunogenicity between different vaccine strategies.

Finally, we presented the methodology of an international randomized phase 2 trial against Ebola, and in particular a methodological and ethical reflection related to the enrollment of study personnel in Ebola vaccine trial in a non-epidemic context.

Methods developed in this thesis will contribute to improve the design and analysis of future vaccine trials, and also could be transposable more widely to other research domains.

Key words: statistical modeling; immunogenicity; vaccine; clinical trials; HIV; Ebola

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Résumé substantiel

Les essais cliniques représentent une étape clé pour le développement d'une nouvelle intervention biomédicale, y compris le développement de nouveaux vaccins. Des défis méthodologiques spécifiques existent dans le développement clinique vaccinal, en raison de la spécificité du développement des vaccins, de la conception des essais cliniques et de la complexité des nouvelles techniques immunologiques pour évaluer l'immunogénicité des vaccins candidats, c'est-à-dire leur capacité à générer des réponses immunitaires chez les participants. Cela nécessite une recherche méthodologique pour définir les méthodes les plus appropriées. Cette thèse porte sur la recherche méthodologique à l'interface de l'épidémiologie clinique et de la biostatistique pour optimiser les méthodes utilisées dans le développement clinique vaccinal, en prenant pour exemple les essais cliniques des vaccins contre le virus de l'immunodéficience humaine (VIH) et contre la maladie à virus Ebola (MVE).

Chapitre 1 : Introduction

Optimiser le développement de nouveaux vaccins

La mise au point de nouveaux vaccins sûrs et efficaces est confrontée à divers challenges tout au long du processus de recherche et de développement. Le développement clinique commence lorsque le vaccin est testé pour la première fois chez l'homme et suit les phases classiques de l'évaluation clinique des médicaments. Cependant il est caractérisé par des questions méthodologiques propres à l'évaluation des vaccins. Pour des raisons de faisabilité et d'éthique, les premières évaluations cliniques humaines d'un vaccin candidat en phases I et II sont basées sur l'étude de l'immunogénicité du vaccin, c'est-à-dire de sa capacité à générer des réponses immunitaires chez les participants. Dans cette thèse, nous nous concentrerons sur les défis méthodologiques de l'évaluation de l'immunogénicité des vaccins dans les essais cliniques de vaccins pour optimiser le développement vaccinal.

Les exemples du développement vaccinal du VIH et celui de la MVE sont utilisés pour illustrer et appliquer ce travail de recherche. Pour ces deux maladies, il existe un besoin urgent de développer des vaccins sûrs et efficaces, et la recherche vaccinale est très active. Le développement de vaccins contre ces deux maladies se heurte notamment à des problèmes méthodologiques communs liés au fait qu'il n'existe à ce jour aucun corrélant immunologique de protection validé qui puisse être utilisé comme marqueur de substitution dans les premières phases des essais cliniques. En l'absence d'un corrélant de protection validé, l'évaluation des vaccins candidats à partir des données d'immunogénicité reste difficile.

Deux exemples : le développement vaccinal du VIH et de la MVE

HIV

Plusieurs essais cliniques prophylactiques et thérapeutiques contre le VIH ont été menés au cours des trente dernières années pour évaluer plusieurs stratégies combinant différents vaccins candidats incluant différentes plateformes vaccinales. Les résultats les plus encourageants sont ceux de l'étude RV144 publiée en 2009, qui a été la première à démontrer l'efficacité partielle - une réduction de 31,2% du risque d'acquisition d'infection à VIH - d'une stratégie de vaccination prophylactique basée sur un critère clinique (acquisition d'infection) [1]. Bien qu'il s'agisse d'un grand pas en avant, ce résultat ne suffit pas pour assurer une protection contre le virus et la recherche de vaccins est toujours en cours.

Le développement d'un vaccin contre le virus de l'immunodéficience humaine (VIH) est confronté à de nombreux défis. En ce qui concerne les défis méthodologiques, un grand nombre de vaccins candidats sont actuellement en cours de développement. De ce fait une conception efficiente des essais cliniques et une évaluation précise des vaccins candidats dans les essais cliniques - surtout dans les premières phases - sont nécessaires pour amener rapidement les meilleurs candidats dans les essais de phase III. Malgré les résultats encourageants de l'essai RV144 a suggéré des anticorps liants contre les boucles variables 1 et 2 de l'enveloppe du VIH et deux sous-ensembles de cellules T CD4+ polyfonctionnelles comme corrélats de protection, ceci reste à démontrer [2-4]. Il n'existe toujours pas de marqueur prédictif confirmé de la protection contre le VIH qui puisse être un marqueur de substitution valide de l'efficacité clinique communément acceptée. L'immunogénicité d'un vaccin contre le VIH peut être représentée par de multiples marqueurs immunologiques et les experts croient maintenant qu'une stratégie prophylactique de vaccination anti-VIH doit générer les deux types de réponse : une réponse humorale - médiée par les cellules B - et une réponse cellulaire des cellules T pour être efficace [5-8].

Maladie à virus Ebola

La recherche clinique vaccinale contre la MVE est très active, notamment relancée pendant l'épidémie majeure ayant touché l'Afrique de l'Ouest en 2014-2016. A ce jour, les options de traitement ne sont que symptomatiques, il n'existe pas de traitement antiviral efficace approuvé. Treize vaccins candidats contre la MVE ont fait l'objet ou font actuellement l'objet d'une évaluation clinique de phase I-III. Les stratégies les plus prometteuses en cours d'évaluation sont le vaccin candidat rVSV-ZEBOV-GP du laboratoire Merk et un vaccin candidat basé sur les composants à vecteur Ad26 (primo-vaccination) et MVA (revaccination) (Ad26.ZEBOV/MVA-BN-Filo) développé par le laboratoire Johnson & Johnson. En outre, deux

vaccins candidats, GamEvac-Combi et Ad5-EBOV sont homologués en Russie et en Chine, respectivement, leurs pays d'origine, sur la base de données d'immunogénicité. La seule étude qui a pu fournir des données sur l'efficacité clinique est l'essai de vaccination *Ebola Ça Suffit* réalisé en Guinée à la fin du foyer 2014-2016 [11]. Cet essai ouvert en anneau randomisé en grappe mené en Guinée a évalué l'efficacité du vaccin rVSV-ZEBOV-GP chez les contacts des cas avec une efficacité clinique estimée à 100 % (IC à 95 % 79,3-100,0, $p=0.0033$). Aucun échantillon de sang n'ayant été prélevé pour mesurer l'immunogénicité, il n'a donc pas été possible de trouver un corrélat protecteur dans cet essai.

À ce jour, il n'existe aucun marqueur de substitution validé pour évaluer l'efficacité des vaccins contre la MVE chez l'humain ; cependant, une étude antérieure menée chez l'animal testant des vaccins recombinants à vecteur VSV ou des vaccins à ADN a révélé une forte corrélation entre le taux d'anticorps (immunoglobuline G contre EBOV-glycoprotéine) observé après vaccination et la survie après infection au virus Ebola [10-15]. Pour cette raison, les essais de phase initiale des essais vaccinaux contre la MVE reposent actuellement principalement sur la quantification de la réponse anticorps par la méthode ELISA après vaccination pour évaluer l'immunogénicité [16], mais d'autres mesures d'immunogénicité sont également effectuées comme analyses secondaires ou exploratoires.

Évaluation de l'immunogénicité des vaccins candidats

Évaluation de l'immunogénicité

L'immunogénicité d'un vaccin peut être représentée par de multiples marqueurs immunologiques humoraux ou cellulaires. Lorsqu'il n'existe aucun marqueur immunologique validé permettant de prédire l'efficacité clinique du vaccin et qui pourrait donc être utilisé comme marqueur de substitution dans le développement clinique, un large éventail de marqueurs immunologiques est mesuré après la vaccination chez les participants aux essais vaccinaux pour évaluer les différents aspects de la réponse immunologique. Les marqueurs mesurés peuvent inclure les niveaux de différents anticorps, les concentrations de molécules sécrétées par ces cellules immunitaires (cytokines), la fréquence de certaines de sous-populations cellulaires, ainsi que le niveau d'expression de dizaines de milliers de gènes dans les cellules. Ainsi, l'évaluation de l'immunogénicité d'un vaccin candidat reste complexe à évaluer et soulève plusieurs questions méthodologiques qui seront détaillées dans les paragraphes suivants.

Considérations méthodologiques

Premièrement, l'analyse des marqueurs d'immunogénicité est compliquée par le fait que des aspects spécifiques doivent être pris en compte pour chaque technique immunologique de dosage et que des critères de positivité validés font défaut ou ont une pertinence biologique inconnue pour de nombreux marqueurs. Par exemple, les réponses des lymphocytes T aux vaccins candidats sont couramment évaluées à d'une technique de cytométrie de flux utilisant la coloration intracellulaire des cytokines (ICS) et l' EliSpot, avec différentes mesures comprenant une réponse de fond (témoin) et des réponses spécifiques avec une ou différentes conditions de stimulation. Les approches statistiques conventionnelles utilisées jusqu'à présent ne tiennent généralement pas compte (ou mal) de toutes les informations et peuvent avoir des performances statistiques sous-optimales en terme d'erreur de type 1 ou de puissance, et parfois même induire des biais. L'évaluation critique des approches d'analyse statistique existantes et, si nécessaire, le développement de nouvelles approches sont importants pour une meilleure mesure de l'effet du vaccin dans les essais cliniques de phase I et II.

Deuxièmement, les temps de mesure des marqueurs immunologiques après les injections de vaccins dans les essais cliniques sont définis de manière empirique et hétérogène et relativement tardive dans la plupart des essais vaccinaux menés à ce jour. Habituellement, tous les différents marqueurs sont mesurés en même temps et le temps de mesure est fixé empiriquement entre deux et quatre semaines après la dernière injection de vaccin, le nombre d'injections variant souvent entre deux et six sur trois à six mois. Ainsi, la dynamique des marqueurs immunologiques pendant la vaccination reste mal connue et les temps de mesure utilisés peuvent ne pas permettre de mesurer la réponse immunitaire. De plus, certains marqueurs peuvent avoir une réponse précoce, quelques jours après la vaccination, tandis que d'autres ont une réponse plus tardive. La mise en œuvre de mesures répétées (par exemple hebdomadaires ou bihebdomadaires) dans le cadre du suivi d'un essai clinique sur un vaccin permettrait d'obtenir une image de la réponse immunitaire, mais cela n'est pas possible pour des raisons de coût et d'acceptabilité pour les participants. Certains marqueurs immunologiques initialement mesurés pourraient prédire la réponse subséquente mesurée à partir d'autres marqueurs. Comprendre et évaluer la dynamique de l'immunogénicité des vaccins dans les essais cliniques humains est un défi majeur pour améliorer la conception et l'évaluation des vaccins dans les essais cliniques futurs.

Enfin, les facteurs influençant la variabilité de la réponse immunitaire après la vaccination restent mal compris. En plus du type de vaccin, certains déterminants liés aux caractéristiques du vaccin, comme la plateforme vaccinale (vecteur d'administration), la posologie, l'insert viral, le calendrier vaccinal ou la population étudiée, pourraient influencer la

mesure de la réponse immunitaire au vaccin dans les essais cliniques. Ces nombreux facteurs pourraient avoir une incidence sur le niveau de réponse immunitaire mesuré dans un essai clinique et pourraient expliquer les différences de résultats entre les études. Il serait donc intéressant de savoir quels facteurs expliquent la variabilité observée de la réponse immunitaire et, le cas échéant, d'obtenir une estimation quantitative de leur influence pour une meilleure reproductibilité et une standardisation dans les essais cliniques futurs.

Une meilleure compréhension de la dynamique des réponses immunitaires aux vaccins, une meilleure évaluation de cette réponse et de ses déterminants dans les essais cliniques chez l'humain est un défi majeur pour améliorer la conception et l'évaluation des vaccins dans les essais cliniques futurs.

Chapitre 2 : Objectifs

L'objectif général de la thèse était de proposer et de développer des méthodes statistiques pour modéliser les données d'immunogénicité des essais cliniques vaccinaux.

Cela comprenait la modélisation tenant compte des spécificités de la technique de mesure, la modélisation de la réponse vaccinale au cours du temps et la modélisation pour évaluer les déterminants de la réponse vaccinale, en prenant l'exemple de stratégies vaccinales expérimentales avec des vaccins candidats, appliquées au VIH et à la MVE.

Chapitre 3 : Modélisation de la réponse immunitaire cellulaire

Modélisation pour l'analyse de la dynamique des réponses immunitaires

Les temps de mesure optimaux pour les marqueurs immunitaires afin d'évaluer au mieux l'immunogénicité cellulaire des stratégies vaccinales dans les essais cliniques ne sont pas clairement connus et les temps de mesure dans les protocoles d'essais vaccinaux sont le plus souvent définis de façon empirique. Certains marqueurs initialement mesurés peuvent prédire la réponse mesurée à partir d'autres marqueurs. L'identification des marqueurs précoces d'une bonne réponse vaccinale pourrait être utile pour identifier rapidement les stratégies vaccinales prometteuses ou éliminer les stratégies inefficaces, ainsi que pour personnaliser les stratégies de vaccination. Par exemple, la dynamique des lymphocytes T CD8+ produisant l'IFN- γ peut être prévisible à partir de la dynamique des lymphocytes T CD4+ produisant l'IL-2 puisque ceux-ci stimulent la maturation des lymphocytes T CD8+. L'objectif principal de ce travail était d'évaluer la dynamique et les corrélations entre les différents

marqueurs immunitaires cellulaires (lymphocytes T CD4+ produisant IL-2 et lymphocytes T CD8+ produisant IFN-g) pendant la vaccination préventive contre le VIH, comme dans une stratégie de vaccination utilisant un des vaccins candidats (adénovirus recombinant type 5).

Pour cela, nous avons analysé les données de l'essai clinique HVTN 068 qui est l'un des très rares essais cliniques de vaccin prophylactique contre le VIH avec des mesures précoces et répétées de l'immunogénicité chez tous les participants. L'objectif principal de cet essai clinique de phase I / II randomisé, multicentrique, contrôlé et randomisé aux Etats-Unis était d'évaluer la tolérance et l'immunogénicité de deux stratégies de primo-revaccination contre le VIH (rAd5-rAd5 vaccine versus DNA-rAd5). Les principaux résultats, publiés en 2011 [17], ne comportaient pas d'analyse détaillée de la dynamique de la réponse immunologique. Par conséquent, une analyse secondaire des données de cet essai a représenté une excellente occasion d'étudier la dynamique de la réponse immunitaire. Nous avons calculé les corrélations de Spearman entre les marqueurs d'immunogénicité aux différents points de mesure dans le temps. La réponse des lymphocytes T CD8+ IFN-g+ dans le groupe rAd5-rAd5 a été modélisée en fonction de la réponse des lymphocytes T CD4+ IL-2+ et du temps en utilisant des modèles de régression à effets mixtes.

Des corrélations modérées à élevées ($r = 0,48-0,76$) ont été observées dans le groupe rAd5-rAd5 entre la réponse des lymphocytes T CD4+ IL-2+ à la semaine 2 et les réponses des lymphocytes T CD8+ IFN-g+ (semaines 2 à 52). Les modèles de régression ont confirmé cette relation avec une association significative entre les deux marqueurs : pour une augmentation de 1,0 % des lymphocytes T CD4+ IL-2+ CD4+ à la semaine 2 post-prime, une augmentation de 0,3 % des réponses CD8+ IFN-g+ T aux points temporels ultérieurs, incluant ceux post-revaccination, a été observée ($p < 0,01$).

Ces résultats soulignent que l'évaluation de la dynamique des marqueurs de réponse immunitaire est non linéaire et nécessite donc l'utilisation de modèles complexes. Cela à nécessiter en particulier une réflexion approfondie pour permettre d'intégrer le temps dans le modèle. Ces résultats suggèrent au niveau immunologique un rôle précoce et prépondérant des lymphocytes T CD4+ dans la réponse cellulaire au vaccin rAd5-rAd5 et en particulier la stimulation des réponses des lymphocytes T CD8+ cytotoxiques. Au niveau méthodologique, des points de temps de mesure précoces devraient être pris en compte dans les essais cliniques futurs afin de mieux comprendre le rôle des lymphocytes T auxiliaires CD4 précoces et d'évaluer leur rôle prédictif dans la réponse immunitaire aux vaccins.

Modélisation bivariée pour l'analyse de la réponse des lymphocytes T

Les réponses des lymphocytes T aux vaccins candidats sont généralement évaluées par coloration intracellulaire de cytokines (ICS) au moyen d'une technique de cytométrie en flux multiparamétrique. Une approche statistique conventionnelle d'analyse des données ICS consiste à comparer, entre les stratégies vaccinales à un temps donné ou entre les données à J0 et post-vaccination du même schéma selon le schéma de l'essai, les pourcentages de cellules produisant une cytokine d'intérêt après stimulation ex vivo des cellules mononucléaires du sang périphérique (PBMC) par des antigènes vaccinaux, après soustraction de la réponse non spécifique (de cellules non stimulées) de chaque échantillon. La soustraction de la réponse non spécifique vise à saisir la réponse spécifique à l'antigène, mais soulève des questions méthodologiques liées à l'erreur de mesure et à la puissance statistique. L'objectif était de développer une nouvelle approche statistique pour l'analyse des données ICS issues d'essais vaccinaux.

Nous avons développé un modèle de régression linéaire bivariée modélisant les réponses ICS non spécifiques et spécifiques des antigènes. Un modèle a été développé pour s'adapter à la comparaison inter-bras entre deux stratégies vaccinales à un temps donné et un autre pour l'évaluation intra-bras d'une stratégie vaccinale. Le modèle a été implémenté sous les logiciels statistiques SAS et R. Nous avons comparé la performance du modèle en termes de biais et de contrôle des erreurs de type I et II par rapport aux approches conventionnelles, et nous l'avons appliqué à des données simulées ainsi qu'à des données réelles avant et après la vaccination provenant de deux essais vaccinaux récents (ANRS VRI01 mené chez des volontaires sains et VRI02 ANRS 149 LIGHT thérapeutique chez des participants séropositifs). Comme nous avons souhaité mettre notre méthode à la disposition d'un plus grand nombre d'utilisateurs, par exemple des immunologistes qui ne sont pas formés à l'utilisation de logiciels statistiques, nous avons également développé une interface en ligne appelée "VICI" qui permet de télécharger directement des données et de les analyser facilement avec le modèle bivarié.

Les simulations ont démontré que le modèle bivarié était aussi bon que la meilleure des approches conventionnelles (avec ou sans soustraction de la réponse non spécifique) dans tous les scénarios de simulation en termes de performance statistique, alors que les approches conventionnelles n'ont pas fourni de résultats solides dans tous les scénarios. Le modèle a permis de plus d'obtenir de plus des résultats plus détaillés, avec en plus de l'estimation d'intérêt de l'effet du vaccin sur la réponse stimulée (paramètre d'intérêt principal),

l'estimation de l'effet du vaccin sur la réponse non-stimulée et l'estimation de l'effet de la réponse stimulée sur la réponse non-stimulée.

Cette nouvelle méthode d'analyse de l'immunogénicité cellulaire, basée sur la modélisation bivariée, conduit à des résultats plus détaillés et permet une meilleure interprétation de l'immunogénicité vaccinale dans les essais cliniques. L'utilisation d'approches conventionnelles, en particulier la comparaison de la réponse des lymphocytes T après soustraction du bruit de fond, ne devrait plus être recommandée pour l'analyse des données issue des ICS dans les essais vaccinaux. Le modèle bivarié pourrait être utilisé pour analyser tout type de réponse fonctionnelle dans laquelle une réponse non stimulée est mesurée.

Chapitre 4 : Modélisation de la réponse immunitaire humorale

Dans ce chapitre, nous nous sommes concentrés sur la réponse anticorps. Dans les essais cliniques sur le vaccin contre la MVE, l'immunogénicité est également mesurée principalement par la réponse anticorps (immunoglobuline G contre la glycoprotéine EBOV) en raison du résultat antérieur pour le corrélât de protection chez l'animal. Cependant, les facteurs qui influencent cette réponse anticorps sont encore très peu connus malgré que ceux-ci peuvent avoir un impact sur le développement du vaccin. La connaissance de ces facteurs pourrait permettre une meilleure interprétation des résultats des essais cliniques et d'améliorer la conception des futurs essais cliniques en normalisant la prise en compte de ces facteurs. L'objectif principal de cette étude était d'identifier les facteurs associés à la variabilité de la réponse des anticorps dans les études publiées sur les essais vaccinaux contre la MVE. L'objectif secondaire était de quantifier la proportion de variabilité de la réponse anticorps qui s'explique par ces facteurs.

Nous avons réalisé une revue systématique de la littérature à l'aide de PubMed et Scopus pour rechercher les études sur le vaccin préventif contre la MVE menées chez les humains ou les primates non humains (PNH) et publiées jusqu'en février 2018. Pour chaque groupe vaccinal avec une mesure du titre d'anticorps après la vaccination, les données sur la réponse des anticorps et ses potentiels déterminants ont été extraits. Une méta-régression à effets aléatoires a été ensuite menée sur les groupes humains avec au moins 8 individus.

Au total, nous avons examiné 49 études (202 groupes de vaccination, dont 74 groupes humains) avec diverses plateformes vaccinales et inserts antigéniques. Le titre moyen d'anticorps était légèrement plus élevé dans le cas des PNH (310, IC 95 % [293 - 327]) que chez les humains (275, IC 95% [257 - 293]). La plateforme vaccinale ($p < 0,001$) et la souche

virale utilisée pour la détection des anticorps ($p < 0,001$) ont été associées à la réponse des anticorps chez l'humain, mais l'hétérogénéité ajustée restait à 95 %.

Diverses plateformes ont été évaluées chez l'homme, dont Ad26, Ad5, ChimpAd3, ADN, MVA et VSV. En plus des plateformes, la souche virale utilisée pour la détection des anticorps influençait la réponse anticorps. Toutefois, la variabilité est demeurée en grande partie inexpliquée. Par conséquent, la comparaison du vaccin l'immunogénicité nécessite des essais contrôlés randomisés.

Chapitre 5 : Essai clinique PREVAC

Ce chapitre souligne mon implication en tant que méthodologiste sur la plateforme de recherche clinique internationale EUCLID/F-CRIN à Bordeaux sur l'essai clinique PREVAC (*Partnership for Research on Ebola VACCination*), un essai vaccinal de phase II prophylactique randomisé en cours qui évalue l'immunogénicité et la tolérance de trois stratégies vaccinales différentes contre la MVE dans quatre pays d'Afrique de l'Ouest (NCT02876328). J'ai notamment participé à la rédaction des différentes versions du protocole et aux réflexions méthodologiques qui ont été soulevées au fil de l'eau lors de l'implémentation ou du suivi de participants, été impliqué en tant que membre du groupe de travail du laboratoire et du groupe spécifique mis en place pour examiner régulièrement l'analyse de la réponse anticorps mesurée par la technique FANG ELISA (critère de jugement principal de l'essai). J'ai eu l'occasion de participer également à la formation du personnel du site sur les principes de base des essais cliniques vaccinaux avant les premières inclusions.

Deux travaux relatifs à cela sont décrits dans ce chapitre. Le premier est la publication sur le protocole de l'essai PREVAC que j'ai été chargée de rédiger au nom du groupe de l'étude PREVAC en binôme avec Moses Badio. Le deuxième travail porte une question liée au recrutement du personnel de l'étude dans un essai vaccinal contre la MVE dans un contexte non épidémique. Cette question s'est posée lors de la mise en place de PREVAC en Guinée et a fait l'objet d'une réflexion méthodologique et éthique publiée dans *Trials*.

Chapitre 6 : Discussion et conclusion

Cette thèse portait sur les aspects méthodologiques de la recherche clinique complexe, illustrée par la recherche sur le VIH et le vaccin Ebola.

Au total, cette thèse d'Université a plusieurs retombées potentielles :

1. Nous avons montré qu'il faudrait tenir compte des points de temps de mesure précoces dans les essais cliniques futurs afin de mieux comprendre le rôle des lymphocytes T auxiliaires CD4 précoces et d'évaluer leur rôle prédictif dans la réponse immunitaire à un vaccin.
2. La nouvelle approche de modélisation bivariée pour l'analyse de la réponse ICS a montré de très bonnes performances statistiques et devrait être la nouvelle méthode statistique standard pour les analyses ICS dans les essais cliniques vaccinaux.
3. Nous avons montré qu'il existe encore des incertitudes importantes quant aux déterminants de la réponse anticorps post-vaccination préventive contre la MVE. Cela met l'accent sur l'intérêt d'harmoniser les méthodes de mesure de la réponse anticorps et les schémas d'étude. De plus, il indique le besoin d'essais cliniques randomisés à bras multiples sur le vaccin contre la MVE pour une comparaison précise de l'immunogénicité entre les différentes stratégies vaccinales.

Références

1. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, et al. Vaccination with ALVAC and AIDSVAX to Prevent HIV-1 Infection in Thailand. *N Engl J Med.* 2009;361(23):2209-20.
2. Haynes BF, Gilbert PB, McElrath MJ, Zolla-Pazner S, Tomaras GD, Alam SM, et al. Immune-Correlates Analysis of an HIV-1 Vaccine Efficacy Trial. *N Engl J Med.* 2012;366(14):1275-86.
3. Lin L, Finak G, Ushey K, Seshadri C, Hawn TR, Frahm N, et al. COMPASS identifies T-cell subsets correlated with clinical outcomes. *Nat Biotechnol.* 2015;33(6):610-6.
4. Kim JH, Excler JL, Michael NL. Lessons from the RV144 Thai phase III HIV-1 vaccine trial and the search for correlates of protection. *Annu Rev Med.* 2015;66:423-37.
5. Watkins DI, Burton DR, Kallas EG, Moore JP, Koff WC. Nonhuman primate models and the failure of the Merck HIV-1 vaccine in humans. *Nat Med* 2008;14(6):617-21.
6. Cox KS, Clair JH, Prokop MT, Sykes KJ, Dubey SA, Shiver JW, et al. DNA gag/Adenovirus Type 5 (Ad5) gag and Ad5 gag/Ad5 gag Vaccines Induce Distinct T-Cell Response Profiles. *J Virol.* 2008;82(16):8161-71.
7. Ramshaw IA, Ramsay AJ. The prime-boost strategy: exciting prospects for improved vaccination. *Immunol Today.* 2000;21(4):163-65.
8. Woodland DL. Jump-starting the immune system: prime-boosting comes of age. *Trends Immunol.* 2004;25(2):98-104.
9. Henao-Restrepo AM, Camacho A, Longini IM, Watson CH, Edmunds WJ, Egger M, et al. Efficacy and effectiveness of an rVSV-vectored vaccine in preventing Ebola virus disease: final results from the Guinea ring vaccination, open-label, cluster-randomised trial (Ebola Ça Suffit!). *Lancet.* 2017;389(10068):505-518.
10. Pushko P, Bray M, Ludwig GV, Parker M, Schmaljohn A, Sanchez A, et al. Recombinant RNA replicons derived from attenuated Venezuelan equine encephalitis virus protect guinea pigs and mice from Ebola hemorrhagic fever virus. *Vaccine.* 2000;19(1):142-53.
11. Wilson JA, Hart MK. Protection from Ebola virus mediated by cytotoxic T lymphocytes specific for the viral nucleoprotein. *J Virol.* 2001;75(6):2660-4.
12. Wong G, Richardson JS, Pillet S, Patel A, Qiu X, Alimonti J, et al. Immune Parameters Correlate with Protection Against Ebola Virus Infection in Rodents and Nonhuman Primates. *Sci Transl Med;* 2012;4(158):158ra146.
13. Xu L, Sanchez A, Yang Z, Zaki SR, Nabel EG, Nichol ST, et al. Immunization for Ebola virus infection. *Nat Med;* 1998;4(1):37-42.
14. Saphire EO, Schendel SL, Gunn BM, Milligan JC, Alter G. Antibody-mediated protection against Ebola virus. *Nat Immunol.* 2018;19(11):1169-78.
15. Meyer M, Malherbe DC, Bukreyev A. Can Ebola Virus Vaccines Have Universal Immune Correlates of protection? *Trends in Microbiology.* 2019;27(1):8-16.
16. Krause PR, Bryant PR, Clark T, Dempsey W, Henchal E, Michael NL, et al. Immunology of protection from Ebola virus infection. *Sci Transl Med.* 2015;7(286):286ps11-286ps11.
17. De Rosa SC, Thomas EP, Bui J, Huang Y, deCamp A, Morgan C, et al. HIV-DNA priming alters T-cell responses to HIV-adenovirus vaccine even when responses to DNA are undetectable. *J Immunol.* 2011;187(6):3391-401.

Scientific production related to the subject of the thesis

Articles

Lhomme E, Richert L, Moodie Z, Pasin C, Kalams SA, Morgan C et al. Early CD4+ T Cell Responses Are Associated with Subsequent CD8+ T Cell Responses to an rAd5-Based Prophylactic Prime-Boost HIV Vaccine Strategy. PLoS One. 2016;11(4):e0152952.

Gross L, Lhomme E, Pasin C, Richert L, Thiebaut R. Ebola vaccine development: Systematic review of pre-clinical and clinical studies, and meta-analysis of determinants of antibody response variability after vaccination. Int J Infect Dis. 2018;74:83-96.

Lhomme E, Modet C, Augier A, Faye S, Dabakuyo-Yonli TS, Levy-Marchal C, et al; PREVAC study team. Enrolling study personnel in Ebola vaccine trials: from guidelines to practice in a non-epidemic context. Trials. 2019;20(1):422.

Lhomme E, Hejblum B, Lacabaratz C, Wiedemann A, Lelièvre JD, Levy Y, Thiébaud R, Richert L. Analyzing cellular immunogenicity in vaccine clinical trials: a new statistical method including non-specific responses for accurate estimation of vaccine effect. J Immunol Methods. 2019:112711[Epub ahead of print].

Communications in conferences with a peer-review committee

Oral communications

International conference

40th Annual Conference of the International Society for Clinical Biostatistics, ISCB40 2019| Leuven, Belgium

Edouard Lhomme, Boris Hejblum, Christine Lacabaratz, Aurélie Wiedemann, Jean-Daniel Lelièvre, Yves Levy, Rodolphe Thiébaud, Laura Richert. A bivariate model for accurate estimation of cellular immunogenicity in vaccine clinical trials.

UseR!, July 9-12 2019 | Toulouse, France

Boris P Hejblum, Edouard Lhomme, Christine Lacabaratz, Aurélie Wiedemann, Jean-Daniel Lelièvre, Yves Levy, Rodolphe Thiébaud, Laura Richert. VICI: a Shiny app for accurate estimation of Vaccine Induced Cellular Immunogenicity with bivariate modeling.

HIV Research For Prevention, HIVR4P | Madrid 2018

Laura Richert, Aurélie Wiedemann, Odile Launay, Frederic Lucht, Isabelle Poizot-Martin, Christine Lacabaratz, Hakim Hocini, Edouard Lhomme, Claire Bauduin, Mathieu Surenaud, Lydia Guillaumat, Boris Hejblum, Solenne Delahaye, Lucile Hardel, Véronique Rieux, Elodie Rouch, Kalevi Reijonen, Yves Lévy, Rodolphe Thiebaut and Jean-Daniel Lelièvre. T-Cell and Transcriptomic Responses to Prime-Boost Strategies of 3 HIV Vaccines (MVA HIV-B; LIPO-5; GTU-MultiHIV B) - ANRS/INSERM VRI01 Trial.

Christine Lacabaratz, Claire Bauduin, Aurélie Wiedemann, Edouard Lhomme, Craig Fenwick, Emile Foucat, Valérie Boilet, Véronique Rieux, Giuseppe Pantaleo, Jean-Daniel Lelièvre, Rodolphe Thiebaut and Yves Lévy. Prime-boost combination of DNA-GTU and Lipopeptide vaccine followed by supervised treatment interruption (STI) in a therapeutic HIV Phase II randomized trial: VRI02 ANRS 149 LIGHT.

3rd Annual Conference on Ebola Vaccines, Therapeutics, Diagnostics and Survivors Care | Conakry, République de Guinée

Edouard Lhomme, Augustin Augier, Sylvain Faye, Claire Levy-Marchal, Geneviève Chêne, Abdoul Habib Beavogui, Laura Richert, and the PREVAC study group. Enrolling study personnel in Ebola vaccine trials: from guidelines to practice in a non epidemic context.

National conference (France)

13rd Conférence Francophone d'Epidemiologie Clinique (EPICLIN), 2019 | Toulouse, France
Edouard Lhomme, Boris Hejblum, Christine Lacabaratz, Aurélie Wiedemann, Jean Daniel Lelièvre, Yves Levy, Rodolphe Thiébaud, Laura Richert. Evaluation de l'immunogénicité cellulaire dans les essais cliniques vaccinaux : un modèle bivarie pour mieux prendre en compte la réponse non spécifique.

Cécilia Champion, Renaud Vatrinet, Marion Bererd Camara, John McCullough, Ken Awuondo, Yeya dit Sadio Sarro, Edouard Lhomme, Laura Richert et le groupe d'étude PREVAC. Gestion en temps réel d'une base de données biologiques centralisée pour un essai clinique vaccinal de phase II multicentrique en Afrique.

11st Conférence Francophone d'Epidemiologie Clinique (EPICLIN) | Saint-Etienne, France
Lise Mandigny, Edouard Lhomme, Chloé Pasin, Laura Richert, Rodolphe Thiebaut. Facteurs associés à la réponse anticorps après vaccination contre le virus Ebola : revue systématique et méta-analyse.

Céline Colin, Matthew Kirchoff, David Vallee, Greg Thompson, Edouard Lhomme, Rodolphe Thiebaut, Jerome F. Pierson, Deborah Watson Jones, Bailah Leigh, Mark Kieh, Abdoul Habib Beavogui, Yazdan Yazdanpanah, Laura Richert, Deborah Wentworth, Geneviève Chêne, James Neaton et l'équipe projet PREVAC. Opérationnalisation de la randomisation d'un essai vaccinal en population générale africaine exemple de l'essai Prevac (Partnership for Research on Ebola VACCination).

9th Conférence Francophone d'Epidemiologie Clinique (EPICLIN) | Montpellier, France
Edouard Lhomme, Laura Richert, Steve Self, Zoe Moodie, Spyros A. Kalams, Stephen C. De Rosa, Cecilia Morgan, Rodolphe Thiébaud. Dynamique des réponses immunitaires à un vaccin préventif contre le VIH-1 : modélisation à partir d'un essai clinique vaccinal.

Poster communications

International Precision Vaccines Conference | Boston, USA

Lhomme E, Hejblum B, Lacabaratz C, Wiedemann A, Lelièvre JD, Levy Y, Thiébaud R, Richert L. Analyzing cellular immunogenicity in vaccine clinical trials: a new statistical method including non-specific responses for accurate estimation of vaccine efficacy.

Europe Biobank Week 2019 | Lubeck, Germany

Renaud Vatrinet, Cécilia Champion, John McCullough, Yeya dit Sadio Sarro, Awa Traore, Julie Blie, Tuda Otieno, Celine Roy, Patricia Boison, Michael Belson, Suzanne Fleck, Aurélie Wiedemann, Marion Bererd, Njoh Wisedi, Deborah Wentworth, Benjamin Hamze, Bonnie Dighero-Kemp, Djeneba Dabita, Brett Lowe, Marie Hoover, James Neaton, Inmaculada Ortega-Perez, Boni-Maxime Ale, Edouard Lhomme, Eric d'Ortenzio, Lisa Hensley and the PREVAC study team. Specimen management in the PREVAC clinical trial.

Europe Biobank Week 2018 | Antwerp, Belgium

Aurelie Wiedemann, Adam Nouveau, Marie Dechenaud, Christine Lacabaratz, Edouard Lhomme, Eric D'Ortenzio, Abdoul Habib Beavogui, Yves Levy, Yazdan Yazdanpanah, Daniela Fusco. Quality parameters for Peripheral Blood Mononuclear Cells (PBMC) isolation: setting standard indicators (SI) in African low resource-settings (LRS).

Awards related to the subject to the thesis

Award by the Scientific Committee of the 13rd « Conférence Francophone d'Epidémiologie Clinique » (EPICLIN), Toulouse, France, 15-17 May 2019 for the oral communication « Evaluation de l'immunogénicité cellulaire dans les essais cliniques vaccinaux : un modèle bivariée pour mieux prendre en compte la réponse non spécifique ».

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Abbreviations

ADCC: antibody dependent cellular toxicity
ADCP: antibody dependent cellular phagocytosis
APP: Agency for the Protection of Programs
c-ART: Combination antiretroviral therapy
CCHF: Crimean-Congo haemorrhagic fever
DNA: Deoxyribonucleic Acid
DRC: Democratic Republic of Congo
ELISA: Enzyme-Linked Immunosorbent Assay
EVD: Ebola virus disease
FANG: Filovirus Animal Non-Clinical Group
HIV: Human immunodeficiency virus
HVTN: HIV Vaccine Trials Network
ICS: Intracellular cytokine staining
ISPED: Institut de santé publique, d'épidémiologie et de développement | Bordeaux University's School of Public Health
MERS-CoV: Middle East respiratory syndrome coronavirus
PBMC: Peripheral blood mononuclear cell
PREVAC: Partnership for Research on Ebola Vaccination
NHP: Non-human primate
rAd5: recombinant adenoviral serotype 5
RVF: Rift Valley fever
SARS: Severe Acute Respiratory Syndrome
SCHARP: Statistical Center for HIV/AIDS Research and Prevention
SISTM: Statistics In System biology and Translational Medicine
VRI: Vaccine Research Institute
WHO: World Health Organization

Preamble

Clinical trials represent a key step for the development of a new biomedical intervention, including the development of new candidate vaccines. Clinical research is very active for vaccines against various infectious diseases, especially high burden diseases where no active vaccine is yet approved. Specific methodological challenges exist in clinical vaccine, due to specifics of vaccine development, clinical trial design, and complexities of new immunological assays for evaluating immunogenicity of vaccine candidates. This requires methodological research to define the most appropriate methods at the different stages of clinical research. This thesis focuses on methodological research at the interface of clinical epidemiology and biostatistics to optimize methods used in the clinical development of vaccines, using HIV and Ebola vaccine clinical trials as an example.

This 3 years' research work has been carried out in a specific setting and context. After qualifying as a medical doctor specialized in public health in 2016, I had the privilege to access to the position of "*Assistant Hospitalo Universitaire*". This position, which I have held since that date, allows me to work as a methodologist within the Bordeaux University Hospital's Clinical Trial Unit and the EUCLID/FCRIN Clinical Trials Platform as well as assistant professor of Public Health and Epidemiology at the School of Medicine and at the Bordeaux University's School of Public Health (ISPED). As methodologist, I have been involved in many clinical trials, including several vaccine clinical trials and specifically the *Partnership for Research on Ebola Vaccination* (PREVAC), which is an ongoing randomized phase II prophylactic vaccine clinical trial evaluating the safety and immunogenicity of three different vaccine strategies against Ebola in four West African countries (NCT02876328). This work is emphasis in the chapter 5 of the thesis. I have also contributed to the organization of onsite capacity training in vaccine clinical research in Guinea and Burkina Faso for PREVAC and Ebovac 2 trials (NCT02564523) respectively.

In parallel, I have conducted my PhD research work in the Statistics In System biology and Translational Medicine (SISTM) team under the direction of Dr Laura Richert. The SISTM team led by Prof Rodolphe Thiébaud is based at the Bordeaux Population Health Center (Inserm U1219) at the University of Bordeaux. The overall objective of SISTM is to develop statistical methods based on statistical and mechanistic modeling for the integrative analysis of health data, especially those related to clinical immunology and vaccinology to answer specific questions risen in the application field. The main application of interest is the immune response to vaccine, mainly in the context of HIV infection. The methods developed in this

context can be applied in other circumstances but the focus of the team on immunology is important for the relevance of the results and their translation into practice, thanks to a longstanding collaboration with several immunologists and the implication of the team in the Labex Vaccine Research Institute (Hôpital Henri Mondor, Creteil, France) which is a laboratory of Excellence is an extension of ANRS (French research HIV Hepatitis) program to accelerate the vaccines development of against HIV and the hepatitis C. This thesis is part of the emergence of a third axis of the SISTM team on translational vaccinology addressing applied research questions to improve vaccine development and evaluation in future clinical trials.

These three years have been extremely fruitful and all the experience acquired as methodologist and university teacher has been a strength that have greatly enriched the research work carried out in the SISTM team.

I. Introduction

Optimizing development of new vaccines

Vaccination is one of the most important discoveries in medicine. More than two centuries after Edward Jenner discovered its principle, vaccination has now led to the eradication of smallpox, the nearly extinction of polio, the substantially decreased number of cases of measles and rubella and the prevention of millions of deaths from various infectious vaccine-preventable diseases every year worldwide. It is one of the most effective public health tools being the one of most successful disease prevention strategies ever implemented [1,2]. However, there are still a number of infectious diseases with high burden for which an effective preventive vaccine does not exist or is not approved so far.

The development of new safe and effective vaccines faces various challenges through a long research and development process. First comes the pre-clinical development carried out with the *in vitro* screening / identification of relevant antigens, creation of the vaccine concept and preliminary evaluation *in vitro* and in animals. Then, the clinical development begins when the vaccine is first tested in humans and follows the four classical phases of drugs clinical studies evaluation [3]. First of all, phase I trials and then phase II trials are conducted on a small number of volunteers testing the safety of the vaccine and its ability to induce immune responses respectively. Only the most promising vaccines are evaluated in larger-scale phase III clinical trials with a clinical endpoint (protection from the disease) which can determine the obtention of a license to market the product for human use. In randomized phase III trials, the evaluation of a candidate vaccine is judged directly on the clinical efficacy of the vaccines, i.e. the comparison of infection rates after a follow-up period between the vaccinated group and the placebo group. For feasibility and ethical reasons, initial human clinical evaluations of a candidate vaccine in phases I and II are based on the study of the immunogenicity of the vaccine, i.e. its ability to generate immune responses in participants.

The clinical development process is characterized by methodological issues specific to vaccine evaluation. Two cases will be used in this thesis to illustrate and apply this methodological research work: the development of vaccines against human immunodeficiency virus (HIV) and Ebola virus disease (EVD). For both HIV and EVD, there is an urgent need to develop safe and effective vaccines and the vaccine research is very active. The vaccine development is challenging for different reasons. For HIV mainly because of virus properties (such as viral diversity and escape from immune responses) and its pathogenic effects on

immune system; and for EVD because of the emergency to develop an effective vaccine in the context of epidemics like the 2014-2016 West-African outbreak which have strongly accelerated the vaccine clinical research. However, vaccine development against these two diseases also face common methodological issues related to the fact that there is no validated immunological correlate of protection so far that can be used as surrogate marker in early clinical trials phases. A good surrogate marker must be statistically associated with the occurrence of the disease, and could be a mechanistic cause of protection or does not cause protection but nevertheless predicts protection through its (partial) correlation with another immune response(s) that mechanistically protects [4-6] (Figure 1). This means that it is not yet possible to predict these clinical outcomes based on immunogenicity markers, i.e. the measurement of immune responses of vaccinated individuals. For the analysis of such surrogate endpoints, one needs datasets of randomized phase IIB or III trials having demonstrated a protective vaccine effect and in which immune responses (putative surrogates) have been measured.

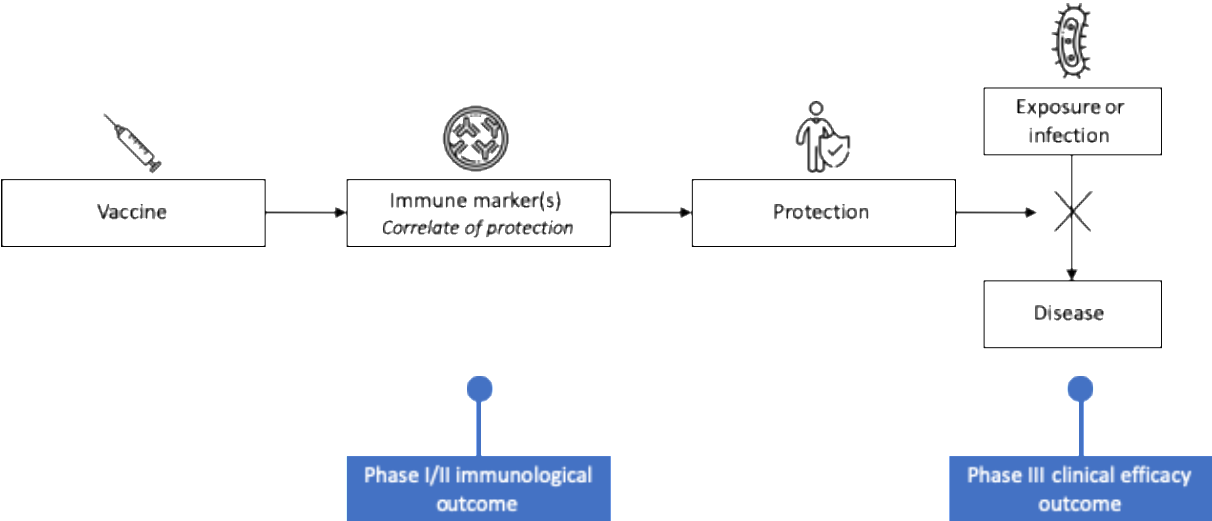


Figure 1. Correlate of protection and outcome in vaccine clinical trials

Moreover, phase III vaccine trials against HIV or Ebola are very difficult to set up. For EVD, direct observation of possible vaccine protection in humans is possible only in an outbreak context, which involves huge operational challenges. Phase III HIV prophylactic vaccine trials are difficult and expensive to conduct because of the low incidence of the disease, requiring thousands of participants in these trials. Thus, decision criteria for clinical vaccine development must largely rely on phase II (and I) data, and the measure of immunogenicity in phase I and phase II vaccine clinical trials is critical to identify the good vaccine candidates. In the absence of a validated correlate of protection, the evaluation of immunogenicity is challenging. In this thesis we will focus on the methodological challenges of

evaluation of vaccine immunogenicity in vaccine clinical trials for optimizing the vaccine development.

Two examples: HIV and Ebola vaccine development

HIV vaccine development

More than thirty years after HIV was identified as the etiological agent of acquired human immunodeficiency syndrome (AIDS), the global HIV epidemic remains one of the major public health challenges and is currently the fourth largest cause of death in the world. In 2018, 1.7 million people were newly infected with the virus and a total of 37.9 million people are living with HIV [7]. Despite remarkable progress in the development of antiretroviral therapies, especially the advent of combined highly active antiviral therapies (c-ART) that contain HIV replication, and recent advances in new prevention methods, the rate of new HIV infections remains very high. The development of a safe, easy-to-use and effective preventive vaccination strategy against HIV is one of the best opportunities in the long term to end the pandemic.

Several prophylactic and therapeutic clinical trials against HIV have been conducted in the last thirty years to evaluate several strategies combining different candidate vaccines that included different vaccine platform: inactivated viruses (adenovirus type 5, ALVAC pox), recombinant viral proteins or subunits (glycoprotein Gp 120, AIDSVAX), deoxyribonucleic acid (DNA). The prophylactic vaccine is intended to provide protection in healthy people, before the contact with the virus, whereas the therapeutic vaccine aims to provide a viral control of the disease in HIV infected patients. To date, only eight phase IIB / III prophylactic vaccine clinical trials have been conducted (Table 1), including two ongoing trials. The most encouraging results were those of the RV144 trial published in 2009 [8], which was the first to demonstrate the partial effectiveness - a 31.2% reduction in the risk of HIV infection acquisition - of a prophylactic vaccination strategy based on a clinical criterion (infection acquisition). While this is a major step forward, it is not enough to provide protection against the virus and vaccine research is still ongoing. Mathematical modeling has demonstrated that the ability to reduce HIV risk by even 50% could be an effective public health approach to the HIV pandemic [9].

The results of the vaccine trials carried out so far suggest the importance of the administration regimen and the concept of heterogeneous prime and boost vaccination to optimize vaccine efficacy. This involves combining several doses of different candidate vaccines in order to optimize immune responses: the injection of a first type of vaccine (prime vaccination) is followed by the injection of a different type (boost). According to current

knowledge, this repetition of antigenic stimulation would make it possible to modulate the intensity, quality and duration of the immune response and thus increase the number of responders to the vaccine [10-13].

Table 1. Description of the different phase IIb/III prophylactic vaccine trials conducted against HIV from 1998 to 2019.

| Trial | Year | Candidate vaccine(s) | Volunteer (N) | Expected immune response | Results |
|------------|-----------|--|---------------|----------------------------------|-------------------|
| VaxGen 004 | 1998-2003 | Protéines gp 120 recombinantes | 5 400 | CD4+ T cell; Antibodies | No efficacy |
| VaxGen 003 | 1999-2003 | Protéines gp 120 recombinantes | 2 500 | CD4+ T cell ; Antibodies | No efficacy |
| Step | 2004-2008 | Adénovirus de type 5 | 3 000 | CD4+ and CD8+ T cell | No efficacy |
| Phambili | 2007-2011 | Adénovirus de type 5 | 3 000 | CD4+ and CD8+ T cell | No efficacy |
| RV 144 | 2004-2009 | ALVAC (Pox)* + AIDSVAX (protéine)** | 16 403 | CD4+ and CD8+ T cell; Antibodies | 31% of efficacy |
| HVTN 505 | 2009-2013 | ADN* + Adénovirus de type 5** | 2 504 | CD4+ and CD8+ T cell; Antibodies | No efficacy |
| HVTN 702 | 2016-2022 | ALVAC-HIV (vCP2438)* + Bivalent Subtype C gp120/MF59** | 5 400 | CD4+ and CD8+ T cell; Antibodies | Not yet published |
| HVTN 705 | 2019 - | Ad26 (mosaic)/ trimeric gp140 | 2600 | CD4+ and CD8+ T cell; Antibodies | Not yet published |

* prime vaccination

** boost vaccination

Development of a human immunodeficiency virus (HIV) vaccine faces many challenges that include the virus life cycle favoring the rapid establishment of hard to clear chronic infections, the high diversity and structure of the envelope glycoprotein limiting the ability to elicit broadly neutralizing antibodies (bnAbs), and the tropism of the virus for T helper cells facilitating infection, spread, and persistence [14]. Moreover, the absence of naturally acquired protection complicates the identification of potential correlates of protection. Regarding methodological challenges, a large number of vaccine candidates - and even larger number of potential prime-boost vaccinations - are currently in the pipeline, thus efficient clinical trial

design with efficient evaluation of vaccine candidates in vaccine clinical trials - especially in early phases - are needed to push quickly the best candidates into phase III trials. Despite encouraging results from the RV144 trial [15,16] suggested binding antibodies against variable loops 1 and 2 of the HIV envelope and two subsets of polyfunctional CD4+ T cells as correlate of protection, whether the RV144 immune correlates of risk will generalize to other populations vaccinated with similar immunogens with different modes and intensity of transmission remains to be demonstrated [17]. There is still no confirmed predictive marker for HIV protection that can be a valid surrogate marker for commonly accepted clinical efficacy. The immunogenicity of a HIV vaccine may be represented by multiple immunological markers and experts now believe that a prophylactic HIV vaccination strategy must generate both type of response: a humoral response - mediated by B cells - and a cellular response from T cells to be effective [15,18,19].

Ebola virus disease

Since its discovery in 1976 in Democratic Republic of Congo (DRC), the Ebola virus has emerged periodically and infected people in several African countries. The 2014–2016 Ebola virus disease outbreak epidemic in West Africa was unprecedented in terms of magnitude, being the first to spread to urban areas and the largest ever recorded. The outbreak was first recognized on 22 March 2014 in Guinea and spread to Sierra Leone and Liberia, infecting more than 28,000 people and leading to more than 11,000 deaths in these three West African countries. More recently, two Ebola outbreaks have been recorded in the DRC in 2018. The second and larger outbreak was declared in North Kivu in August 2018 and is still ongoing with more than 3083 confirmed and probable EVD, resulting in 2136 deaths, with an overall case fatality rate of 67% [20]. The genus Ebolavirus includes 5 species: Zaire, Sudan, Reston, Tai Forest, and Bundibugyo; the most virulent being the Zaire species. Several strains of the Zaire species have been identified, including Mayinga (1976), Kikwit (1995), Zaire (1976), and Makona (2014). The 2014-2016 West-African outbreak was linked to the new Makona strain, as well as the ongoing outbreak in the DRC [21].

Epidemics of MVE are characterized by a very high mortality rate ranging from 50% to 90%. To date, treatment options are only symptomatic, there is no approved effective antiviral treatment available [22]. However, to limit the spread of EVD, implementation of effective and coordinated public health measures, relying on case management and other prevention measures, and risk communication, social mobilization, and community engagement initiatives, can be implemented. In August 2019, preliminary results from an ongoing therapeutic trial in Democratic Republic of Congo (DRC) evaluating the effectiveness of four drugs in patients with Ebola infection who are in treatment centers (PALM trial,

ClinicalTrials.gov identifier: NCT03719586) reported a partial efficacy of two drugs containing monoclonal antibodies. If this is a big step forward in the fight against Ebola, they alone will not stop Ebola. An effective preventive vaccine would allow reducing the transmission of the disease, preventing the massive occurrence of cases and preventing significant disease-related mortality. The 2014-2016 outbreak highlighted the urgent need for the development of an effective vaccine and led to accelerated vaccine development against EVD. Clinical research is very active and continued after the epidemic in non-epidemic context, and is also currently ongoing in the DRC outbreak.

The development of Ebola vaccine follows the different usual stages before it can be approved for marketing. Before the clinical phases in humans, the treatment is first tested in animals in preclinical studies. In the case of EVD, the most commonly used animal model is the NPH because it is the one that most closely reproduces the symptoms and pathophysiology observed in humans [23]. As of July 31, 2019, 46 completed clinical trials, three active and not recruiting, and four recruiting Ebola vaccine clinical studies are registered on ClinicalTrials.gov. Thirteen candidate Ebola vaccines candidate vaccine have undergone or are currently undergoing phase I-III clinical evaluation. The most promising strategies currently undergoing the evaluation process are the rVSV-ZEBOV-GP candidate vaccine from Merck and a prime/boost candidate vaccine based on Ad26- and MVA-vectored components (Ad26.ZEBOV/MVA-BN-Filo) developed by Johnson and Johnson. Moreover, two candidate vaccines, GamEvac-Combi and Ad5- EBOV are licensed in the Russian Federation and China respectively, their countries of origin, based on immunogenicity data.

The only study that has been able to provide data on clinical efficacy is the *Ebola Ça Suffit* vaccination trial performed in Guinea at the end of the 2014-2016 outbreak [24]. This open label cluster randomized ring vaccination trial in Guinea evaluated vaccine effectiveness in case contacts, where clusters of contacts of Ebola cases were randomized for immediate or delayed vaccination with vesicular stomatitis virus based vaccine expressing the glycoprotein of a Zaire Ebolavirus (rVSV-ZEBOV). Vaccine efficacy was estimated to be 100% (95% CI 79.3-100.0, $p=0.0033$) in individuals vaccinated in the immediate group compared with those eligible and randomized to the delayed group. There were no blood samples collected for immunogenicity measurement, so it was not possible to look for a protective correlate in this trial. Ring vaccination with rVSVΔG-ZEBOV-GP is also currently being used as part of the control efforts for the DRC North Kivu outbreak and more than 150 000 individuals (contacts, contacts of contacts and Health care workers/ Front line workers) have been vaccinated.

Phase III vaccine clinical trials, as *Ebola Ca Suffit* trial where a direct observation of a clinical protection, can be performed only in an epidemic context. However, for early clinical phases and all clinical studies in general performed after the West African outbreak in a non-epidemic context where there is no longer any active transmission of the virus, the evaluation of vaccine is based on immunogenicity (and safety). To date, there is no validated surrogate marker to evaluate the efficacy of Ebola vaccines in humans, however, a previous study conducted in monkeys testing recombinant VSV vector vaccines or DNA vaccines found a strong correlation between the level of antibodies (immunoglobulin G against EBOV glycoproteine) observed following vaccination and survival after infection with the Ebola virus [25-30]. For this reason, early phase trials of vaccine trials against EVD currently rely mainly on the quantification of the antibody response by the ELISA method after vaccination to assess immunogenicity [31] but other immunogenicity measurements are also performed as secondary or exploratory analyses. Indeed, the correlate could vary according to the time point of the measurement post-vaccination and the type of vaccine. In another preclinical study using a recombinant adenovirus vaccine, the cellular response seemed to explain more primate survival [32]. The protection in the *Ebola ça suffit* trial seems to start earlier than the induction of the antibody response (so there is probably an early protection correlate that is not the antibody response).

Immunogenicity evaluation of candidate vaccines

Immune responses to vaccines

The role of a preventive vaccine is to present the immune system with a component of an infectious agent called an antigen to induce a specific immune response against this agent. In response to contact with a pathogen, an innate immune response is developed and then a specific adaptive immune response against the agent involved. The innate response is non-specific and constitutes an immediate response of the organism to contact with the pathogen. This response involves the inflammatory reaction, phagocytic cells (neutrophils, monocytes and macrophages), and NK ("Natural Killer") cells. The adaptive immune response is implemented after the innate response and is able to generate a specific response against an antigen, and to maintain an immunological memory.

Cellular and humoral immunity are the two parts of adaptive immunity (figure 2). Humoral immunity is mediated by antibodies, which are produce by B cells. Present in the blood and mucous secretions, these antibodies recognize the extracellular antigen, neutralize the infection and eliminate microbes through different mechanisms. Antibodies are made up of proteins called immunoglobulins. Cellular immunity is mediated by T cells. Intracellular

germs, such as HIV, survive and proliferate inside host cells where they are inaccessible to circulating antibodies. T cells recognize the antigens of intracellular microbes and allow the destruction of these microbes or infected cells. They are made up of several populations with different functions, the two main ones being CD4 + auxiliary T cells and cytotoxic CD8+ T cells. In response to antigenic stimulation, T cells proliferate and differentiate into effector cells whose functions are mediated largely by small proteins called cytokines (eg. IL-2, IFN γ , TNF α). Once activated with CD4+ T cells, CD8+ T cells proliferate and differentiate into cytotoxic CD8+ T cells capable of killing cells producing foreign antigens such as those infected with HIV [33]. CD4+ T lymphocytes are a major player in the immune response by orchestrating the CD8+ T cells response and humoral response. A multitude of cellular subpopulations exist for these different lymphocytes with functions that are not yet well known, but knowledge is constantly improving.

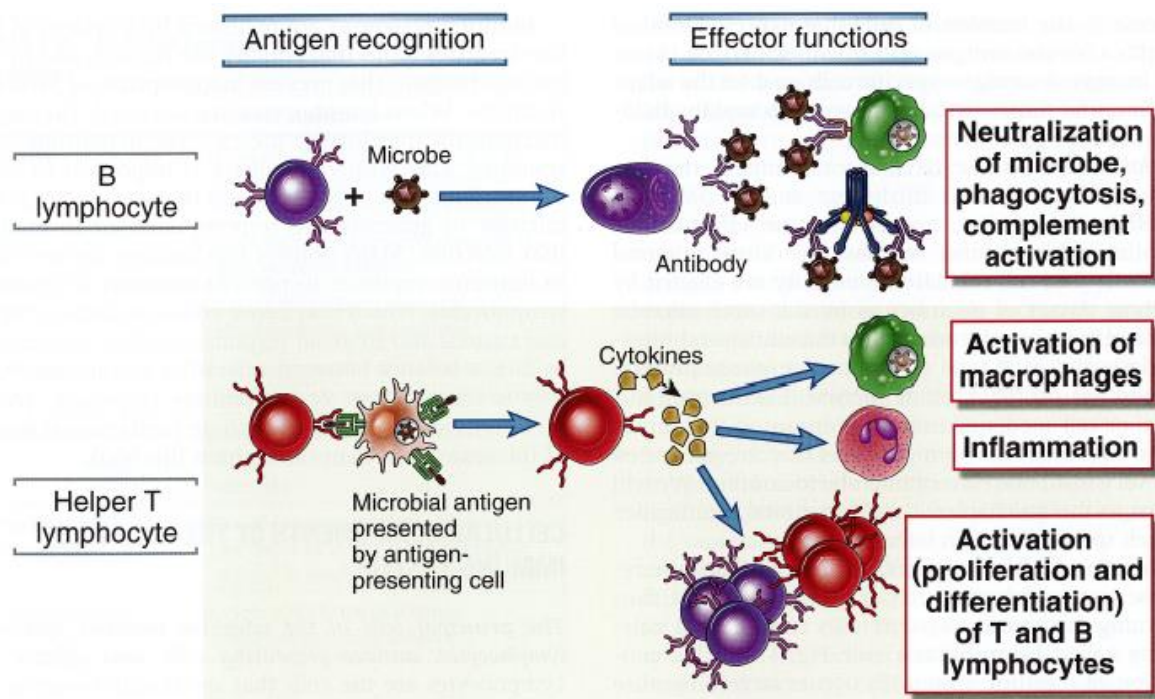


Figure 2. Description of humoral and cellular immunogenicity responses. Adapted from Abbas AK et al. Cellular and molecular immunology, ed.8. © Elsevier 2014

Evaluation of immunogenicity

The immunogenicity of a vaccine can be represented by multiple humoral or cellular immunological markers. When no validated immunological marker exists that would predict the clinical efficacy of the vaccine and could therefore be used as a surrogate marker in clinical development, a broad spectrum of immunological markers is measured after vaccination in participants in vaccine trials to evaluate in detail the different aspects of the immunological

response. The markers measured can include the levels of different antibodies, the concentrations of molecules secreted by these immune cells (cytokines), the frequency of hundreds of cell subpopulations, as well as the level of expression of tens of thousands of genes in cells. With the development of high-throughput immunological assays, the data for one participant and blood sample increases in number and complexity. Thus, the evaluation of the immunogenicity of a candidate vaccine remains complex to evaluate and raise several methodological issues that will be detailed in the following paragraphs.

Methodological considerations

First, the analysis of immunogenicity markers is complicated by the fact that specific aspects need to be taken into account for each assay technique and that validated positivity criteria are lacking or have an unknown biological relevance for many markers. For instance, T-cell responses to vaccine candidates are commonly assessed by T cell assay such as intracellular cytokine staining (ICS) and EliSpot, with different measures including a background response (control) and specific responses with different stimulation conditions. Conventional statistical approaches do usually not take into account all the information and can have sub-optimal statistical performances, and sometimes even induce bias. The critical appraisal of existing statistical analyses approaches and if needed the development of novel approaches is important for a better measure of the vaccine effect in phase I and II vaccine clinical trials.

Second, the measurement times of the immunological markers after vaccine injections in clinical trials are defined empirically and heterogeneous and relatively late in most vaccine trials conducted to date. Usually all the different markers are measured at the same time and the measurement time is set empirically between two and four weeks after the last vaccination injection, the number of injections often varying between two and six over three to six months. Thus, the dynamics of immunological markers during vaccination remains poorly known and the measurement times used can miss the measure of immune response. Moreover, some markers can have an early response, a few days after the vaccination, while others a later response. The implementation of repeated measurements (eg. weekly or bi-weekly) in the follow up of a vaccine clinical trial would make it possible to capture the pic of the immune response but this is not feasible for reasons of cost and acceptability for participants. Some immunological markers initially measured could predict the subsequent response measured from other markers, such as the yellow fever vaccine, for which the measurement of the initial innate response predicts the subsequent antibody response [34]. Understanding and

assessing the dynamics of vaccine immunogenicity in human clinical trials is a major challenge for improving vaccine design and evaluation in future clinical trials.

Finally, factors influencing the variability of the immune response after vaccination remain poorly understood. In addition to the type of vaccine, certain determinants related to vaccine characteristics such as vaccine platform (delivery vector), dosage, viral insert, vaccine schedule, or study population could influence the measure of the immune response to the vaccine in clinical trials. These many factors could have an impact on the level of immune response measured in a clinical trial, and could explain differences in results between studies. It would therefore be interesting to know which factors explain the observed variability of the immune response, and if so, to obtain a quantitative estimate of their influence for a more reproducibility and standardization in future vaccine clinical trials.

A better understanding of the dynamics of immune responses to vaccines, a better assessment of this response and its determinants in human clinical trials is a major challenge for improving vaccine design and evaluation in future clinical trials.

References

1. Koff WC, Burton DR, Johnson PR, Walker BD, King CR, Nabel GJ et al. Accelerating next-generation vaccine development for global disease prevention. *Science*. 2013;340(6136):1232910.
2. D'Argenio DA, Wilson CB. A decade of vaccines: Integrating immunology and vaccinology for rational vaccine design. *Immunity*. 2010;33(4):437-40
3. Singh K, Mehta S. The clinical development process for a novel preventive vaccine: An overview. *J Postgrad Med*. 2016;62(1):4-11.
4. Plotkin SA. Correlates of Protection Induced by Vaccination. *Clin Vaccine Immunol*. 2010;17(7):1055-65.
5. Plotkin SA, Gilbert PB. Nomenclature for Immune Correlates of Protection After Vaccination. *Clin Infect Dis*. 2012;54(11):1615-7.
6. WHO. Correlates of vaccine-induced protection: methods and implications. [Internet]. [cited October 7 2019]. Available from: https://apps.who.int/iris/bitstream/handle/10665/84288/WHO_IVB_13.01_eng.pdf
7. UNAIDS. UNAIDS data 2019. [Internet] [cited October 7 2019]. Available from: https://www.unaids.org/sites/default/files/media_asset/2019-UNAIDS-data_en.pdf
8. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, et al. Vaccination with ALVAC and AIDSVAX to Prevent HIV-1 Infection in Thailand. *N Engl J Med*. 2009;361(23):2209-20.
9. Medlock J, Pandey A, Parpia AS, Tang A, Skrip LA, Galvani AP. Effectiveness of UNAIDS targets and HIV vaccination across 127 countries. *Proc Natl Acad Sci U S A*. 2017;114(15):4017-22.
10. Watkins DI, Burton DR, Kallas EG, Moore JP, Koff WC. Nonhuman primate models and the failure of the Merck HIV-1 vaccine in humans. *Nat Med* 2008;14(6):617-21.
11. Cox KS, Clair JH, Prokop MT, Sykes KJ, Dubey SA, Shiver JW, et al. DNA gag/Adenovirus Type 5 (Ad5) gag and Ad5 gag/Ad5 gag Vaccines Induce Distinct T-Cell Response Profiles. *J Virol*. 2008;82(16):8161-71.
12. Ramshaw IA, Ramsay AJ. The prime-boost strategy: exciting prospects for improved vaccination. *Immunol Today*. 2000;21(4):163-65.
13. Woodland DL. Jump-starting the immune system: prime–boosting comes of age. *Trends Immunol*. 2004;25(2):98-104.
14. Robinson HL. HIV/AIDS Vaccines: 2018. *Clin Pharmacol Ther*. 2018;104(6):1062-73.
15. Haynes BF, Gilbert PB, McElrath MJ, Zolla-Pazner S, Tomaras GD, Alam SM, et al. Immune-Correlates Analysis of an HIV-1 Vaccine Efficacy Trial. *N Engl J Med*. 2012;366(14):1275-86.
16. Lin L, Finak G, Ushey K, Seshadri C, Hawn TR, Frahm N, et al. COMPASS identifies T-cell subsets correlated with clinical outcomes. *Nat Biotechnol*. 2015;33(6):610-6.
17. Kim JH, Excler JL, Michael NL. Lessons from the RV144 Thai phase III HIV-1 vaccine trial and the search for correlates of protection. *Annu Rev Med*. 2015;66:423-37.
18. Barouch DH. Challenges in the Development of an HIV-1 Vaccine. *Nature*. 2008;455(7213):613-19.
19. Girard MP, Osmanov S, Assossou OM, Kieny M-P. Human immunodeficiency virus (HIV) immunopathogenesis and vaccine development: A review. *Vaccine*. 2011;29(37):6191-218.

20. WHO. Ebola virus disease – Democratic Republic of the Congo. [Internet] [cited October 7 2019]. Available from: <https://www.who.int/csr/don/03-october-2019-ebola-drc/en/>
21. McMullan LK, Flint M, Chakrabarti A, Guerrero L, Lo MK, Porter D, et al. Characterisation of infectious Ebola virus from the ongoing outbreak to guide response activities in the Democratic Republic of the Congo: a phylogenetic and in vitro analysis. *Lancet Infect Dis.* 2019;19(9):1023-1032.
22. Kaner J, Schaack S. Understanding Ebola: the 2014 epidemic. *Global Health* 2016;12(1):53
23. Bente D, Gren J, Strong JE, Feldmann H. Disease modeling for Ebola and Marburg viruses. *Dis Model Mech.* 2009;2(1-2):12-7.
24. Henao-Restrepo AM, Camacho A, Longini IM, Watson CH, Edmunds WJ, Egger M, et al. Efficacy and effectiveness of an rVSV-vectored vaccine in preventing Ebola virus disease: final results from the Guinea ring vaccination, open-label, cluster-randomised trial (Ebola Ça Suffit!). *Lancet.* 2017;389(10068):505-518.
25. Pushko P, Bray M, Ludwig GV, Parker M, Schmaljohn A, Sanchez A, et al. Recombinant RNA replicons derived from attenuated Venezuelan equine encephalitis virus protect guinea pigs and mice from Ebola hemorrhagic fever virus. *Vaccine.* 2000;19(1):142-53.
26. Wilson JA, Hart MK. Protection from Ebola virus mediated by cytotoxic T lymphocytes specific for the viral nucleoprotein. *J Virol.* 2001;75(6):2660-4.
27. Wong G, Richardson JS, Pillet S, Patel A, Qiu X, Alimonti J, et al. Immune Parameters Correlate with Protection Against Ebola Virus Infection in Rodents and Nonhuman Primates. *Sci Transl Med;* 2012;4(158):158ra146.
28. Xu L, Sanchez A, Yang Z, Zaki SR, Nabel EG, Nichol ST, et al. Immunization for Ebola virus infection. *Nat Med;* 1998;4(1):37-42.
29. Saphire EO, Schendel SL, Gunn BM, Milligan JC, Alter G. Antibody-mediated protection against Ebola virus. *Nat Immunol.* 2018;19(11):1169-78.
30. Meyer M, Malherbe DC, Bukreyev A. Can Ebola Virus Vaccines Have Universal Immune Correlates of protection? *Trends in Microbiology.* 2019;27(1):8-16.
31. Krause PR, Bryant PR, Clark T, Dempsey W, Henchal E, Michael NL, et al. Immunology of protection from Ebola virus infection. *Sci Transl Med.* 2015;7(286):286ps11-286ps11.
32. Sullivan NJ, Hensley L, Asiedu C, Geisbert TW, Stanley D, Johnson J, et al. CD8+ cellular immunity mediates rAd5 vaccine protection against Ebola virus infection of nonhuman primates. *Nat Med.* 2011;17(9):1128-31.
33. Abbas AK, Lichtman AH, Pillai S. 6th Edition. *Cellular and molecular immunology.* Philadelphia: Elsevier; 2010.
34. Pulendran B. Learning immunology from the yellow fever vaccine: innate immunity to systems vaccinology. *Nat Rev Immunol.* 2009;9(10):741-47.

II. Objectives and outline of the thesis

The general objective of the thesis is to propose and develop statistical methods to model immunogenicity data from vaccine clinical trials.

This includes modeling taking into account the specificities of the measurement technique, modeling of the vaccine response over time, and modelling to assess the determinants of the vaccine response, as an example of experimental vaccine strategies with candidate vaccines, applied to HIV and EVD.

Chapter 3 of the thesis covers the work on the modeling of the cellular immune response. In the first part of this chapter we present a work that addresses the modelling of the dynamics of the immune responses over time, with an application on an HIV vaccine trial called HVTN 068, which was a special trial due to its number of repeated measurements throughout the trial. In the second part, we propose a new statistical method for the analysis of functional T-cell responses in vaccine trials, using modelling for a more accurate estimation of the vaccine effect in the presence of “background” (control) measurements.

In Chapter 4, we change focus and address the modelling of the antibody response. This part presents a systematic review and meta-analysis of the determinants of antibody responses, in the context of Ebola vaccine trials.

In Chapter 5, we will open the scope of this thesis beyond the methodological aspects related to immunogenicity with the presentation of a phase 2 randomized international Ebola vaccine trial and a specific methodological reflection we had while setting up this trial on the enrollment of study personnel in a non-epidemic context.

III. Modelling cellular immune responses

In this chapter, we will focus on the cellular immunogenicity. T-cell adaptive immune responses are an important immunological read-out in diverse experimental settings. Measuring the amount of T-cell for a specific pathogen may be useful to follow the course of an infection and to monitor how the immune system responds to an infection. In vaccine clinical research, the vaccine specific T-cell response is a measure of the immunological efficacy of a given vaccine and is part of the large panel of immunological measured in phase I and II vaccine trials especially when there is no correlate of protection. In vaccine clinical trials, the cellular response of a vaccine candidate is usually evaluated by the measure of the CD4+ and CD8+ T cell responses producing cytokines of interest. To properly evaluate the cellular response to vaccine, these markers should be measured at relevant time points after vaccination to response to the objective of the trial – which could be an evaluation of the durability of the response or the early response post-vaccination -, and the vaccine effect should be analyzed with appropriate unbiased statistical methods.

In the first part of the chapter, we will address the methodological issue regarding the definition of the right timing of measurement post-vaccination in vaccine clinical trials, and in the second part the development of an unbiased statistical analysis method, using in both cases modelling approaches.

1. Modelling for analyzing the dynamics of the immune responses

Measurement time points in vaccine clinical trials

Optimal measurement time points for immune markers to best assess the cellular immunogenicity of vaccine strategies in clinical trials are not clearly known and time points in vaccine trial protocols are most often defined empirically. However, it is necessary to make a well-considered choice of the time required to measure the immune response. Too many time points are sources of significant cost and poor feasibility of the clinical trial; therefore, few time points must be performed but these must be chosen in a relevant way so as not to miss the dynamic of interest. The definition of optimal times requires therefore a good knowledge of the dynamics of immune markers during vaccination. Some markers initially measured may predict the subsequent response measured from other markers, such as the yellow fever vaccine, for which the measurement of the initial innate response predicts the subsequent antibody response [1] or identification of early gene expression signature for predicting Ebola vaccine efficacy [2]. Identifying early markers of a good vaccine response could be useful for quickly

identifying promising vaccine strategies or eliminating ineffective strategies. This could also be useful in personalizing vaccination strategies, for instance offering an early boost to those who respond poorly to a first vaccination. Understanding and assessing the dynamics of cellular immunogenicity in human clinical trials is a major challenge for improving vaccine design and evaluation in future clinical trials, especially in HIV vaccine trials where the cellular immunogenicity could have an important role in the vaccine response. For example, the dynamics of CD8+ T lymphocytes producing IFN-g may be predictable or at least influenced by the dynamics of CD4+ T lymphocytes producing IL-2 since the latter stimulate the maturation of CD8+ T lymphocytes. This is a hypothesis as it is not granted that the markers observed in the whole blood captured well the mechanism that occurs in the lymph nodes. The evaluation of the dynamics of immune response markers is non-linear and therefore requires the use of complex models. This requires in particular a thorough reflection to allow time to be integrated into the model.

HVTN 068 trial

HVTN 068 trial is one of the very rare prophylactic HIV vaccine trial with early and repeated measurements of immunogenicity in all participants. The main objective of this randomized, multicenter, controlled, Phase I / II clinical trial in the U.S. was to evaluate the safety and immunogenicity of two different prime-boost vaccination strategies against HIV: a prime vaccination with recombinant adenovirus serotype 5 (rAd5) vaccine versus primary vaccination with DNA vaccine, followed for both groups by a boost vaccination with rAd5 vaccine. The primary results, published in 2011 [3], did not include a detailed analysis of the dynamics of the immunological response. Therefore, a secondary analysis of the data from this trial represented a great opportunity to study the dynamics of the immune response.

Hypothesis and objective

Studying the correlations between different post-vaccination immunological markers for HIV and their dynamics could help determine the optimal measurement times for the different markers. We hypothesize that an early response of CD4+ T lymphocytes producing IL-2 could predict a later response of CD8+ T lymphocytes producing IFN-g.

The main objective of this work was to evaluate the dynamics and correlations between the different cellular immune markers (CD4+ T lymphocytes producing IL-2 and CD8+ T lymphocytes producing IFN-g) during preventive HIV vaccination, as in a vaccination strategy using one of the candidate vaccines (rAd5).

This work has been performed in collaboration with the HIV Vaccine Trials Network (HVTN) which is the world's largest publicly-funded international collaboration focused on the development of vaccines to prevent HIV/AIDS and which conducted the HVTN 068 trial and SCHARP (Statistical Center for HIV/AIDS Research and Prevention; U Washington), both based at the Fred Hutchinson Cancer Research Center in Seattle. This has been published in Plos ONE.

RESEARCH ARTICLE

Early CD4+ T Cell Responses Are Associated with Subsequent CD8+ T Cell Responses to an rAd5-Based Prophylactic Prime-Boost HIV Vaccine Strategy

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Data Availability Statement: The data used in this analysis are owned by the clinical research sites and managed by the HIV Vaccine Trials Network (HVTN). In accordance with Network policy, other researchers can request permission to access the data in the same manner it was granted to the authors. Requests for access to study data should be sent to the HVTN: vn.research@hvtn.org or by completion of a form on the HVTN website: <http://www.hvtn.org/en/science/submit-idea-proposal/idea-submission.html>. Use of this data requires approval by the National Institutes

Abstract

Introduction

Initial evaluation of a candidate vaccine against HIV includes an assessment of the vaccine's ability to generate immune responses. However, the dynamics of vaccine-induced immune responses are unclear. We hypothesized that the IFN- γ producing cytotoxic CD8+ (CD8+ IFN- γ +) T cell responses could be predicted by early IL-2 producing CD4+ (CD4+ IL-2+) helper T cell responses, and we evaluated this hypothesis using data from a phase I/II prophylactic HIV vaccine trial. The objective was to assess the dynamics and correlations between CD4+ IL-2+ T cell and CD8+ IFN- γ + T cell responses after vaccination with a recombinant adenoviral serotype 5 (rAd5) HIV vaccine.

Methods

We analyzed data from the HVTN 068 HIV vaccine trial, which evaluated the immunogenicity of two different strategies for prime and boost vaccination (rAd5-rAd5 vaccine versus DNA-rAd5) in 66 healthy volunteers. Spearman correlations between immunogenicity markers across time-points were calculated. CD8+ IFN- γ + T cell response in the rAd5-rAd5 arm was modeled as a function of CD4+ IL-2+ T cell response and time using mixed effects regression models.

Results

Moderate to high correlations ($r = 0.48-0.76$) were observed in the rAd5-rAd5 arm between the CD4+ IL-2+ T cell response at week 2 and later CD8+ IFN- γ + T cell responses (weeks

of Allergy and Infectious Diseases (NIAID), the NIAID Vaccine Research Center (VRC), and the HVTN. If approved, data from this study will be provided by grant of access to a secure web portal. Resulting publications need to abide by HVTN publication requirements, including review with the NIAID, the VRC and the HVTN prior to publication.

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2–52). Regression models confirmed this relationship with a significant association between the two markers: for a 1.0% increase in CD4+ IL-2+ T cells at week 2 post-prime, a 0.3% increase in CD8+ IFN- γ + T cell responses across subsequent time points, including post-boost time points, was observed ($p < 0.01$).

Conclusion

These results suggest an early and leading role of CD4+ T cells in the cellular response to the rAd5-rAd5 vaccine and in particular the stimulation of cytotoxic CD8+ T cell responses. These results could inform better timing of CD4+ T cell measurements in future clinical trials.

Introduction

More than thirty years after the identification of the human immunodeficiency virus (HIV) as the etiological agent of acquired immunodeficiency syndrome (AIDS), the global HIV epidemic remains one of the major global health challenges [1,2]. The development of a safe and efficacious prophylactic vaccine strategy against HIV constitutes an opportunity to control the pandemic.

Several clinical trials have been conducted in recent years to evaluate prophylactic strategies combining different candidate vaccines, but showed at best modest efficacy [3–8]. Previous trials have especially highlighted the importance of the vaccine regimen, in particular the concept of prime and boost strategy to optimize vaccine efficacy [3,9,10].

Although correlates of protection in HIV vaccine trials are not yet validated, both cellular and humoral immune responses are likely required to achieve sufficient protective vaccine efficacy against HIV acquisition. The assessments of these immune responses therefore play a major role in phase I-II HIV vaccine trials and in the decision to proceed to large scale efficacy trials.

One goal of vaccine-induced cellular responses is to stimulate CD8+ cytotoxic responses able to effectively fight against the virus and to control the infection. IL-2 producing CD4+ T cells play a role in the generation of this response, as they stimulate the differentiation of CD8+ cytotoxic effector cells and memory cells. They also help for the differentiation of B cells [11,12]. However, little is known about the relationships between the different markers of immunologic response in HIV vaccine recipients, and little data are available on the dynamics of immunologic responses over time.

The time points at which different immunogenicity markers are measured in phase I and II HIV vaccine trials vary but are usually selected between two and four weeks after the final vaccine injection [13]. A better understanding of the dynamics of vaccine-induced immune responses would help facilitate a sampling strategy that can target optimal time points for each immunogenicity marker, to learn more about the vaccine's immunogenicity and increase the efficiency of clinical trials.

We hypothesized that certain immunogenicity markers which are measured early after vaccination could predict the subsequent immune responses of other markers: in particular, the dynamics of IFN- γ producing cytotoxic CD8+ T cells could be predicted by the early dynamics of IL-2 producing CD4+ helper T cells, which are known to stimulate the maturation of CD8+ T cells [14–16].

The main objective of this work was to determine the dynamics and correlations between different cellular immunogenicity markers (IFN- γ producing cytotoxic CD8+ T cells and IL-2 producing CD4+ helper T cells) during a prophylactic HIV vaccine strategy, using the example

of a phase I trial of prime-boost vaccine strategies with rAd5 and DNA candidate vaccines. The secondary objective was to determine the correlations between cellular and humoral (Env-binding antibodies) immunogenicity markers.

Methods

Study population

This study was based on individual data from HVTN (HIV Vaccine Trials Network) 068 (<http://www.clinicaltrials.gov>; NCT00270218), a phase I placebo-controlled multicenter trial comparing two different prophylactic HIV vaccine strategies in healthy HIV-uninfected volunteers. The first group (rAd5-rAd5 group) received recombinant adenovirus type 5 (rAd5) vector vaccine as a prime (week 0) and a rAd5 boost (week 24). The second group (DNA-rAd5 group) received two DNA vaccine primes (weeks 0 and 4), and a rAd5 boost (week 24). The vaccines encoded HIV-1 Env glycoprotein (clades A/B/C) and clade B Gag/Pol fusion gene.

Sixty-six participants (aged 18–50 years and lacking detectable pre-existing Ad5-neutralizing antibodies) were randomized, with 30 active vaccine recipients and three placebo recipients in each of the two groups. The institutional review committee at each clinical site approved the protocol prior to study initiation, and all participants provided a written informed consent to participate in this study. Details of this trial have been previously reported [17].

The main interest in using HVTN 068 data for the present analysis was that early and repeated immunogenicity measurements were available following each vaccine injection (Fig 1).

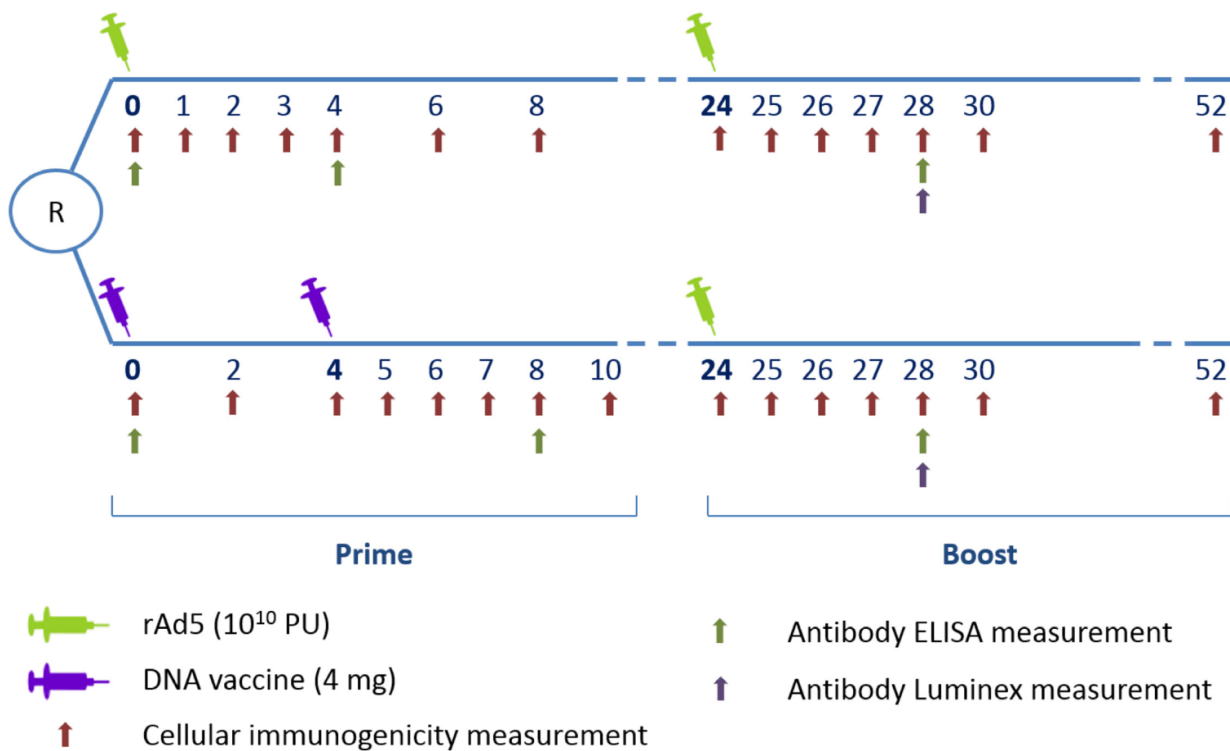


Fig 1. HVTN 068 trial design with repeated immunogenicity measurements—cellular and antibody responses—during the 52 weeks of follow-up.

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In the present analysis, only participants who received active vaccine were analyzed (60 participants).

Immunogenicity measurements

Immunogenicity measurements were performed in batches in a central laboratory. At each cellular immunogenicity sampling time point (see Fig 1), a validated eight-color intracellular cytokine staining (ICS) assay [18] and a ten-color ICS assay were used to measure cytokine production following 6 hours stimulation of peripheral blood mononuclear cells with Env, Gag or Pol potential T cell epitope (PTE) peptide pools. After gating, percentages of CD4+ and CD8+ T-cells producing IL-2 or IFN- γ , respectively, were assessed using FlowJo (Trestar) or LabKey Flow software. The gating hierarchy is shown in Appendix A in S1 File. The present analyses focused on Env-specific responses because few Gag and Pol responses were detected [17].

Env binding antibody titers were measured using ELISA at three time points (at baseline, and four weeks after the prime and the boost vaccine injections) and using the multiplex bead array (Luminex) assay four weeks after the boost injection as previously described [17,19].

Statistical analysis

All analyses were conducted separately for the DNA-rAd5 group and rAd5-rAd5 group, respectively. Associations between background-unsubtracted percentages of IFN- γ producing cytotoxic CD8+ T cells (CD8+ IFN- γ +) and IL-2 producing CD4+ (CD4+ IL-2+) helper T cells were assessed at each time point using Spearman correlations. We used background-unsubtracted responses for the main analysis of immune responses from HIV-uninfected individuals since with current knowledge it cannot be excluded that background responses may have some biological relevance [20]. Background-subtracted responses were analyzed in robustness analyses. Correlations between CD8+ IFN- γ + T cells and CD4+ IL-2+ helper T cells and antibody responses (ELISA and Luminex) were also assessed. Multiplicity-adjusted p-values were calculated by using the Benjamini and Hochberg adaptive false discovery rate (FDR) method [21]. FDR-adjusted p-values < 0.10 were considered statistically significant.

Further modeling analyses were performed in vaccine groups with relevant correlations in the descriptive analyses. Modeling of the dynamics of the immune responses was performed using mixed effects regression models [22]. The mixed effects model assumptions were verified. The distribution of CD4+ IL-2+ and CD8+ IFN- γ + were not entirely Gaussian but the use of these models is considered robust to moderate deviations from normality assumptions.

First, CD8+ IFN- γ + T cells response was modeled as a function of time (expected visit day) using spline functions to fit the effect of follow-up time covering both prime- and boost injections. The set of values of time was divided into (k+1) intervals with nodes to define the limits of the intervals. In each interval, the relationship between the percentage of CD8+ IFN- γ + T cell and time was modeled by a degree three polynomial [23,24]. Random intercepts and random slopes on each spline were used with an unstructured covariance matrix to take into account inter-participant variability.

Then, CD8+ IFN- γ + T cells response was modeled as a function of both time and CD4+ IL-2+ helper T cell response in order to take into account the correlation between these two markers and to study more precisely the role of CD4+ IL-2+ helper T cells response in CD8+ IFN- γ + response. The effect of time on the CD8+ IFN- γ + response was included in this model using the spline functions previously defined.

Several models were compared to identify the one with the best fit using the Akaike information criterion (AIC) (smaller is better):

The first model (model 1 in Appendix B in [S1 File](#)) included the CD4+ IL-2+ response at each time as explanatory variables, and was adjusted for time using cubic B-splines.

In model 2 (model 2 in Appendix B in [S1 File](#)), CD4+ IL-2+ response was introduced as two explanatory variables: one taking values of observed CD4+ IL-2+ before week 2 (from baseline to week 1), and the second taking two values: 0 before week 2, and then the value observed at week 2 at each visit until the end of the follow-up. The first variable allowed for taking into account the CD4+ IL-2+ response before week 2, and the second variable the specific CD4+ IL-2+ response at week 2 on all following CD8+ IFN- γ + measures. This choice, guided by the results of the descriptive correlation analyses, was made to more precisely study the association of the specific measure of CD4+ IL-2+ at week 2 on the entire subsequent CD8+ IFN- γ + responses.

An additional exploratory analysis was performed to evaluate the robustness of the previous model using imputed CD4+ IL-2+ responses at theoretical time points around week 2 (at day 10 and day 17, respectively) obtained by linear interpolation.

All statistical analyses were done on available data using SAS (SAS Institute, Inc., Cary, NC) version 9.3 and R version 3.2.1.

Results

Of the 30 participants randomized to each regimen, 26 rAd5-rAd5 participants and 28 DNA-rAd5 participants completed all vaccinations. The kinetics of their cellular immune responses—CD4+ IL-2+ T cells and CD8+ IFN- γ + T cells, respectively—after Env peptide stimulation are shown in [Fig 2](#).

A heat map of the Spearman correlations between CD4+ IL-2+ T cell and CD8+ IFN- γ + T cell responses between all time points in the rAd5-rAd5 group is presented in [Fig 3](#). In this group, moderate to high correlations (Spearman correlation coefficients ranging from 0.48 to 0.76) were observed between the percentage of CD4+ IL-2+ T cells at week 2 and the various measures of CD8+ IFN- γ + T cells from week 2 until the end of the trial ([Appendix C in S1 File](#)). In contrast, no relevant correlations between CD4+ IL-2+ and CD8+ IFN- γ + responses were found in the DNA-rAd5 group ([Appendixes D and E in S1 File](#)), and no significant correlations were found between CD4+ IL-2+ and antibody responses in either of the two groups. Robustness analyses using background-subtracted responses showed concordant results.

A complementary analysis of the correlation between CD4+ IL-2+ T cell and CD8+ IFN- γ + T cell responses in the rAd5-rAd5 group using pooled Env, Gag and Pol peptides stimulation is presented in [Appendix F in S1 File](#). Although the numbers of CD4+IL2+ at week 2 are still correlated (>0.5) with the CD8 response post-boost, the message is less clear than for Env only, as there are higher correlations between post boost CD4 and CD8 responses.

Guided by the results of the descriptive correlation analyses, regression modeling was performed only in the rAd5-rAd5 group using Env peptide stimulation data. The first model (model 1) fitted CD8+ IFN- γ as a function of CD4+ IL-2+ response at the same time point, and an additional effect of time using splines. In this model, CD4+ IL-2+ response was not significantly associated ($p = 0.28$) with CD8+ IFN- γ response ([Table 1](#)).

Model 2 was the best model with the lowest (i.e. best) AIC ([Table 1](#)). In this model, CD4+ IL-2+ responses observed at week 2 post-prime were significantly associated with CD8+ IFN- γ + response at all later time points, including time points after the boost: for an increase of 0.10% in the percentage of CD4+ IL-2+ at week 2, the percentage of CD8+ IFN- γ increased an average of 0.03% throughout follow-up ($p < 0.01$). In contrast, the values of CD4+ IL-2+ before week 2 were not significantly associated with CD8+ IFN- γ + response ($p = 0.82$).

In additional exploratory models with imputed CD4+ IL-2+ responses at day 10 and day 17 (obtained by linear interpolation, instead of using the week 2 measurement), CD4+ IL-2+

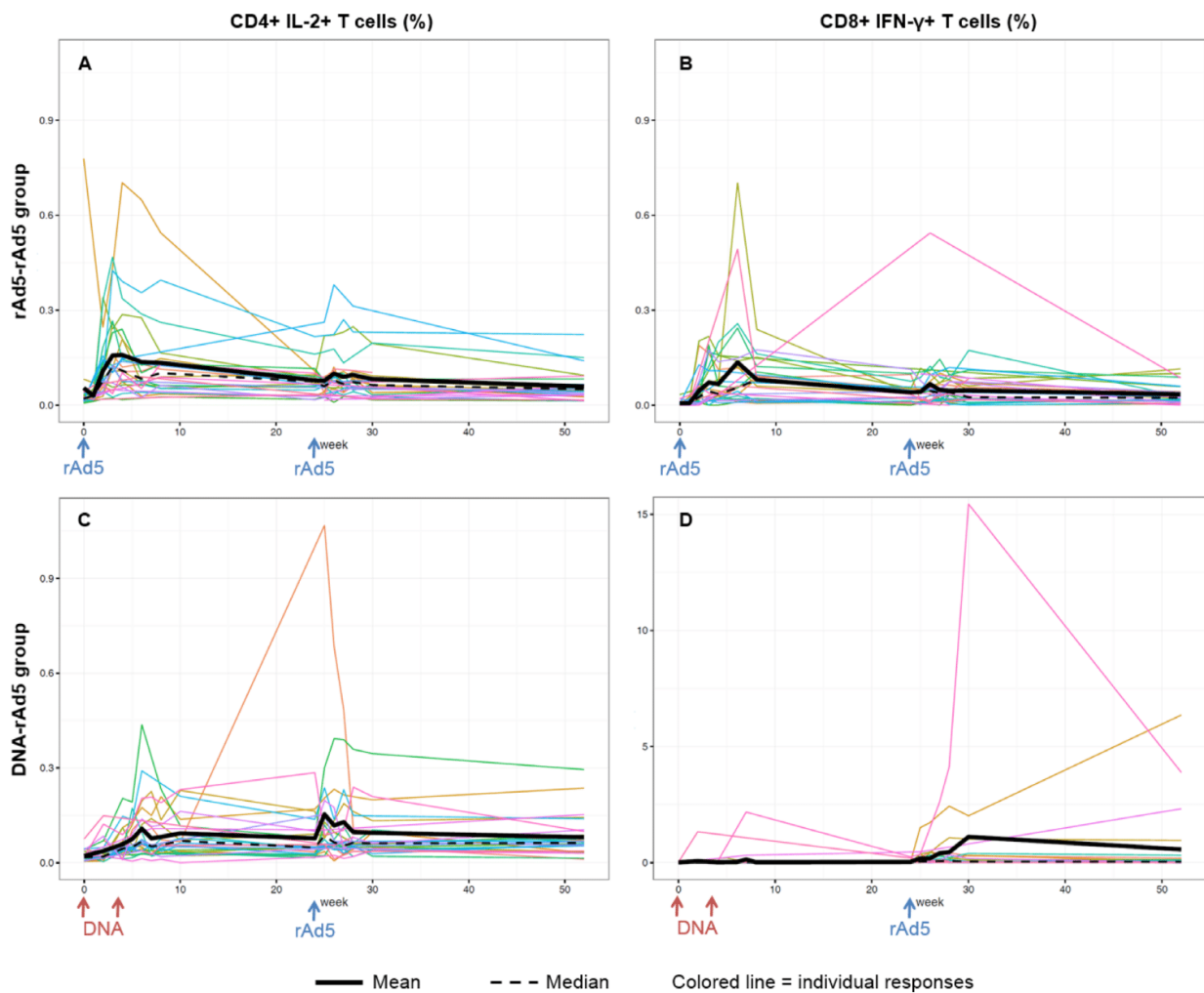


Fig 2. Kinetics of IL-2 producing CD4+ helper T cells (CD4+ IL-2+ T cells) and IFN- γ producing cytotoxic CD8+ T (CD8+ IFN- γ + T cells) responses over time after Env ex-vivo stimulation of PBMC in the rAd5-rAd5 and DNA-rAd5 groups respectively, HVTN 068 trial. (A) CD4+ IL-2+ T cells responses in the rAd5-rAd5 group. (B) CD8+ IFN- γ + T cells responses in the rAd5-rAd5 group. (C) CD4+ IL-2+ T cells responses in the DNA-rAd5 group. (D) CD8+ IFN- γ + T cells responses in the DNA-rAd5 group.

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T cells were also significantly associated with CD8+ IFN- γ + response ($p < 0.01$), but had higher AICs.

Discussion

We found a significant association in the rAd5-rAd5 group between the measurement of CD4+ IL-2+ responses 2 weeks following the prime and subsequent CD8+ IFN- γ + T cell responses, including responses after the boost. This result emphasizes the role of early CD4+ helper cells in stimulating the response of CD8+ cytotoxic T cells [11,12]. IL-2 signals from CD4+ T cells affect CD8+ T cells during all stages of an immune response, including primary expansion, contraction,

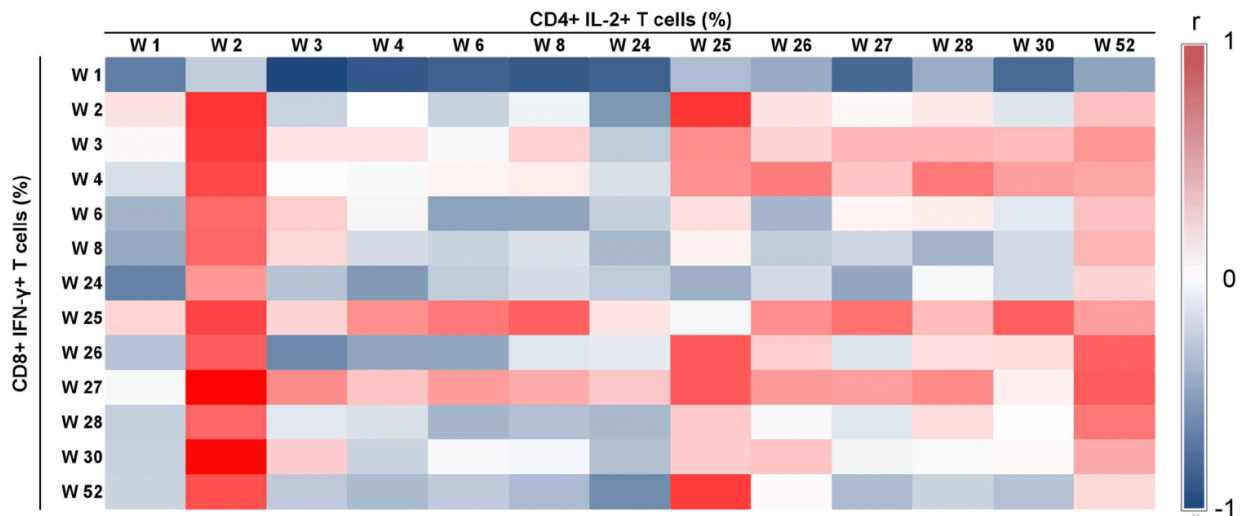


Fig 3. Heat map of Spearman correlation coefficients (r) between IL-2 producing CD4+ helper T cells (CD4+ IL-2+ T cells) and IFN-γ producing cytotoxic CD8+ T cells (CD8+ IFN-γ+ T cells) in the rAd5-rAd5 group (Env ex-vivo stimulation of PBMC), HVTN 068 trial.

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memory generation and secondary expansion [25–27]. One could hypothesize that the initial CD8+ T cell response may have little dependence on the CD4+ T cells during the first two weeks after prime vaccination, but that the production of IL-2 by antigen-stimulated CD4+ T cells intervenes to stimulate and sustain the CD8+ T cell response beyond that time.

The association with the measurement specifically at week 2 is novel in a HIV vaccine trial. First, this trial led to the opportunity to find this association because it featured early sampling times that are rarely performed. Similar trials typically have initial sample times that are a month or more after the prime, or even after the boost [28–31]. Second, we explored the effect of values of CD4+ T cell counts at several other time points based on the observed dynamics. Our finding of a specific association with a measure at week 2 indicates that adding this early sampling time point to future trials could be of interest at least for the measure of CD4+ T cell responses.

Table 1. Modeling of IFN-γ producing cytotoxic CD8+ T cells (CD8+ IFN-γ+ T cells) as a function of IL-2 producing CD4+ helper T cells (CD4+ IL-2+ T cells) and time (model 1 and model 2) in the rAd5-rAd5 group, HVTN 068 trial.

| Models | CD4+ IL2+ Tcell (each time) | | CD4+ IL2+ Tcell (day 0—day 7) | | CD4+ IL2+ Tcell (day 14) | | Time (splines) | AIC |
|---------|-----------------------------|---------|-------------------------------|---------|--------------------------|---------|----------------|--------|
| | β | p-value | β | p-value | β | p-value | p-value | |
| Model 1 | 0.004 | 0.28 | - | - | - | - | <0.01 | -846.2 |
| Model 2 | - | - | -0.001 | 0.82 | 0.031 | <0.01 | <0.01 | -860.7 |

Model 1: % of CD8+ IFN-γ modelled as a function of % of CD4+ IL-2+ response at each time, adjusted for time (splines).

Model 2: % of CD8+ IFN-γ modelled as a function of % of CD4+ IL-2+ response introduced as two explanatory variables (one taking values of observed % of CD4+ IL-2+ before week 2 and the second taking two values: 0 before week 2, and then the value observed at week 2 at each visit until the end of the follow-up), adjusted for time (splines)

The shown regression coefficients (β) correspond to the estimated increase in % of CD8+ IFN-γ+ T cells per 0.1% increase in CD4+ IL-2+. For an increase of 0.10% in the percentage of CD4+ IL-2+ at week 2, the percentage of CD8+ IFN-γ increased of 0.03% throughout follow-up (p<0.01). In contrast, the values of CD4+ IL-2+ before week 2 were not significantly associated with CD8+ IFN-γ+ response (p = 0.82).

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The main analyses focused on Env-specific responses because few Gag and Pol responses were detected [17]. However, we also looked at the correlation between the two cellular immunogenicity markers using pooled Env, Gag and Pol peptides stimulation. The message was less clear than for Env only, although the numbers of CD4+IL2+ at week 2 are still correlated (>0.5) with the CD8 response post-boost. This result could suggest a specific role of Env peptide stimulation in the early immune response post-prime compared to other peptides.

We found no significant correlations between cellular responses and antibody responses in this dataset. One might have expected a significant correlation because CD4+ helper T cells are also important for providing help to B cells in support of the humoral response [32]. Several hypotheses could explain the lack of significant correlations in our dataset. First, few sampling time points of serum antibody responses were available during follow-up, limiting the correlation analyses to a small number of time points. One cannot exclude the possibility that relevant correlation may exist for time points not observed in this trial. Second, these are measured markers in the blood and therefore may not represent the dynamics of immune response markers at other relevant tissue sites for vaccine-induced immune responses.

Immune responses to the vaccine strategies were different between the two arms of HVTN 068 trial, and no correlations between the CD4+ IL-2+ and CD8+ IFN- γ + responses were observed in the group with the DNA vaccine prime. Indeed, few responses were detectable by intracellular cytokine staining following the DNA prime injections in this group, whereas responses to the boost were similar or higher than the ones in the rAd5-rAd5 group [17]. Previous studies have shown that DNA vaccines alone are not highly immunogenic in humans [33], but that they prime the responses for a heterologous boost. The immune effects elicited by a DNA prime may be too subtle to be detected by intracellular cytokine staining or may rely on a different immune mechanism than the ones usually assessed in clinical trials. In vitro expansion of CD4+ T cells could be relevant to evaluate the initial response in this type of situation but its association with the CD8 response remained to be demonstrated in this setting. Other immunological markers (e.g. transcriptome) may be helpful to better examine subtle effects of DNA vaccine primes [17]. Moreover, newer DNA vaccination strategies such as the process of electroporation, which facilitates the entrance of the DNA in cells, shows markedly better CD4+ and CD8+ vaccine responses [34]. These strategies might even be better for the modeling of DNA vaccines.

To our knowledge this is the first study modeling the immune response dynamics to a prophylactic HIV vaccine strategy in humans. Before generalizing our findings, validation in external datasets would be of interest. However, existing HIV vaccine clinical trial datasets with CD4+ cell response measurements early after the prime are scarce, and we have thus far not had the opportunity to perform an external validation of our hypothesis.

In conclusion, this study highlighted the early role of the CD4 helper T cell response, as measured by CD4+ IL-2+ T cells, in the cellular response to an Ad5-Ad5 vaccine strategy, and in particular in the stimulation of subsequent cytotoxic CD8 T cell responses. Early sampling time points should be considered in future clinical trials to better understand the role of the early CD4 helper T cells and to evaluate their predictive role in the immune response to vaccines.

Supporting Information

S1 File.
(PDF)

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Author Contributions

Conceived and designed the experiments: EL LR ZM CP SK CM SS SDR RT. Performed the experiments: EL LR ZM CP SK CM SS SDR RT. Analyzed the data: EL LR CP RT. Contributed reagents/materials/analysis tools: EL LR ZM CP SK CM SS SDR RT. Wrote the paper: EL LR ZM CP SK CM SS SDR RT.

References

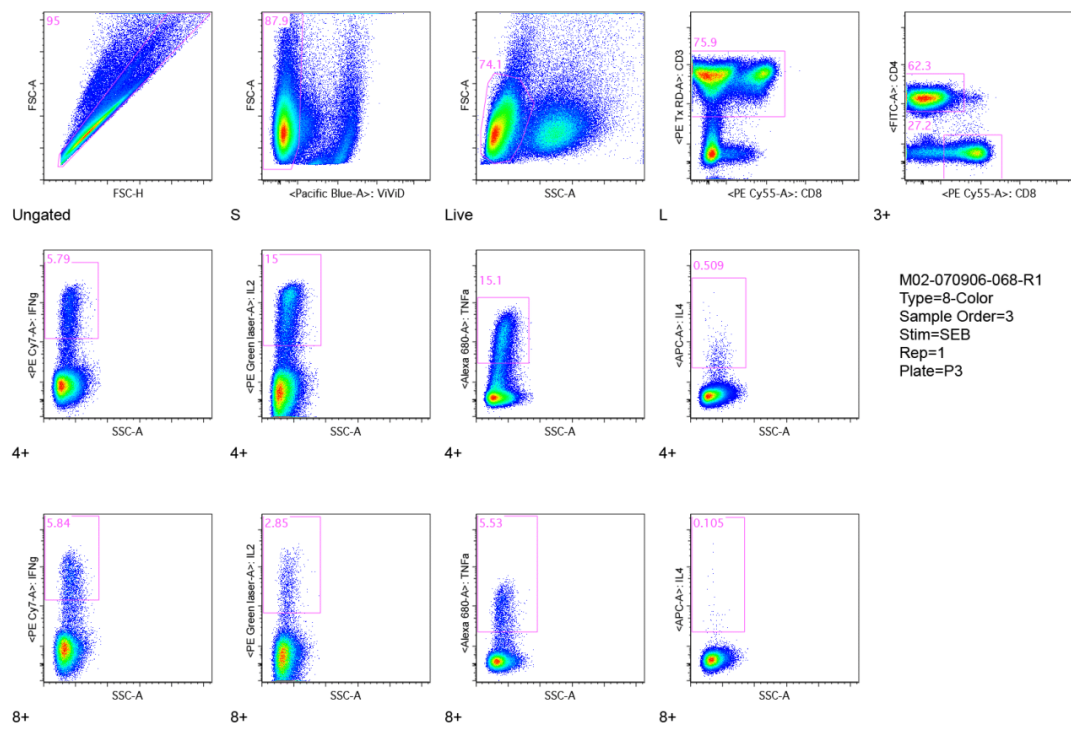
1. Barré-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest J, et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* 1983; 220(4599): 868–71. PMID: [6189183](#)
2. Joint United Nations Programme on HIV/AIDS (UNAIDS). UNAIDS report on the global AIDS epidemic. Geneva, Switzerland: 2013.
3. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. *N Engl J Med* 2009; 361(23): 2209–20. doi: [10.1056/NEJMoa0908492](#) PMID: [19843557](#)
4. Buchbinder SP, Mehrotra DV, Duerr A, Fitzgerald DW, Mogg R, Li D, et al. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. *Lancet* 2008; 372(9653): 1881–93. doi: [10.1016/S0140-6736\(08\)61591-3](#) PMID: [19012954](#)
5. Flynn NM, Forthal DN, Harro CD, Judson FN, Mayer KH, Para MF. Placebo-controlled phase 3 trial of a recombinant glycoprotein 120 vaccine to prevent HIV-1 infection. *J Infect Dis* 2005; 191(5): 654–65. PMID: [15688278](#)
6. Gray GE, Allen M, Moodie Z, Churchyard G, Bekker LG, Nchabeleng M, et al. Safety and efficacy of the HVTN 503/Phambili study of a clade-B-based HIV-1 vaccine in South Africa: a double-blind, randomised, placebo-controlled test-of-concept phase 2b study. *Lancet Infect Dis* 2011; 11(7): 507–15. doi: [10.1016/S1473-3099\(11\)70098-6](#) PMID: [21570355](#)
7. Hammer SM, Sobieszczek ME, Janes H, Karuna ST, Mulligan MJ, Grove D, et al. Efficacy Trial of a DNA/rAd5 HIV-1 Preventive Vaccine. *N Engl J Med* 2013; 369(22): 2083–92. doi: [10.1056/NEJMoa1310566](#) PMID: [24099601](#)
8. Pitisuttithum P, Gilbert P, Gurwith M, Heyward W, Martin M, van Griensven F, et al. Randomized, double-blind, placebo-controlled efficacy trial of a bivalent recombinant glycoprotein 120 HIV-1 vaccine among injection drug users in Bangkok, Thailand. *J Infect Dis* 2006; 194(12): 1661–71. PMID: [17109337](#)
9. Gilbert PB, Berger JO, Stablein D, Becker S, Essex M, Hammer SM, et al. Statistical interpretation of the RV144 HIV vaccine efficacy trial in Thailand: a case study for statistical issues in efficacy trials. *J Infect Dis* 2011; 203(7): 969–75. doi: [10.1093/infdis/jiq152](#) PMID: [21402548](#)
10. Robb ML, Rerks-Ngarm S, Nitayaphan S, Pitisuttithum P, Kaewkungwal J, Kunasol P, et al. Risk behaviour and time as covariates for efficacy of the HIV vaccine regimen ALVAC-HIV (vC1521) and AIDSVAX B/E: a post-hoc analysis of the Thai phase 3 efficacy trial RV 144. *Lancet Infect Dis* 2012; 12(7): 531–7. doi: [10.1016/S1473-3099\(12\)70088-9](#) PMID: [22652344](#)
11. Bevan MJ. Helping the CD8+ T-cell response. *Nat Rev Immunol* 2004; 4(8): 595–602. PMID: [15286726](#)
12. Seder RA, Darrah PA, Roederer M. T-cell quality in memory and protection: implications for vaccine design. *Nat Rev Immunol* 2008; 8(4): 247–58. doi: [10.1038/nri2274](#) PMID: [18323851](#)
13. Richert L, Lhomme E, Fagard C, Lévy Y, Chêne G, Thiébaud R. Recent developments in clinical trial designs for HIV vaccine research. *Hum Vaccin Immunother* 2015; 11(4): 1022–9. doi: [10.1080/21645515.2015.1011974](#) PMID: [25751670](#)
14. Streeck H, Nixon DF. T cell immunity in acute HIV-1 infection. *J Infect Dis* 2010; 202: S302–8. doi: [10.1086/655652](#) PMID: [20846037](#)
15. Ling Munier CM, Kelleher AD, Kent SJ, De Rose R. The role of T cell immunity in HIV-1 infection. *Curr Opin Virol* 2013; 3(4): 438–46. doi: [10.1016/j.coviro.2013.05.009](#) PMID: [23747036](#)
16. Perreau M, Levy Y, Pantaleo G. Immune response to HIV. *Curr Opin HIV AIDS* 2013; 8(4): 333–40. doi: [10.1097/COH.0b013e328361faf4](#) PMID: [23743723](#)

17. De Rosa SC, Thomas EP, Bui J, Huang Y, deCamp A, Morgan C, et al. HIV-DNA priming alters T-cell responses to HIV-adenovirus vaccine even when responses to DNA are undetectable. *J Immunol* 2011; 187(6): 3391–401. doi: [10.4049/jimmunol.1101421](https://doi.org/10.4049/jimmunol.1101421) PMID: [21844392](https://pubmed.ncbi.nlm.nih.gov/21844392/)
18. Horton H, Thomas E, Stucky J, Frank I, Moodie Z, Huang Y, et al. Optimization and Validation of an 8-Color Intracellular Cytokine Staining (ICS) Assay to Quantify Antigen-Specific T Cells Induced by Vaccination. *J Immunol Methods* 2007; 323(1): 39–54. PMID: [17451739](https://pubmed.ncbi.nlm.nih.gov/17451739/)
19. Goepfert PA, Tomaras GD, Horton H, Montefiori D, Ferrari G, Deers M, et al; NIAID HIV Vaccine Trials Network. Durable HIV-1 antibody and T-cell responses elicited by an adjuvanted multi-protein recombinant vaccine in uninfected human volunteers. *Vaccine* 2007; 25(3):510–8. PMID: [17049679](https://pubmed.ncbi.nlm.nih.gov/17049679/)
20. Su LF, Kidd BA, Han A, Kotzin JJ, Davis MM. Virus-specific CD4(+) memory-phenotype T cells are abundant in unexposed adults. *Immunity* 2013; 38: 373–83. doi: [10.1016/j.immuni.2012.10.021](https://doi.org/10.1016/j.immuni.2012.10.021) PMID: [23395677](https://pubmed.ncbi.nlm.nih.gov/23395677/)
21. Benjamini Y, Hochberg Y. On the Adaptive Control of the False Discovery Rate in Multiple Testing with Independent Statistics. *J Educ Behav Stat* 2000; 25: 60–83.
22. Jacqmin-Gadda H, Sibillot S, Proust C, Molina JM, Thiébaud R. Robustness of the linear mixed model to misspecified error distribution. *Computational Statistics and Data Analysis* 2007; 51: 5142–54.
23. Verbeke G, Molenberghs G. *Linear mixed models for longitudinal data*. New-York, Springer; 2000.
24. Harrell FE. *Regression Modeling Strategies: With Applications to Linear Models, Logistic Regression, and Survival Analysis*. New York: Springer; 2001.
25. Boyman O, Cho JH, Sprent J. The role of interleukin-2 in memory CD8 cell differentiation. *Adv Exp Med Biol*. 2010; 684:28–41. PMID: [20795538](https://pubmed.ncbi.nlm.nih.gov/20795538/)
26. Boyman O, Sprent J. The role of interleukin-2 during homeostasis and activation of the immune system. *Nat Rev Immunol* 2012; 12(3):180–90. doi: [10.1038/nri3156](https://doi.org/10.1038/nri3156) PMID: [22343569](https://pubmed.ncbi.nlm.nih.gov/22343569/)
27. Lai YP, Lin CC, Liao WJ, Tang CY, Chen SC. CD4+ T cell-derived IL-2 signals during early priming advances primary CD8+ T cell responses. *PLoS One* 2009; 4(11): e7766. doi: [10.1371/journal.pone.0007766](https://doi.org/10.1371/journal.pone.0007766) PMID: [19901991](https://pubmed.ncbi.nlm.nih.gov/19901991/)
28. García F, Bernaldo de Quirós JC, Gómez CE, Perdiguero B, Nájera JL, Jiménez V, et al. Safety and immunogenicity of a modified pox vector-based HIV/AIDS vaccine candidate expressing Env, Gag, Pol and Nef proteins of HIV-1 subtype B (MVA-B) in healthy HIV-1-uninfected volunteers: A phase I clinical trial (RISVAC02). *Vaccine* 2011; 29(46):8309–16. doi: [10.1016/j.vaccine.2011.08.098](https://doi.org/10.1016/j.vaccine.2011.08.098) PMID: [21907749](https://pubmed.ncbi.nlm.nih.gov/21907749/)
29. Kalams SA, Parker S, Jin X, Elizaga M, Metch B, Wang M, et al. NIAID HIV Vaccine Trials Network. Safety and Immunogenicity of an HIV-1 Gag DNA Vaccine with or without IL-12 and/or IL-15 Plasmid Cytokine Adjuvant in Healthy, HIV-1 Uninfected Adults. *PLoS One* 2012; 7(1):e29231. doi: [10.1371/journal.pone.0029231](https://doi.org/10.1371/journal.pone.0029231) PMID: [22242162](https://pubmed.ncbi.nlm.nih.gov/22242162/)
30. Frey SE, Peiperl L, McElrath MJ, Kalams S, Goepfert PA, Keefer MC, et al. Phase I/II Randomized Trial of Safety and Immunogenicity of LIPO-5 Alone, ALVAC-HIV (vCP1452) Alone, and ALVAC-HIV (vCP1452) Prime/LIPO-5 Boost in Healthy, HIV-1-Uninfected Adult Participants. *Clin Vaccine Immunol* 2014; 21(11):1589–99. doi: [10.1128/CVI.00450-14](https://doi.org/10.1128/CVI.00450-14) PMID: [25253665](https://pubmed.ncbi.nlm.nih.gov/25253665/)
31. Currier JR, Ngauy V, de Souza MS, Ratto-Kim S, Cox JH, Polonis VR, et al. Phase I Safety and Immunogenicity Evaluation of MVA-CMDR, a Multigenic, Recombinant Modified Vaccinia Ankara-HIV-1 Vaccine Candidate. *PLoS One* 2010; 5(11):e13983. doi: [10.1371/journal.pone.0013983](https://doi.org/10.1371/journal.pone.0013983) PMID: [21085591](https://pubmed.ncbi.nlm.nih.gov/21085591/)
32. Agnellini P, Wiesel M, Schwarz K, Wolint P, Bachmann MF, Oxenius A. Kinetic and mechanistic requirements for helping CD8 T cells. *J Immunol* 2008; 180(3): 1517–25. PMID: [18209047](https://pubmed.ncbi.nlm.nih.gov/18209047/)
33. Jin X, Morgan C, Yu X, DeRosa S, Tomaras GD, Montefiori DC, et al. Multiple factors affect immunogenicity of DNA plasmid HIV vaccines in human clinical trials. *Vaccine* 2015; 33(20): 2347–53. doi: [10.1016/j.vaccine.2015.03.036](https://doi.org/10.1016/j.vaccine.2015.03.036) PMID: [25820067](https://pubmed.ncbi.nlm.nih.gov/25820067/)
34. Kalams SA, Parker SD, Elizaga M, Metch B, Edupuganti S, Hural J, et al. Safety and comparative immunogenicity of an HIV-1 DNA vaccine in combination with plasmid interleukin 12 and impact of intramuscular electroporation for delivery. *J Infect Dis* 2013; 208(5): 818–29. doi: [10.1093/infdis/jit236](https://doi.org/10.1093/infdis/jit236) PMID: [23840043](https://pubmed.ncbi.nlm.nih.gov/23840043/)

Early CD4+ T cell responses are associated with subsequent CD8+ T cell responses to a rAd5-based prophylactic prime-boost HIV vaccine strategy

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Supporting Information



Appendix A. Example of gating hierarchy for 8-color ICS assay.

The upper row shows the series of gates to identify CD4+ and CD8+ T cells. In order, cells are gated for singlets, live cells, lymphocytes, CD3+ T cells and then CD4+ and CD8+ T cells. The middle and lower rows show the cytokine gates for CD4+ and CD8+ T cells, respectively. This is an example for the SEB positive control. The numbers in the gates show the percent of the gated cells of the parent gate.

Appendix C. Spearman correlations between IFN- γ producing cytotoxic CD8+ T cells (CD8+ IFN- γ + T cells) and IL-2 producing CD4+ helper T cells (CD4+ IL-2+ T cells) across follow-up in the rAd5-rAd5 group (Env ex-vivo stimulation of PBMC), HVTN 068 trial

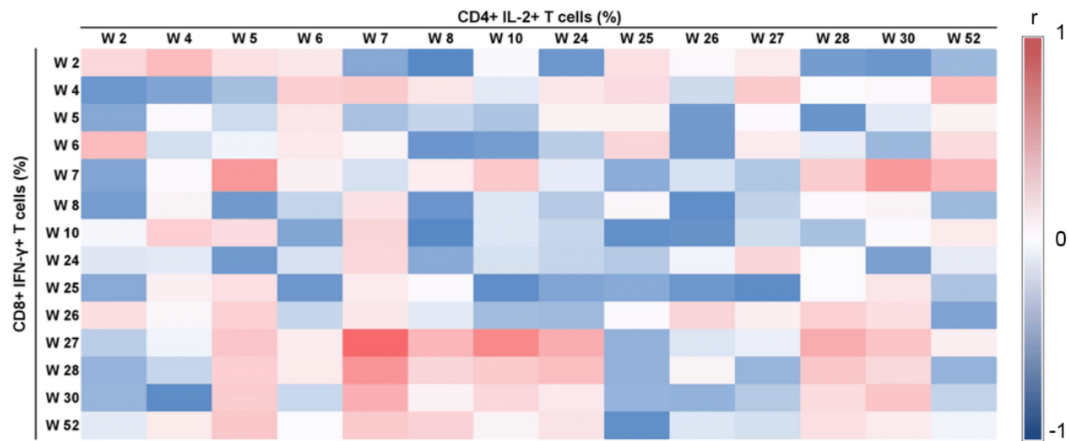
| CD8+ IFN- γ + T cells (%) | CD4+ IL-2+ T cells (%) | | | | | | | | | | | | | |
|----------------------------------|------------------------|-------|-------------|-------|-------|-------|-------|-------|-------------|------|-------|------|-------|------|
| | Prime | | | | | | Boost | | | | | | | |
| | W 1 | W 2 | W 3 | W 4 | W 6 | W 8 | W24 | W 25 | W 26 | W 27 | W 28 | W 30 | W 52 | |
| Prime | | | | | | | | | | | | | | |
| W 1 | r | -0.01 | 0.17 | -0.14 | -0.10 | -0.08 | -0.09 | -0.08 | 0.14 | 0.10 | -0.06 | 0.10 | -0.06 | 0.07 |
| | p-value | 0.89 | 0.63 | 0.69 | 0.73 | 0.74 | 0.73 | 0.74 | 0.72 | 0.73 | 0.79 | 0.73 | 0.79 | 0.75 |
| W 2 | r | 0.34 | 0.67 | 0.19 | 0.28 | 0.18 | 0.25 | 0.04 | 0.66 | 0.33 | 0.30 | 0.33 | 0.23 | 0.40 |
| | p-value | 0.39 | 0.02 | 0.55 | 0.39 | 0.61 | 0.47 | 0.82 | 0.06 | 0.36 | 0.45 | 0.36 | 0.55 | 0.26 |
| W 3 | r | 0.30 | 0.65 | 0.33 | 0.33 | 0.27 | 0.37 | 0.16 | 0.50 | 0.36 | 0.42 | 0.42 | 0.41 | 0.48 |
| | p-value | 0.45 | 0.02 | 0.33 | 0.33 | 0.47 | 0.35 | 0.64 | 0.26 | 0.33 | 0.33 | 0.26 | 0.33 | 0.18 |
| W 4 | r | 0.22 | 0.63 | 0.28 | 0.28 | 0.31 | 0.32 | 0.22 | 0.49 | 0.52 | 0.40 | 0.53 | 0.46 | 0.44 |
| | p-value | 0.56 | 0.04 | 0.41 | 0.41 | 0.44 | 0.42 | 0.56 | 0.26 | 0.16 | 0.35 | 0.13 | 0.29 | 0.26 |
| W 6 | r | 0.11 | 0.56 | 0.37 | 0.27 | 0.07 | 0.07 | 0.17 | 0.34 | 0.12 | 0.30 | 0.32 | 0.24 | 0.40 |
| | p-value | 0.71 | 0.18 | 0.38 | 0.48 | 0.74 | 0.74 | 0.63 | 0.44 | 0.70 | 0.44 | 0.43 | 0.50 | 0.33 |
| W 8 | r | 0.09 | 0.57 | 0.36 | 0.21 | 0.18 | 0.22 | 0.12 | 0.31 | 0.16 | 0.19 | 0.11 | 0.20 | 0.42 |
| | p-value | 0.74 | 0.16 | 0.40 | 0.58 | 0.60 | 0.55 | 0.70 | 0.48 | 0.63 | 0.60 | 0.71 | 0.58 | 0.32 |
| Boost | | | | | | | | | | | | | | |
| W 24 | r | -0.00 | 0.48 | 0.15 | 0.05 | 0.17 | 0.21 | 0.16 | 0.10 | 0.20 | 0.08 | 0.27 | 0.20 | 0.37 |
| | p-value | 0.92 | 0.26 | 0.68 | 0.81 | 0.63 | 0.58 | 0.63 | 0.74 | 0.58 | 0.74 | 0.48 | 0.58 | 0.39 |
| W 25 | r | 0.36 | 0.63 | 0.37 | 0.49 | 0.53 | 0.58 | 0.33 | 0.27 | 0.50 | 0.55 | 0.41 | 0.58 | 0.46 |
| | p-value | 0.45 | 0.09 | 0.37 | 0.26 | 0.26 | 0.26 | 0.48 | 0.45 | 0.26 | 0.26 | 0.33 | 0.26 | 0.26 |
| W 26 | r | 0.15 | 0.59 | 0.01 | 0.08 | 0.08 | 0.23 | 0.24 | 0.59 | 0.37 | 0.23 | 0.34 | 0.35 | 0.58 |
| | p-value | 0.67 | 0.10 | 0.89 | 0.74 | 0.74 | 0.55 | 0.53 | 0.16 | 0.32 | 0.58 | 0.36 | 0.39 | 0.11 |
| W 27 | r | 0.28 | 0.76 | 0.50 | 0.39 | 0.47 | 0.44 | 0.39 | 0.59 | 0.47 | 0.47 | 0.50 | 0.31 | 0.59 |
| | p-value | 0.48 | 0.02 | 0.26 | 0.33 | 0.26 | 0.29 | 0.36 | 0.26 | 0.27 | 0.26 | 0.26 | 0.44 | 0.16 |
| W 28 | r | 0.18 | 0.57 | 0.23 | 0.21 | 0.11 | 0.14 | 0.12 | 0.38 | 0.27 | 0.23 | 0.35 | 0.29 | 0.54 |
| | p-value | 0.61 | 0.10 | 0.26 | 0.50 | 0.71 | 0.67 | 0.70 | 0.33 | 0.44 | 0.56 | 0.33 | 0.46 | 0.14 |
| W 30 | r | 0.18 | 0.75 | 0.38 | 0.19 | 0.27 | 0.27 | 0.14 | 0.38 | 0.39 | 0.26 | 0.28 | 0.29 | 0.44 |
| | p-value | 0.61 | 0.02 | 0.37 | 0.61 | 0.45 | 0.47 | 0.68 | 0.41 | 0.33 | 0.48 | 0.47 | 0.44 | 0.29 |
| W 52 | r | 0.19 | 0.62 | 0.16 | 0.13 | 0.16 | 0.13 | 0.01 | 0.65 | 0.29 | 0.13 | 0.18 | 0.14 | 0.35 |
| | p-value | 0.61 | 0.05 | 0.60 | 0.66 | 0.63 | 0.68 | 0.88 | 0.06 | 0.42 | 0.69 | 0.58 | 0.67 | 0.29 |

FDR-adjusted p-values < 0.10 were considered statistically significant and are presented in bold.

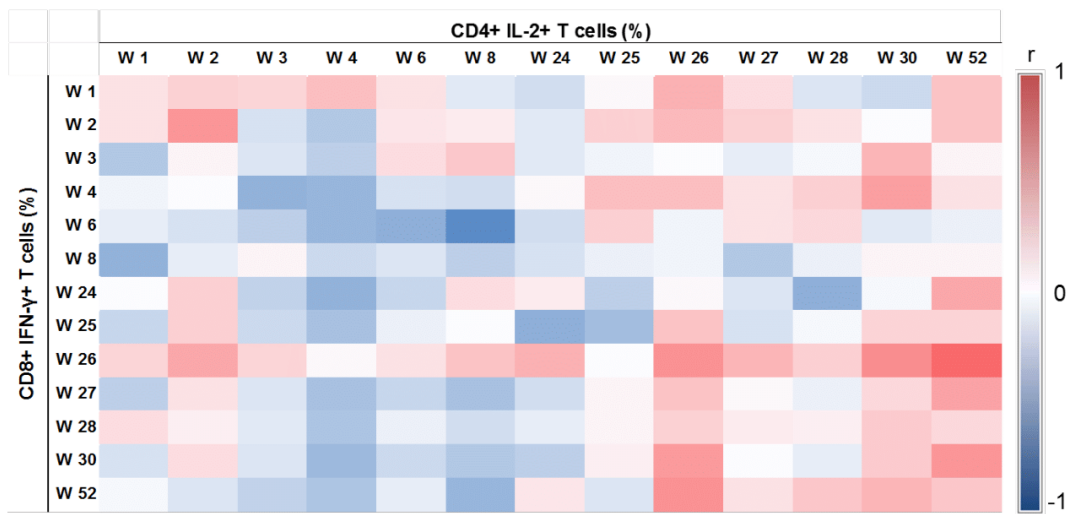
Appendix D. Spearman correlations between IFN- γ producing cytotoxic CD8+ T cells (CD8+ IFN- γ + T cells) and of IL-2 producing CD4+ helper T cells (CD4+ IL-2+ T cells) across follow-up in the DNA-rAd5 group (Env ex-vivo stimulation of PBMC), HVTN 068 trial

| CD8+ IFN- γ + T cells (%) | CD4+ IL-2+ T cells (%) | | | | | | | | | | | | | | |
|----------------------------------|------------------------|-------|-------|-------|-------|------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | Prime | | | | | | Boost | | | | | | | | |
| | W 2 | W 4 | W 5 | W 6 | W 7 | W 8 | W 10 | W 24 | W 25 | W 26 | W 27 | W 28 | W 30 | W 52 | |
| Prime | | | | | | | | | | | | | | | |
| W 2 | r | 0.27 | -0.38 | -0.25 | -0.22 | 0.04 | 0.00 | 0.14 | -0.02 | -0.25 | -0.17 | -0.21 | 0.03 | 0.02 | -0.06 |
| | <i>p-value</i> | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 |
| W 4 | r | 0.02 | -0.04 | -0.07 | -0.31 | -0.3 | -0.23 | -0.13 | -0.22 | -0.26 | -0.11 | -0.33 | -0.16 | -0.17 | -0.38 |
| | <i>p-value</i> | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 |
| W 5 | r | 0.04 | -0.11 | 0.11 | -0.23 | -0.0 | -0.10 | -0.08 | -0.19 | -0.19 | -0.03 | -0.16 | -0.02 | -0.13 | -0.19 |
| | <i>p-value</i> | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 |
| W 6 | r | -0.38 | 0.12 | -0.14 | -0.22 | 0.18 | 0.01 | -0.03 | -0.09 | -0.29 | 0.02 | -0.21 | 0.13 | -0.07 | -0.27 |
| | <i>p-value</i> | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 |
| W 7 | r | -0.04 | 0.17 | 0.50 | 0.19 | 0.11 | 0.20 | 0.33 | 0.13 | 0.05 | -0.12 | 0.08 | 0.31 | 0.49 | 0.39 |
| | <i>p-value</i> | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 |
| W 8 | r | -0.03 | -0.18 | 0.02 | -0.10 | 0.24 | 0.02 | 0.12 | 0.08 | 0.18 | 0.01 | -0.10 | 0.16 | 0.18 | 0.06 |
| | <i>p-value</i> | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 |
| W 10 | r | -0.15 | -0.31 | 0.26 | 0.04 | 0.28 | 0.00 | 0.12 | 0.10 | 0.01 | -0.02 | -0.11 | 0.07 | 0.16 | 0.21 |
| | <i>p-value</i> | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 |
| Boost | | | | | | | | | | | | | | | |
| W 24 | r | -0.13 | -0.13 | -0.02 | -0.12 | 0.28 | 0.05 | 0.11 | 0.10 | -0.09 | -0.14 | -0.29 | 0.15 | 0.03 | -0.13 |
| | <i>p-value</i> | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 |
| W 25 | r | -0.05 | 0.20 | 0.24 | -0.02 | 0.20 | -0.16 | -0.01 | 0.04 | 0.05 | 0.02 | 0.00 | 0.15 | 0.23 | 0.08 |
| | <i>p-value</i> | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 |
| W 26 | r | -0.25 | 0.18 | 0.30 | 0.10 | 0.22 | 0.13 | -0.07 | -0.07 | 0.17 | 0.28 | 0.19 | 0.30 | 0.25 | -0.04 |
| | <i>p-value</i> | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 |
| W 27 | r | 0.09 | 0.14 | 0.34 | 0.21 | 0.67 | 0.40 | 0.55 | 0.42 | 0.05 | 0.12 | 0.13 | 0.43 | 0.35 | 0.19 |
| | <i>p-value</i> | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 |
| W 28 | r | -0.06 | 0.10 | 0.31 | 0.21 | 0.51 | 0.29 | 0.32 | 0.37 | 0.05 | 0.18 | 0.06 | 0.33 | 0.27 | 0.05 |
| | <i>p-value</i> | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 |
| W 30 | r | -0.06 | 0.01 | 0.32 | 0.11 | 0.42 | 0.19 | 0.28 | 0.21 | 0.06 | 0.05 | 0.08 | 0.26 | 0.34 | 0.10 |
| | <i>p-value</i> | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 |
| W 52 | r | -0.13 | 0.21 | 0.34 | 0.16 | 0.32 | 0.30 | 0.18 | 0.23 | -0.01 | 0.12 | 0.11 | 0.24 | 0.20 | 0.14 |
| | <i>p-value</i> | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 |

FDR-adjusted p-values < 0.10 were considered statistically significant and are presented in bold.



Appendix E. Heat map of Spearman correlation (r) between IL-2 producing CD4+ helper T cells (CD4+ IL-2+ T cells) and IFN- γ producing cytotoxic CD8+ T cells (CD8+ IFN- γ + T cells) in the DNA-rAd5 group (Env ex-vivo stimulation of PBMC), HVTN 068 trial.



Appendix F. Heat map of Spearman correlation (r) between IL-2 producing CD4+ helper T cells (CD4+ IL-2+ T cells) and IFN- γ producing cytotoxic CD8+ T cells (CD8+ IFN- γ + T cells) in the rAd5-rAd5 group (pooled Env, Gag and Pol ex-vivo stimulation of PBMC), HVTN 068 trial.

2. Bivariate modelling for analyzing the T-cell response

Recent technological advances in flow cytometry have transformed the field of immunology. A large number of parameters are quantified at the level of the single cell. Intracellular cytokine staining (ICS) assay is now an important tool for characterizing subsets of antigen-specific T cell subsets capable of simultaneously producing multiple effector cytokines and other functional markers (polyfunctional T cells). In vaccine trials, this tool is used to measure post-vaccination T cell response, as in the HVTN 068 trial where the T cell response was evaluated by ICS at each time point. Statistical tools currently used to analyze are based on traditional comparison tests that are not taken into account the complexity of these immune responses. New statistical approaches adapted the specificity of immunological assay are needed.

In this part, we will focus on data analysis of the ICS assay during vaccine clinical trials and the improvement the analysis of these immunological assay for a better evaluation of the vaccine effect in vaccine clinical trials. To properly understand the methodological issues related to the analysis of these data, it is necessary to describe how this technique works.

ICS: the common methods for evaluating the T-cell response

Although the IFN γ ELISpot assay was historically used to measure the T-cell responses to vaccine candidates, the intracellular cytokine staining (ICS), a flow cytometry assay involving cryopreserved peripheral blood mononuclear cells (PBMCs) of the participants, is now used more commonly. ICS assay allows simultaneously characterization of the phenotype of individual cells and the production of intra-cellular cytokines after *ex vivo* stimulation by antigen (such as HIV proteins or pools of overlapping peptides for an HIV protein in HIV vaccine trials) [4-6]. Cytokines are small protein, glycoprotein or peptide molecules that, through cell signaling, allow intricate cellular communication. The overview of the ICS process is presented in Figure 3. The first step is an *in vitro* stimulation. For a sample (PBMCs) of one participant at one time point, the sample is divided in several wells. Some wells are stimulated by pools of antigen peptide (eg. Gag, Pol, Env in HIV vaccine trials), and other are not stimulated and are used as negative control. After stimulation, whole PBMCs are labeled with fluorophore-conjugated antibodies against phenotypic (e.g. CD4, CD3, CD8) and functional (e.g. IFN γ , IL2, TNF α) markers. The expression of each marker on each labeled cell is measured via flow cytometry, wherein cells pass in single-file through a flow cell and lasers of different wave lengths excite the fluorophores on the markers. A series of filters and detectors measure the emitted photons from the different fluorophores, providing a measure of intensity proportional to the amount of each protein expressed by each cell. After acquisition, data are

processed and distinct cell populations of interest identified via a process termed “gating”, which describes identifying thresholds in multivariate space that classify each marker as either ‘positive’ (expressed) or ‘negative’ (not expressed). Then, the data are exported and the results are reported as percentages of CD4+ and CD8+ T cells producing a cytokine of interest.

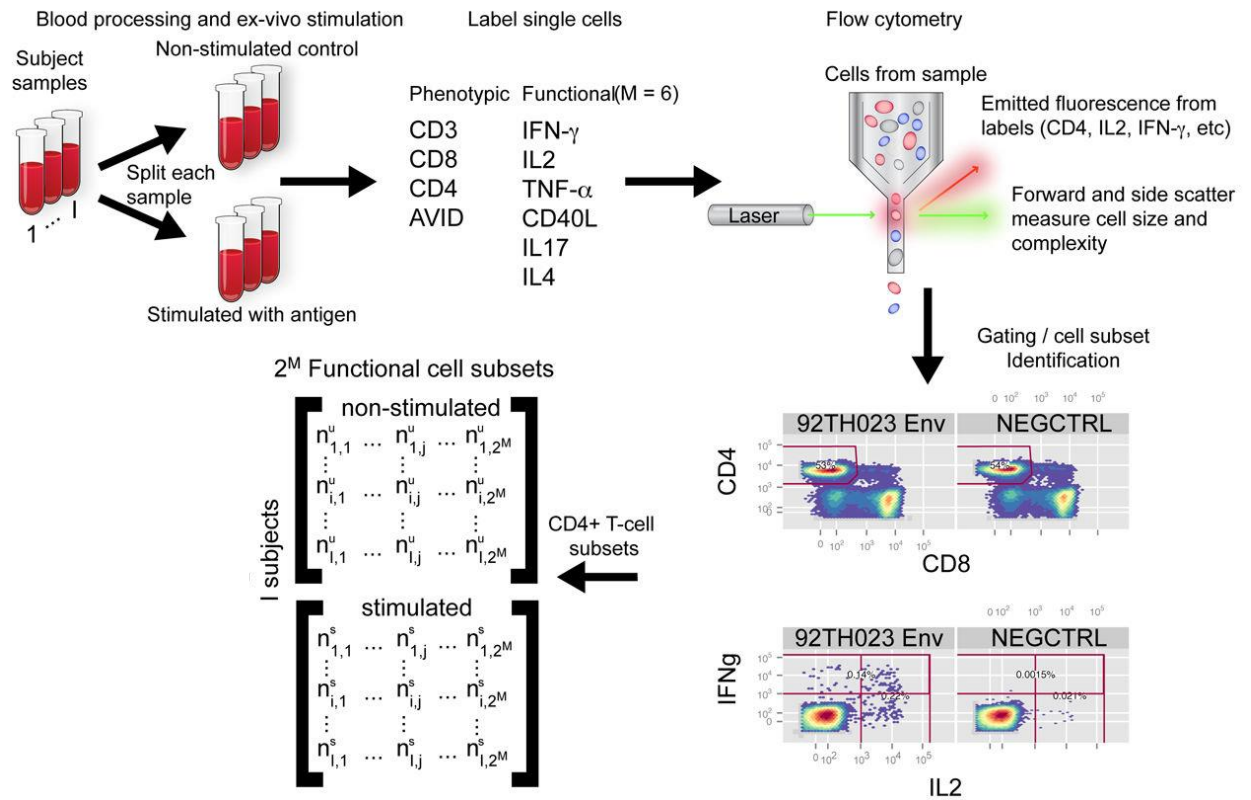


Figure 3. Overview of an intracellular cytokine staining (ICS) experiment. Adapted from Lin and al, Nat Biotechnol. 2015.

For instance, in the case of evaluation of an HIV vaccine candidate, the cellular responses can be measured by enumerating IFN γ -, IL2-, and TNF α -producing CD4+ and CD8+ T cells by flow cytometry, after stimulation of PBMCs with pools of the HIV peptides contained in the vaccine sequence, e.g., Gag, Nef, Pol, and/or Env [7-10]. An example of an ICS dataset is presented in Table 2. We can see that for one sample of one participant at one time point, we have the CD4+ Tcell response producing a cytokine of interest (IFN γ , IL2, or TNF α) after stimulation by antigen Nef, and in mirror the same response for the non-stimulated aliquots. In summary, for each time point of each participants, the dataset for each subject I at each time point Y_i^{NS} the non-stimulated response and Y_i^S the specific stimulated response by antigen(s) of interest.

Table 2. Example of a ICS dataset for one sample A of one participant at one time point with the CD4+ T cell response producing IFN γ , IL2 and TNF α after stimulation by Nef (antigen).

| Sample | Stimulation | T-cell | Population | Percentage |
|---------------|--------------------|---------------|-------------------|-------------------|
| A | Nef | CD4 | IFN γ | 0.0285 |
| A | Nef | CD4 | IL2 | 0.1985 |
| A | Nef | CD4 | TNF α | 0.018 |
| ... | ... | ... | ... | ... |
| A | Non stimulated | CD4 | IFN γ | 0.014 |
| A | Non stimulated | CD4 | IL2 | 0.268 |
| A | Non stimulated | CD4 | TNF α | 0.006 |
| ... | ... | ... | ... | ... |

Statistical methods for analyzing cellular immune responses in vaccine clinical trials

A variety of approaches exist for the statistical analysis of ICS data. Several authors have proposed statistical methods for the creation of qualitative binary criteria [11-12]. These methods report the results as proportion of vaccine recipients with a detectable or “positive” response (percentage of “responders”). In absence of correlate of protection identified, the binary response variable can be defined using either empirical thresholds on a relative or absolute scale (e.g., positive response if higher than threefold the non-stimulated cells or at least 0.05% of T cells), or relying on a statistical approach, or a combination of both. For instance, a Fisher exact test on count data (number of flow cytometer events) is often used to compare the proportion of cytokine-producing stimulated and non-stimulated cells within a given sample, yielding a multiple testing-adjusted p-value per sample that is used as a statistical positivity criterion [11]. This is the case, for instance, in the HVTN 068 trial where the cellular response (CD4+ and CD8+ T cell producing IFN- γ , IL-2, TNF- α and IL-4) was measured by ICS assay, for each condition of stimulation (Gag, Nef, Pol, Env). ICS assay positivity for the primary paper was calculated based on comparisons between stimulated and negative control responses via a one-sided Fisher's Exact Test and the resulting multiplicity-adjusted p-values were used to determine positivity, with $p \leq 1 \times 10^{-5}$ indicating a positive response. They also completed the results with quantitative comparisons of the magnitudes. Qualitative criteria have the advantage of being easy to implement and interpret. However, as well described by Richert and al [13], in the absence of formally identified thresholds of

positivity, the biological meaning of such binary criteria remains unknown. In addition, a binary variable induces a loss of precision and information and therefore decreases the statistical power compared to using the full distribution of the marker.

Thus, use of quantitative endpoints for the analysis of ICS responses is relevant and allows to take into account the information contained in the whole distribution of the immunogenicity marker. The gold standard (*i.e.*, conventional statistical approaches) for the analysis of quantitative ICS data is: i) to subtract the response observed in non-stimulated cells from each stimulated condition of a given sample (Y_i^S minus Y_i^{NS} for each sample of each participant i), and ii) to perform a standard inter- or intra-arm comparison of the distribution of percentages of cells producing the cytokine(s) of interest depending of the design of the trial. This can be achieved, for instance, using Student's *t*-test to compare two means of cytokine-producing cells at a given time point between trial arms (or a non-parametric equivalent test as Wilcoxon rank sums test), or the corresponding tests for paired data for intra-group (“before-after immunization”) comparisons. The background subtraction in step i) is aimed at capturing the antigen-specific response, but it can result in biased estimates and induces an inflation of type-I errors (a type-I error occurs when a researcher rejects a null hypothesis when it is true), and reduce the statistical power by increasing the measurement error or biasing the observed distribution. Moreover, although measurement errors and random biological variations are likely to contribute to the observed response in non-stimulated cells (often called “background”), it cannot be excluded that this non-specific response has some biological relevance [14,15], via bystander activation for instance, and should not be ignored in the data analysis. In addition, from a statistical point of view, the existence (or absence) of a correlation between non-specific and specific responses is significant [16]. The conventional approaches do not take this into account, which may lead to erroneous results.

We propose a new statistical method for the analysis of cellular immune responses in vaccine trials using a bivariate regression model that guarantees accurate estimation of the vaccine effect. This work was divided in several steps: we first specified the statistical model and implemented it in SAS and R softwares. Then, we confirmed model performances with simulated data, to compare bias control, type-I-error control and statistical power against common approaches. We have also used the model on real data from two phase II vaccine clinical trials against HIV, ANRS VRI01 in healthy volunteers and therapeutic VRI02 ANRS 149 LIGHT in HIV-infected participants. Finally, we also developed an online interface that we called VICI for *accurate estimation of Vaccine Induced Cellular Immunogenicity with bivariate modeling* and that allows researchers without a strong statistical background to use this method. A specific section with the description of the interface is detailed after the manuscript

below. The manuscript has been submitted to the *Journal of Immunological Methods* in August 2019 and is currently under review.

To illustrate the analyses carried out in this work, the data of two HIV vaccine trials have been used: ANRS/Inserm VRI01 and VRI02 ANRS 149 LIGHT. Since the two trials are only briefly described in the manuscript further below, a slightly more detailed description of the design of these two clinical trials detailed provided here:

ANRS VRI01

ANRS/INSERM VRI01 is an open-label phase I/II randomized multicenter trial of 3 prophylactic candidate vaccines used as prime or boost (ClinicalTrials.gov Identifier: NCT02038842): MVA HIV-B (coding for Gag, Pol, Nef); LIPO-5 (5 lipopeptides from Gag, Pol, Nef); and DNA GTU-MultiHIV B (coding for Rev, Nef, Tat, Gag, gp160 clade B). A total of 92 healthy adult volunteers were randomly allocated in a 1:1:1:1 ratio at trial entry to four parallel arms with the following prime-boost strategies (Figure 4):

- Arm 1. MVA HIV-B primes at W0 and W8 + LIPO-5 boosts at W20 and W28,
- Arm 2. LIPO-5 primes at W0 and W8 + MVA HIV-B boosts at W20 and W28,
- Arm 3. GTU-MultiHIV B primes at W0, W4 and W12 + LIPO-5 boosts at W20 and W28,
- Arm 4. GTU-MultiHIV B primes at W0, W4 and W12 + MVA HIV-B boosts at W20 and W28.

The co-primary objectives were i) to evaluate the safety of MVA HIV-B and ii) to discard vaccine strategies with an insufficient level of immunogenicity, defined by HIV-specific IFN- γ -EliSpot responses, among 4 HIV prophylactic prime-boost combinations (MVA HIV-B/LIPO-5; LIPO-5/MVA HIV-B; GTU-MultiHIV B/LIPO-5; GTU-MultiHIV B/MVA HIV-B). Secondary objectives included to assess for each prime-boost combination the type of vaccine-induced T cell response with the production of cytokine (IFN- γ , IL-2, TNF- α) by HIV-specific CD8+ and CD4+ T cells, measured by intracellular cytokine staining following stimulation with HIV-1 peptide pools at week 30.

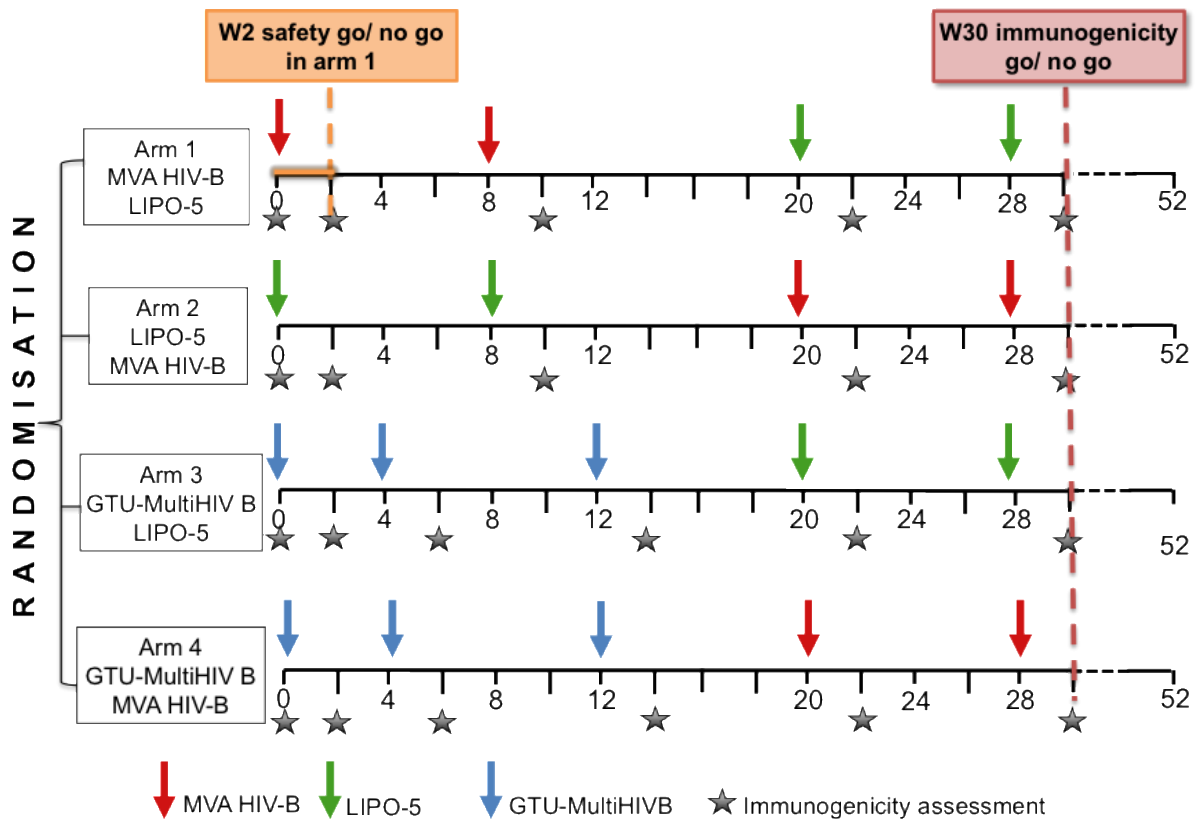


Figure 4. Design of the ANRS/Inserm VRI01 phase I/II randomized trial.

VRI02 ANRS 149 LIGHT

VRI02 ANRS 149 LIGHT is a multicenter, double blind, 2 parallel group randomized phase II trial evaluating the efficacy of a therapeutic prime-boost strategy against HIV versus placebo (ClinicalTrials.gov identifier: NCT01492985). The design of the trial is detailed in Figure 5. A total of 103 HIV-1 infected patients under combination antiretroviral therapy (c-ART) with T lymphocyte $\geq 600/\text{mm}^3$ were included in 18 centers in France and randomized (2:1 ratio) to receive 3 dose of DNA vaccine (GTU-MultiHIVB coding for Rev, Nef, Tat, epitopes from Gag and gp160 HAN2 B clade) at W0, W4 and W12 followed by two doses of LIPO-5 vaccine (5 long peptides from Gag/Pol/Nef) at W20 and W24 or vaccine placebos. At week 36 c-ART was interrupted until week 48 or $\text{CD4} \leq 350/\text{mm}^3$. The main hypothesis was that immune responses in vaccinated patients may be associated with a better control of viral replication following c-ART interruption as compared to placebo-vaccinated patients.

The primary endpoint is based on the maximum value of plasma HIV-1 RNA (in log10 copies/mL) observed in each participant during the ART interruption period between W36 and W48. Secondary outcomes included the evaluation of immunogenicity measured by ICS at

week 28. HIV-specific responses (IFN γ , IL-2, TNF α) against HIV peptides (Gag, Nef, Pol/Env) were assessed by ICS at week 0 and week 28 in 57 vaccine and 32 placebo participants (per protocol group of participants who all receive the complete vaccine strategy and interrupted c-ART).

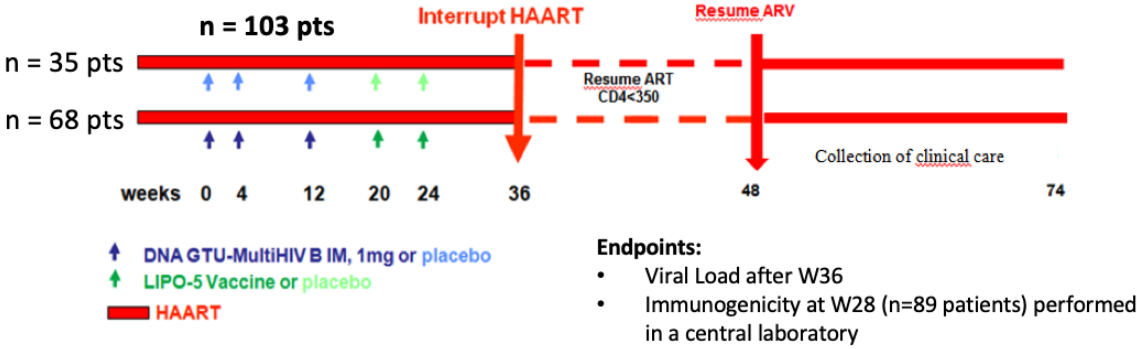


Figure 5. Design of the ANRS VRI02 Light trial.

Analyzing cellular immunogenicity in vaccine clinical trials: a new statistical method including non-specific responses for accurate estimation of vaccine effect

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Abstract

Evaluation of immunogenicity is a key step in the clinical development of novel vaccines. T-cell responses to vaccine candidates are typically assessed by intracellular cytokine staining (ICS) using multiparametric flow cytometry. A conventional statistical approach to analyze ICS data is to compare, between vaccine regimens or between baseline and post-vaccination of the same regimen depending on the trial design, the percentages of cells producing a cytokine of interest after *ex vivo* stimulation of peripheral blood mononuclear cells (PBMC) with vaccine antigens, after subtracting the non-specific response (of unstimulated cells) of each sample. Subtraction of the non-specific response is aimed at capturing the specific response to the antigen, but raises methodological issues related to measurement error and statistical power. We describe here a new statistical approach to analyze ICS data from vaccine trials.

We propose a bivariate linear regression model for estimating the non-specific and antigen-specific ICS responses. We benchmarked the performance of the model in terms of both bias and control of type-I and -II errors in comparison with conventional approaches, and applied it to simulated data as well as real pre- and post-vaccination data from two recent HIV vaccine trials (ANRS VRI01 in healthy volunteers and therapeutic VRI02 ANRS 149 LIGHT in HIV-infected participants).

The model was as good as the conventional approaches (with or without subtraction of the non-specific response) in all simulation scenarios in terms of statistical performance, whereas the conventional approaches did not provide robust results across all scenarios. The proposed model estimated the T-cell responses to the antigens without any effect of the non-specific response on the specific response, irrespective of the correlation between the non-specific and specific responses.

This novel method of analyzing T-cell immunogenicity data based on bivariate modelling is more flexible than conventional methods, and so yields more detailed results and enables accurate interpretation of vaccine-induced response.

Keywords: Flow cytometry; intracellular cytokine staining; vaccine; immunogenicity; clinical trials

Highlight

- Evaluation of vaccine immunogenicity is a key step in the clinical development of vaccines; the T-cell responses to vaccine candidates are typically assessed by intracellular cytokine staining using flow cytometry.
- Conventional approaches for analyzing T-cell responses do not take into consideration the relationship between the specific response and the background response and can compromise statistical performance, particularly in terms of the type-I error rate and statistical power.
- We propose a new modeling approach that considers all measured data and is more flexible than conventional methods, which yields more detailed results (vaccine effect on the non-stimulated response and non-stimulated response effect on stimulated response) and enables accurate interpretation of vaccine-induced response.

1. Introduction

In clinical development of vaccines, assessing the ability of the candidate vaccine to generate immune responses is an important objective of phase I and II clinical trials. Only candidate vaccines with sufficient immunogenicity are subjected to phase III clinical trials. Immunogenicity can be a secondary objective of phase III vaccine trials, to investigate potential correlates of protection.

In phase I and II vaccine trials, a variety of immunological markers, including the vaccine-induced T-cell responses, are typically assayed (1-4). T-cell responses to vaccine candidates are commonly assessed by intracellular cytokine staining (ICS), a flow cytometry assay involving cryopreserved peripheral blood mononuclear cells (PBMCs). ICS assay allows characterization of subsets of specific cytokine-producing T cells after *ex vivo* antigenic stimulation (5,7). For example, in the case of evaluation of an HIV vaccine candidate, the cellular responses can be measured by enumerating IFN γ -, IL2-, and TNF α -producing CD4+ and CD8+ T cells by flow cytometry, after stimulation of PBMCs with pools of the HIV peptides contained in the vaccine sequence, *e.g.*, Gag, Nef, Pol, and/or Env (8-11).

Different approaches exist for the statistical analysis of ICS data. Methods based on qualitative binary criteria can be used to report the proportion of vaccine recipients with a detectable or “positive” response (percentage of “responders”). The binary response variable can be defined using either empirical thresholds on a relative or absolute scale (*e.g.*, positive response if higher than threefold the non-stimulated cells or at least 0.05% of T cells), relying on a statistical approach, or a combination of both. For instance a Fisher exact test with multiplicity adjustment on count data (number of flow cytometer events) is often used to compare the proportion of cytokine-producing stimulated and non-stimulated cells within a given sample, yielding a multiple testing-adjusted *p*-value per sample that is used as a statistical positivity criterion (5). However, in the absence of formally identified thresholds of positivity (correlate of protection),

the biological meaning of such binary criteria remains unknown. In addition, a binary variable induces a loss of precision and information and therefore decreases the statistical power compared to using the full distribution of the marker (12). Quantitative criteria have the advantage of being easy to implement and interpret, without any positivity criteria to define. Thus, use of quantitative endpoints for the analysis of ICS responses is relevant. The gold standard (*i.e.*, conventional statistical approaches (1-4)) for the analysis of quantitative ICS data is: i) to subtract the response observed in non-stimulated cells from each stimulated condition of a given sample and ii) to perform a standard inter- or intra-arm comparison of the distribution of percentages of cells producing the cytokine(s) of interest. This can be achieved, for instance, using Student's *t*-test to compare two means of cytokine-producing cells at a given time point between trial arms (or for non-parametric data a rank test), or the corresponding tests for paired data for intra-group ("before-after immunization") comparisons. The background subtraction in step i) is aimed at capturing the antigen-specific response, but it can result in biased estimates and induce type-I errors (a type-I error occurs when a researcher rejects a null hypothesis when it is true), and reduce the statistical power by increasing the measurement error or biasing the observed distribution.

Moreover, although measurement errors and random biological variations are likely to contribute to the observed response in non-stimulated cells (often called "background"), it cannot be excluded that this non-specific response has some biological relevance (13,14), via bystander activation for instance, and should not be ignored in the data analysis. In addition, from a statistical point of view, the existence (or absence) of a correlation between non-specific and specific responses is significant (15). The conventional approaches do not take this into account, which may lead to erroneous results.

We propose a new statistical method for the analysis of cellular immune responses in vaccine trials using a bivariate regression model for a more accurate estimation of the vaccine effect.

2. Materials and Methods

2.1. Statistical model

We propose a bivariate linear model, which is an extension of the univariate regression models frequently used in biomedicine. While a univariate model allows estimation of the effects of one or several explanatory (independent) variables on a single response (dependent) variable, a bivariate model simultaneously includes two response markers as dependent variables (16). As for univariate linear regression models, a maximum-likelihood approach can be used to estimate the effects of the explanatory variables on the outcome in a bivariate model. Using standard linear modeling techniques, standard errors are approximated through the (restricted) maximum likelihood framework with the observed Fisher information estimate, and a Wald test is then performed to obtain p-values (17,18). Regression coefficients (so-called “betas”) are thus estimated, along with their confidence intervals and corresponding *p*-values, while simultaneously modeling the vaccine effect on the non-specific and specific responses assayed by ICS.

The model considers the non-stimulated cell response and the raw stimulated cell response(s) as the dependent variables. These responses are modeled according to the vaccine effect as the main explanatory variable, and the stimulated cell response is additionally adjusted for the non-stimulated cell response. This adjustment allows consideration of the potential correlation between these responses, which is not possible with current conventional approaches. The model provides an estimation of the vaccine effect on the non-stimulated cell response and of the effect of the non-stimulated cell response on the stimulated cell response (Figure 1).

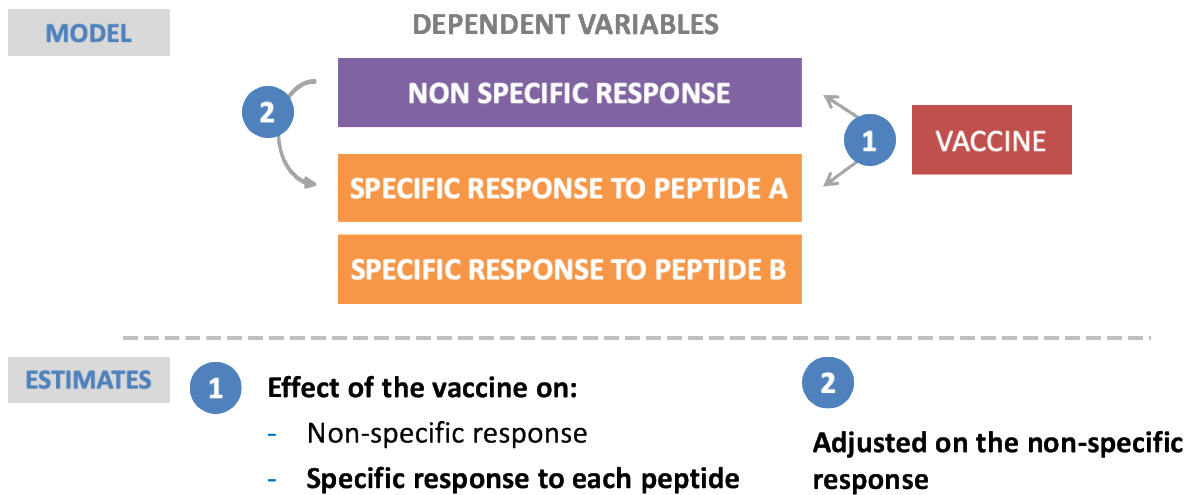


Figure 1. Bivariate linear model for estimating the non-specific and antigen-specific responses measured by ICS following stimulation *in vitro* with peptides A and B. The model is represented as a directed acyclic graph.

Within this framework, we explicitly propose two statistical models, depending on whether the vaccine trial is comparing multiple arms or not. The first model (1) is for a transversal between-trial arm comparison at a given time point, *i.e.*, in a comparative randomized trial comparing two vaccine regimens or an experimental vaccine *vs.* placebo. The second model (2) was developed for a within-arm comparison (comparison of post-vaccination *vs.* baseline for a single vaccine strategy), *i.e.*, non-comparative single- or multi-arm phase I/II vaccine trials of several strategies one by one (19,20).

The mathematical equations specifying each of the two models are described below.

Let $Y_i = \begin{bmatrix} Y_i^{NS} \\ Y_i^{S_k} \end{bmatrix}$, be the response vector for subject i , with Y_i^{NS} the non-stimulated cell

response, and $Y_i^{S_k}$ the n^k vector of the stimulated cell response where k indexes the different antigen stimulations. We define the two bivariate linear models presented above as:

Model (1) for inter-arm comparison at one time point

$$\begin{cases} Y_i^{NS} = \beta_0^{NS} + \beta_1^{NS} V_i + \varepsilon_i^{NS} \\ Y_i^S = \beta_0^S + \beta_1^S V_i + \beta_2^S Y_i^{NS} + \varepsilon_i^S \end{cases}$$

Where V_i is the variable indicating the vaccine arm, and β and ε , respectively, are the parameters and the errors of the model. This formulation implies the following probability distribution for the responses and errors:

$$Y_i^{NS} = N(\beta_0^{NS} + \beta_1^{NS} V_i, \sigma^{NS}) \text{ and } \varepsilon_i^{NS} \sim N(0, \sigma^{NS})$$

$$Y_i^S = N(\beta_0^S + \beta_1^S V_i + \beta_2^S Y_i^{NS}, \sigma^S) \text{ and } \varepsilon_i^S \sim N(0, \sigma^S)$$

The parameter of interest is β_1^S that is the arm effect (vaccine versus placebo) on the stimulated response, adjusted on the non-stimulated response.

Model (2) for intra-arm comparison (post-vaccination vs. baseline)

$$\begin{cases} Ydiff_i^{NS} = \beta_0^{NS} + \varepsilon_i^{NS} \\ Ydiff_i^S = \beta_0^S + \beta_1^S Ydiff_i^{NS} + \varepsilon_i^S \end{cases}$$

where $Ydiff_i^{NS} = Y_i^{NS}(T1) - Y_i^{NS}(T0)$ and $Ydiff_i^S = Y_i^S(T1) - Y_i^S(T0)$, β and ε are, respectively, the parameters and the errors of the model. This formulation implies the following probability distribution for the responses and errors:

$$Ydiff_i^{NS} = N(\beta_0^{NS} + \beta_1^{NS} V_i, \sigma^{NS}) \text{ and } \varepsilon_i^{NS} \sim N(0, \sigma^{NS})$$

$$Ydiff_i^S = N(\beta_0^S + \beta_1^S V_i + \beta_2^S Ydiff_i^{NS}, \sigma^S) \text{ and } \varepsilon_i^S \sim N(0, \sigma^S)$$

The parameter of interest is β_0^S that is the vaccination effect on the stimulated response, adjusted on the non-stimulated response.

We provide an implementation of the model in both SAS (using Proc Mixed) and R (using the nlme package). The SAS and R codes are provided in Appendix A. In addition, we built a user-friendly graphical interface that allows analysis of ICS data with the bivariate model and visualization of the results. The tool is implemented as an R-Shiny application and is available on the Internet (21).

2.2. Simulation study

Simulated datasets enable assessment of the performance of the proposed model in various scenarios where the truth is known (contrary to real-world data). Synthetic datasets were generated under various scenarios chosen to compare the behavior of the proposed model with the conventional approaches in different situations: a similar vaccine effect on stimulated cell response between arms to evaluate the risk of type-I error (*i.e.*, the risk of concluding that a vaccine effect exists when it does not); the vaccine effect on the stimulated cell response to evaluate the statistical power (1-beta, or type II error); the presence and absence of correlations between the stimulated and non-stimulated responses; and with or without a vaccine effect on the non-stimulated response.

The various scenarios and parameters used to generate the data are described in Appendix B – Table 1. For each scenario, 1,000 simulations were run with three different sample sizes, respectively, 15, 30, and 60 participants per arm. Bias, type-I error control, the statistical power, and coverage rate of the bivariate model in each scenario were compared with those of two quantitative conventional approaches based on Student's *t*-test or paired *t*-test (for inter- and intra-arm comparisons, respectively) using (i) the raw stimulated cell response or (ii) the stimulated cell response after subtraction of the non-stimulated cell response. Relative bias was calculated as follows:

$$BIAS(\%) = \frac{DIFF_{obs} - DIFF_{theo}}{DIFF_{theo}}$$

where $DIFF_{obs}$ is the observed mean of the difference between stimulated responses (between time points in model (1); between arms in model (2)) and $DIFF_{theo}$, the corresponding theoretical difference (known in simulations). Type-I error was calculated as the percentage of simulations with significant vaccine effect ($p < 0.05$) among scenarios with no true vaccine effect. Statistical power was calculated as the percentage of simulations with significant vaccine effect ($p < 0.05$) among scenarios with true vaccine effect. The values and parameters used to

generate the unstimulated cell response and the vaccine effect were based on the magnitude of the cellular responses measured in the ANRS/INSERM VRI01 trial. All simulations were performed using SAS v. 9.4 (SAS Institute, Cary, NC).

2.3. Application to real data

We applied our modeling approach to analyze data from two HIV vaccine trials—VRI02 ANRS 149 LIGHT (NCT01492985) and ANRS/INSERM VRI01 (NCT02038842).

VRI02 ANRS 149 LIGHT evaluated a prime-boost combination of DNA-GTU and Lipopeptide vaccine followed by supervised treatment interruption (STI) in a therapeutic HIV phase II randomized trial. A total of 103 HIV-1-infected participants on c-ART were randomized (2:1 ratio) to receive three doses of DNA GTU-MultiHIV B (encoding Rev, Nef, Tat, Gag, and gp160) at week (W) 0, W4 and W12 followed by two doses of LIPO-5 vaccine containing long peptides from Gag, Pol, and Nef at W20 and W24, or a placebo. The HIV-specific CD4+ and CD8+ T-cell responses (IFN γ , IL2, TNF α) to HIV peptide pools (Gag, Nef, and Pol/Env) were assessed by ICS at W0 and W28 in 57 vaccinated and 32 placebo participants.

ANRS/INSERM VRI01 was a prophylactic open-label phase I/II randomized multicenter trial of the immunogenicity and safety of three candidate vaccines used as prime or boost: MVA HIV-B (encoding Gag, Pol, and Nef); LIPO-5 (five lipopeptides from Gag, Pol, and Nef); and DNA GTU-MultiHIV B (encoding Rev, Nef, Tat, Gag, and gp160 clade B). Healthy adult volunteers were randomized to four parallel groups: G1 received MVA at W0/8 + LIPO-5 at W20/28; G2, LIPO-5 at W0/8 + MVA at W20/28; G3, DNA at W0/4/12 + LIPO-5 at W20/28; and G4, DNA at W0/4/12 + MVA at W20/28. HIV-specific CD4+ and CD8+ T-cell responses (IFN γ , IL2, TNF α) were analyzed after stimulation of PBMC by HIV antigens (Gag, Nef, and Pol/Env peptide pools) using ICS at W0, W30 and W52. Only participants from

G1, G2 and G4 were included (n = 62) by ICS because frequency of W30 IFN γ -ELISpot responders was 0% in G3. Details on the ICS are provided in Appendix C.

Model (1) for inter-group comparison (vaccine vs. placebo) at W28 (primary endpoint post-vaccination) and model (2) for intra-group comparison (W0–W28 in the vaccine group) were applied to VRI02 ANRS 149 LIGHT ICS data. For the ANRS/INSERM VRI01 trial, only model (2) was used for intra-group comparisons (W0–W30 [primary endpoint post-vaccination] in each vaccine group) as no formal comparison between vaccine regimens was planned in this trial. Each model was run for each HIV-specific CD4+ and CD8+ T-cell response (IFN γ , IL2, and TNF α) and included the non-stimulated and the three stimulation conditions (Gag, Nef, and Pol/Env) as dependent variables (*i.e.*, a total of four response variables in the multivariate model).

3. Results

3.1. Simulation study

The statistical performance was similar for models (1) and (2) in terms of control of bias, type-I error, and statistical power. A summary of the statistical performance of models (1) and (2) is shown in Figure 2 and Appendix B – Figure 1, respectively, and detailed results for model (1) and model (2) are provided in Appendix B (Figures 2–7 for model (1) and Figures 8–13 for model (2)).

The type-I error rate was controlled at $\approx 5\%$ in all scenarios by the model as well as the conventional approach using the raw stimulated cell response. For the conventional method with subtraction of the non-stimulated cell response, the type-I error rate was not controlled ($> 20\%$) when there was a vaccine effect on the non-stimulated cell response (Figure 2C, upper panel). Regarding the control of bias and statistical power (lower panel), the performance of the two conventional approaches varied across the scenarios: the raw stimulated cell response

performed better than the background-subtracted response in the absence of a correlation (Figure 2A) and, more importantly, in cases of a vaccine effect on the non-stimulated cell response (Figure 2C). In contrast, a conventional approach with background subtraction was better in cases of correlations between the non-stimulated and stimulated cell responses (Figure 2 B). The proposed modeling approach had excellent performance in all scenarios, at least as good as the respective conventional approach. The exception were cases of a vaccine effect on the non-stimulated response, in which the conventional approach without background-subtraction had slightly higher statistical power (Figure 2C). Good 95% coverage rates were obtained in all scenarios for the three methods, except for the conventional approach with background-subtraction where the 95% coverage rate was weak in case of a vaccine effect on the non-stimulated response (Appendix B – Table 2).

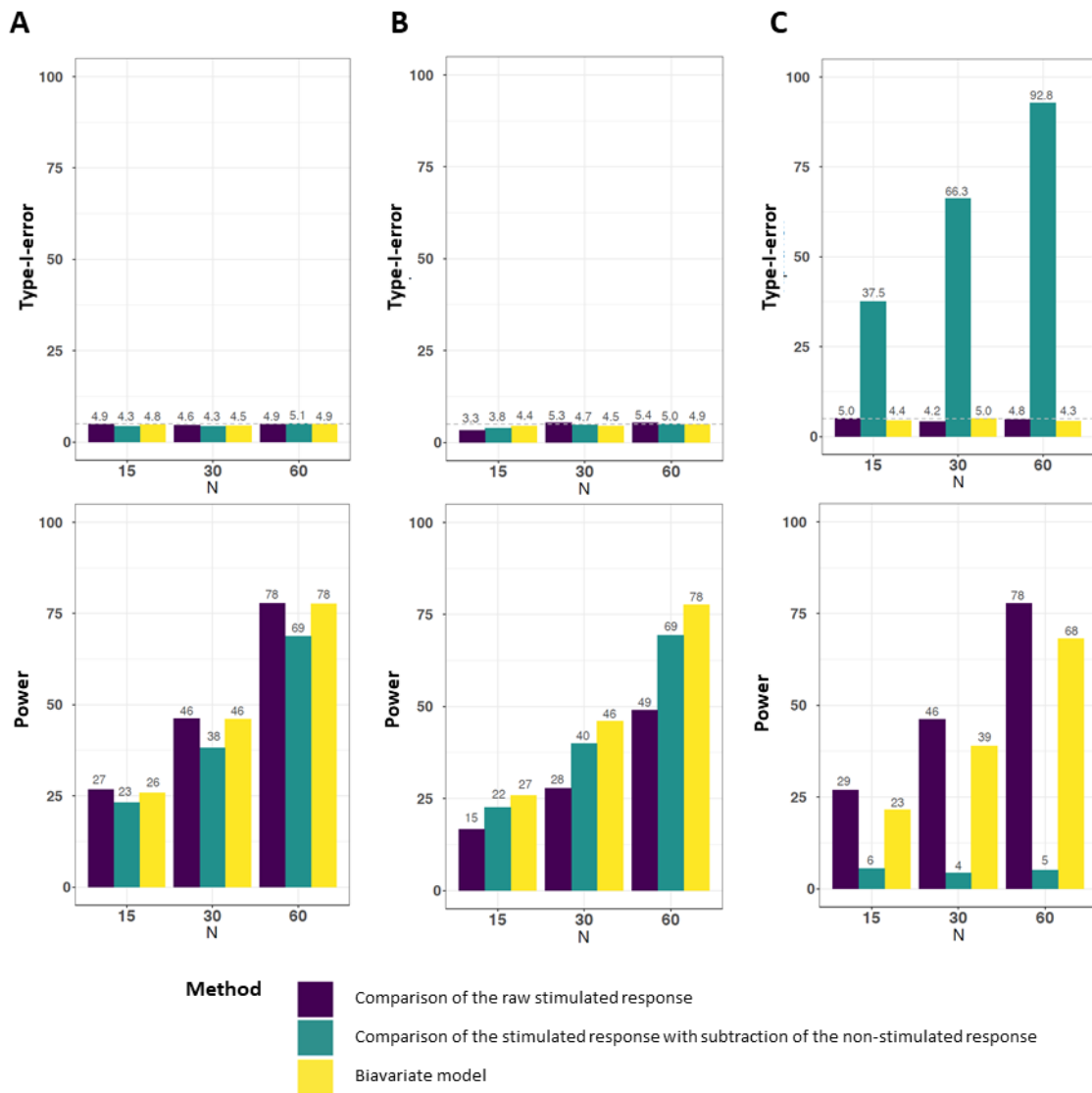


Figure 2: Evaluation of the performance of model 1 in terms of control of type 1 error and statistical power of the bivariate model for evaluating the effect of the vaccine (arm-vaccine versus placebo) compared to conventional approaches (with or without subtraction of the non-stimulated cell response) via simulations (1000 simulations per scenario).

To have good statistical performance, a model must control the type-I error at the nominal testing level (conventionally 5%) and the power must be as high as possible.

A: Scenarios with no correlation between stimulated and non-stimulated cell response and no vaccine effect on the non-stimulated cell response

B: Scenarios with correlation ($p=0.70$) between stimulated and non-stimulated cell response

C: Scenarios with a vaccine effect on the non-stimulated cell response

3.2. Application to real-world data

3.2.1. VRI02 ANRS 149 LIGHT

We applied the proposed model to evaluation of cellular immunogenicity at W28 for inter-arm comparison in the vaccine ($n=57$) and placebo arms ($n=32$)—model (1)—and intra-

arm comparison in the vaccine and placebo arms—model (2). The estimated vaccine effect on cells stimulated by Gag, Nef, and Pol/Env using models 1 and 2 and the respective conventional approaches are presented in Figure 3.

Regarding the inter-arm comparison (Figure 3A), differences were observed between the two conventional approaches: a significant change in the frequency of CD4⁺ T cells producing TNF α , IL2, and IFN γ in response to the Pol/Env peptide pool was found in the vaccine arm *vs.* the placebo arm at W28 using the approach with subtraction of the non-stimulated cell response. In contrast, the approach without subtraction found only a significant change in the frequency of CD4⁺ T cells producing IFN γ in response to the same peptides. The bivariate modelling approach found specific CD4⁺ T cells producing IL2 in response to the Gag peptide pool. No significant change in CD8⁺ T-cell responses was found with the model or the conventional approaches with and without subtraction of the non-stimulated cell response. Regarding the intra-arm comparison in the vaccine arm (Figure 3B, upper panel), the results were similar for the three methods between W0 and W28—CD8⁺ T-cell responses to Nef and Pol/Env and CD4⁺ T-cell responses to the three peptides. A significant vaccine effect on CD8⁺ T cells producing IL2 was detected by bivariate modelling and the conventional approach with subtraction of the non-specific response but not with the non-subtracted conventional approach. In the placebo arm (Figure 3B, lower panel), the three methods yielded discordant results. Whereas no significant CD4⁺ and CD8⁺ T-cell responses were observed with the modeling approach, several significant cellular responses were found using the conventional approaches, particularly with subtraction of the unstimulated cell response. This result may be linked to an increased frequency of type-I errors with conventional approaches, as suggested by numerical simulation results (Appendix B – Figure 1 for instance).

Estimates of the vaccine effect on the non-stimulated cell response and the association of the non-stimulated response with the stimulated cell responses are shown in Appendix D. No

significant vaccine effect on the non-stimulated cell response was observed with model 1 (Appendix D, Figure 1) or model 2 (Appendix D, Figure 2). However, a significant association between the non-stimulated cell response and the stimulated cell responses was found for several CD4+ and CD8+ T-cell responses in both models. For example, estimates (standard deviation) from model 1 (inter-arm comparison at W28) for the analysis of the CD4+ IL2+ response after Pol/Env stimulation were 0.010 (0.033) for the vaccine effect on the stimulated cell responses ($p = 0.002$), and 1.097 (0.154) for the effect of the non-stimulated response on the stimulated response ($p < 0.001$).

Regarding conventional approaches without subtraction, the average difference (standard deviation) in raw stimulated responses between the two arms was 0.006 (0.018) ($p = 0.14$; standardized effect: 0.328). With the conventional approach with subtraction of the non-stimulated response, the average difference (standard deviation) in raw stimulated responses between the two arms was 0.010 (0.014) ($p = 0.003$; standardized effect: 0.684). For this example, the correlation coefficient between non-stimulated response and stimulated response was 0.57, which is a moderate correlation explaining why the conventional approach with subtraction of the non-stimulated cell response was closer of the modelling result.

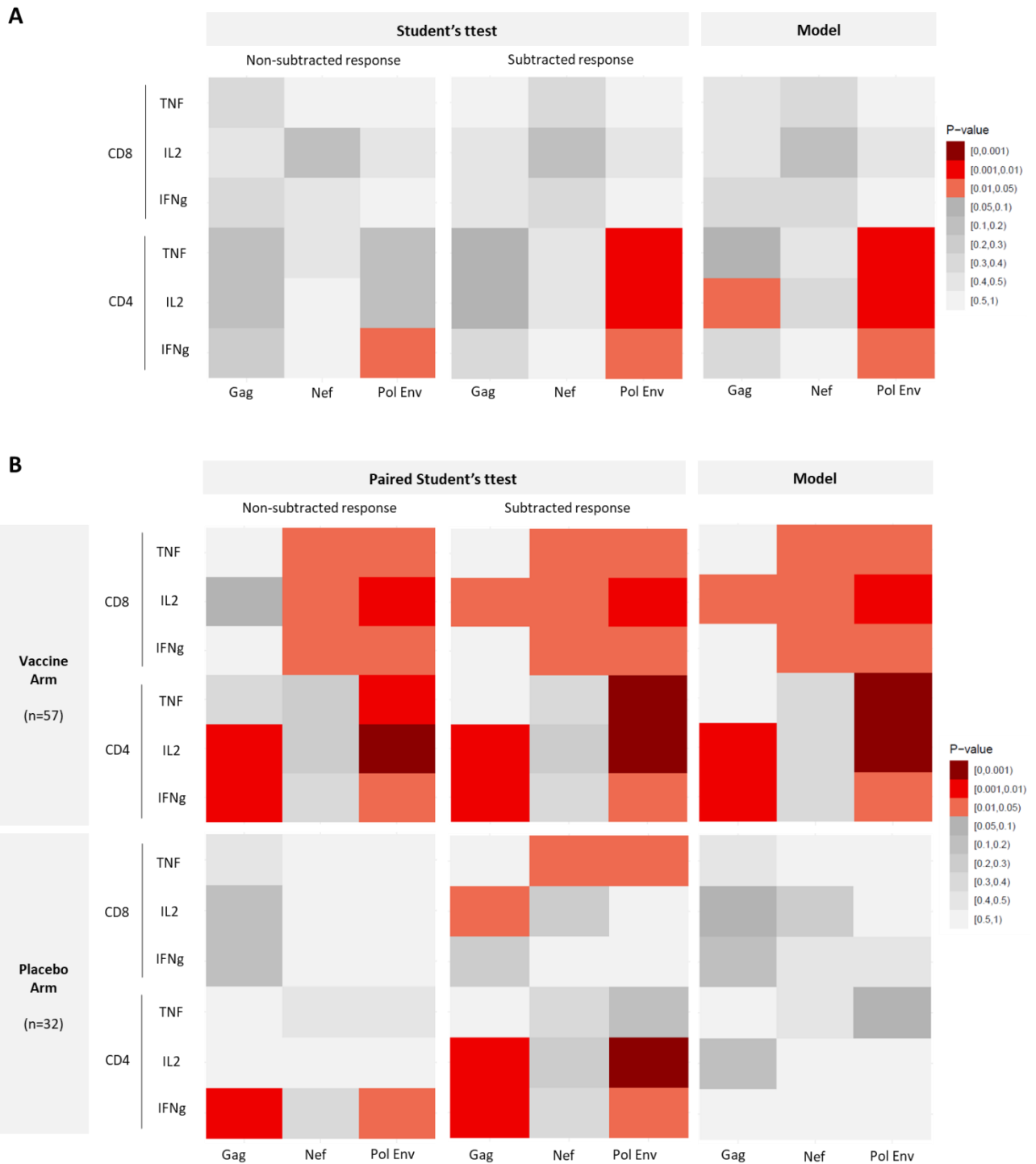


Figure 3. Heatmap of the p-values of the vaccine effect on the CD4+ and CD8+ T-cell responses measured by ICS in the VRI02 ANRS 149 LIGHT trial arms. The three approaches were (1) comparison of the raw stimulated cell response, (2) comparison of the specific response with subtraction of the non-stimulated cell response, and (3) a bivariate model with the non-stimulated cell responses and stimulated cell responses as dependent variables.

A. Inter-arm (vaccine *vs.* placebo) comparisons.

B. Intra-arm (week 28 *vs.* baseline) comparisons in the vaccine and placebo arms.

3.2.2. ANRS/INSERM VRI01

We applied the proposed model to evaluate cellular immunogenicity at W30 in the MVA-LIPO (n= 21), LIPO-MVA (n= 21), and GTU-MVA (n= 20) arms. Estimates of the vaccine effects on the Gag, Nef, and Pol/Env responses in each arm (model 2) and the respective conventional approaches are shown in Figure 4.

As expected, some discrepancies were observed between the two conventional approaches for measuring the vaccine effect. The modelling approach resolved this uncertainty, and yielded more robust results that were sometimes closer to one conventional approach, sometimes closer to the other.

Estimates of the vaccine effect on the non-stimulated response and the effect of the non-stimulated response on the stimulated responses are presented in Appendix E – Figure 1. In the analysis of VRI02 ANRS 149 LIGHT, no significant vaccine effect on non-stimulated cell responses was observed. A significant association of the non-stimulated response with the stimulated responses was found for several CD4+ and CD8+ T-cell responses.

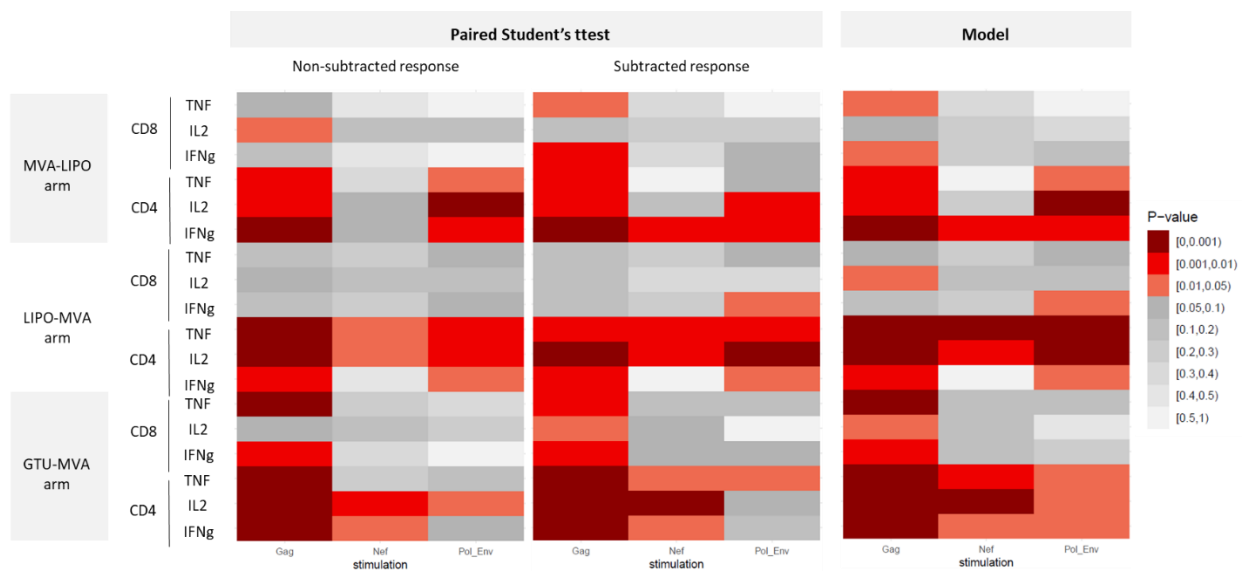


Figure 4. Heatmap of the p -values of the intra-arm analysis of the vaccine effect on the CD4+ and CD8+ T-cell responses measured by ICS in the ANRS/INSERM VRI01 trial arms. The three approaches were (1) comparison of the raw stimulated response between W30 and baseline, (2) comparison of the stimulated response with subtraction of the non-stimulated

response between W30 and baseline, and (3) a bivariate model with the non-stimulated responses and the stimulated responses as dependent variables.

4. Discussion

We present a multivariate modelling approach to analyze the cellular immune response of vaccine candidates during vaccine clinical trials. Simulations showed that the bivariate model effectively controlled the type-I error in all assessed scenarios with different population sizes, while its statistical power was at least as good as the conventional approaches in all scenarios. Controlling type-I errors is fundamental for the consistency of research. A high frequency of type-I errors leads to an apparently statistically significant result that is not reproducible in further studies. This is one of the determinants of the very high prevalence of false-positive results (22). The lack of statistical power is also a drawback, especially in the context of early phase vaccine clinical trials in which the number of subjects is restricted. Conventional approaches based on comparison of the background-subtracted response by t-test are the most frequently used (1-4) but did not control type-I error and had low statistical power for the vaccine effect on the non-stimulated cell response. Comparison of the data without subtraction of the non-stimulated response had the highest power for the scenario of a vaccine effect on the non-stimulated responses, but exhibited little statistical power in cases of correlations between the non-stimulated and stimulated cell responses.

The use of real data from prophylactic and therapeutic HIV vaccine trials showed the feasibility of the modelling approach. Not surprisingly, divergent results among the three analysis approaches (modelling and two conventional approaches) were obtained for some ICS responses. This demonstrated that the proposed model yields robust results and provides information on the correlation between the non-stimulated and stimulated cell responses. The validity of the proposed modelling approach could not be tested with real data but an *in silico* study demonstrated the drawbacks of conventional methods (15). The systematic use of one of

the conventional approaches leads to erroneous results. The originality of our approach lies in the simultaneous modeling of the non-stimulated and stimulated responses, unlike conventional approaches. This enables assessment of the vaccine effect on stimulated cell response adjusted for the non-stimulated cell response as well as of the vaccine effect on the non-stimulated cell response without loss of statistical power. The strength of the model compared to conventional approaches is its good statistical performance irrespective of the relationship between the vaccine and the non-stimulated response and between the non-stimulated response and the stimulated response. The conventional approaches do not take these relationships into account. In addition, the bivariate model is easily extendable to multivariate models with more than two dependent variables, allowing consideration of more than one antigen, while a large number of tests must be performed using conventional approaches, leading to a risk of type-I error. Furthermore, while controlling for statistical errors, the model provides more biological information on the effect of variables on the background or on the specific response independently of the intervention. Notably, the same modelling approach can be used to explore any variable that modifies the effect of interest (e.g., the vaccine) on the ICS response. As an example, it could be used to evaluate whether the vaccine induced a similar ICS response in women and men through an interaction term.

In a previous paper, Gilbert et al (15) proposed an ANCOVA regression model for analyzing immunogenicity of candidate vaccines in randomized clinical trials, that could improve statistical power by adjusting on participants baseline characteristics (especially the non-stimulated response at baseline). The ANCOVA approach suffers from a huge drawbacks in comparison of our bivariate modelling approach: the measurement error of the non-stimulated response is not taken into account. Hence, this could lead to a bias toward the null with an underestimation of the effect of this unstimulated variable (23). Direct comparisons with ANCOVA have been performed with the others approaches in different scenarios

ANCOVA showed inferior statistical performances in terms of statistical power and an increase of the type-I-error (appendix F).

One potential drawback of our approach is the fact that it is a fully parametric method making assumptions. It is assuming that residuals are normally (i.e., Gaussian) distributed with constant variance, which could not be the case. However, there are several arguments for thinking that the consequences on the usefulness of the method are limited. First, the conditional distribution could be more likely Gaussian than the marginal one tested with the *t*-test. Second, the mixed models are quite robust to misspecification of residual distribution (24,25). Realistic non-gaussian simulation results suggest some robustness of the approach to the linear model (see Appendix G). Therefore, we think that parametric assumptions should not limit the use of the approach if the sample size is adequate (>20 individuals).

Although our modeling approach is more complex than conventional approaches based on basic statistical tests (Student's *t*-test), this should not prevent its practical use. To facilitate its implementation, the code for SAS and R software is provided in the Appendix. In addition, we developed an R-shiny application with an interactive and user-friendly web interface that enables immunologists to analyze their data with no statistical software or experience required. The application provides simple and clear interpretation of the results and the output can be directly integrated into publications.

In conclusion, our novel method of analyzing T-cell immunogenicity data, based on bivariate modelling, enables consideration of all available information with more flexibility than conventional methods, leading to more accurate and more detailed results, enhancing interpretation of the vaccine effect on T-cell function. Our multivariate model is an alternative to conventional approaches and should be recommended for ICS in vaccine trials. The bivariate model could be used to analyze any type of functional response in which a non-stimulated cell response is measured.

Acknowledgments

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Declarations of interest: none

References

1. Kibuuka H, Berkowitz NM, Millard M, Enama ME, Tindikahwa A, Sekiziyivu AB, et al. Safety and immunogenicity of Ebola virus and Marburg virus glycoprotein DNA vaccines assessed separately and concomitantly in healthy Ugandan adults: a phase 1b, randomised, double-blind, placebo-controlled clinical trial. *The Lancet* 2015;385(9977):1545-54.
2. Guardo A, Gómez CE, Díaz-Brito V, Pich J, Arnaiz JA, Perdiguero B, et al. Safety and vaccine-induced HIV-1 immune responses in healthy volunteers following a late MVA-B boost 4 years after the last immunization. *PLoS One* 2017;12(10).
3. Lévy Y, Thiébaud R, Montes M, Lacabaratz C, Sloan L, King B, et al. Dendritic cell-based therapeutic vaccine elicits polyfunctional HIV-specific T-cell immunity associated with control of viral load. *Eur J Immunol* 2014;44(9):2802-10.
4. Luabeya AKK, Kagina BMN, Tameris MD, Geldenhuys H, Hoff ST, Shi Z, et al. First-in-human trial of the post-exposure tuberculosis vaccine H56:IC31 in Mycobacterium tuberculosis infected and non-infected healthy adults. *Vaccine* 2015;33(33):4130-40.
5. Horton H, Thomas E, Stucky J, Frank I, Moodie Z, Huang Y, et al. Optimization and Validation of an 8-Color Intracellular Cytokine Staining (ICS) Assay to Quantify Antigen-Specific T Cells Induced by Vaccination. *J Immunol Methods* 2007;323(1):39-54.
6. De Rosa SC. Vaccine applications of flow cytometry. *Methods* 2012;57(3):383-91.
7. Lin L, Finak G, Ushey K, Seshadri C, Hawn TR, Frahm N, et al. COMPASS identifies T-cell subsets correlated with clinical outcomes. *Nat Biotechnol* 2015;33(6):610-6.
8. Churchyard GJ, Morgan C, Adams E, Hural J, Graham BS, Moodie Z, et al. A phase IIA randomized clinical trial of a multiclade HIV-1 DNA prime followed by a multiclade rAd5 HIV-1 vaccine boost in healthy adults (HVTN204). *PLoS ONE* 2011;6(8):e21225.
9. Tung FY, Tung JK, Pallikkuth S, Pahwa S, Fischl MA. A therapeutic HIV-1 vaccine enhances anti-HIV-1 immune responses in patients under highly active antiretroviral therapy. *Vaccine* 2016;34(19):2225-32.
10. Janes HE, Cohen KW, Frahm N, De Rosa SC, Sanchez B, Hural J, et al. Higher T-Cell Responses Induced by DNA/rAd5 HIV-1 Preventive Vaccine Are Associated With

- Lower HIV-1 Infection Risk in an Efficacy Trial. *J Infect Dis* 2017;215(9):1376-85.
11. Kalams SA, Parker SD, Elizaga M, Metch B, Edupuganti S, Hural J, et al. Safety and Comparative Immunogenicity of an HIV-1 DNA Vaccine in Combination with Plasmid Interleukin 12 and Impact of Intramuscular Electroporation for Delivery. *J Infect Dis* 2013;208(5):818-29.
 12. Richert L, Thiebaut R, BiostatisticsBioinformatics Division of the ANRS Vaccine Research Institute. Is the current use of « positivity » thresholds meaningful for evaluating HIV-vaccine immunogenicity endpoints? *AIDS* 2013;27(8):1362-5.
 13. Liu AY, De Rosa SC, Guthrie BL, Choi RY, Kerubo-Bosire R, Richardson BA, Kiarie J, Farquhar C, Lohman-Payne B High background in ELISpot assays is associated with elevated levels of immune activation in HIV-1-seronegative individuals in Nairobi. *Immun Inflamm Dis* 2018;6(3):392-401.
 14. Su LF, Kidd BA, Han A, Kotzin JJ, Davis MM. Virus-specific CD4+ memory phenotype T cells are abundant in unexposed adults. *Immunity* 2013;38(2):373-83.
 15. Gilbert PB, Sato A, Sun X, Mehrotra DV. Efficient and robust method for comparing the immunogenicity of candidate vaccines in randomized clinical trials. *Vaccine* 2009;27(3):396-401.
 16. Thiébaut R, Jacqmin-Gadda H, Chêne G, Leport C, Commenges D. Bivariate linear mixed models using SAS proc MIXED. *Computer Methods and Programs in Biomedicine* 2002;69(3):249-56.
 17. Carroll R J, Ruppert D. Transformation and Weighting in Regression. Springer. 2013
 18. Pinheiro J C, Douglas M B. Mixed-Effects Models in S and S-PLUS. Springer. 2000
 19. Moodie Z, Rossini AJ, Hudgens MG, Gilbert PB, Self SG, Russell ND. Statistical evaluation of HIV vaccines in early clinical trials. *Contemporary Clinical Trials* 2006;27(2):147-60.
 20. Richert L, Lhomme E, Fagard C, Lévy Y, Chêne G, Thiébaut R. Recent developments in clinical trial designs for HIV vaccine research. *Hum Vaccin Immunother* 2015;11(4):1022-9.
 21. VICI: accurate estimation of Vaccine Induced Cellular Immunogenicity by bivariate modelling [Internet]. <https://shiny-vici.apps.math.cnrs.fr/>
 22. Ioannidis JP. Why most discovered true associations are inflated. *Epidemiology*. 2008;19(5):640-8.
 23. Stefansky L A. The effects of measurement error on parameter estimation. *Biomctrika* 1985;71(3)
 24. Jacqmin-Gadda H, Sibillot S, Proust C, Molina J-M, Thiébaut R. Robustness of the linear mixed model to misspecified error distribution. *Computational Statistics & Data Analysis* 2007;51(10):5142-54.
 25. Krzywinski M, Altman N. Multiple linear regression. *Nat Methods* 2015;12(12):1103-4.

Appendix A: Model specification and software codes

SAS code

Model (1)

Below an example of SAS code for a bivariate model for an inter-arm comparison at one time point post vaccination where STIMULATION is a binary variable indicating the response variable concerned (STIMULATION = 0 for Y_i^{NS} and STIMULATION = 1 for Y_i^S); ID_PATIENT a single identification number for each patient, VACCINE a qualitative variable for the treatment group; Y_NS with Y_NS = 0 when STIMULATION = 0 and the value of the non-stimulated response when STIMULATION = 1.

```
proc mixed data = DATATABLE;  
class ID_PATIENT STIMULATION ;  
model Y = STIMULATION STIMULATION*VACCINE STIMULATION* Y_NS / cl noint ;  
repeated /type=VC grp=STIMULATION sub=ID_PATIENT ;  
run ;
```

Model (2)

Below an example of SAS code for a bivariate model for an intra-arm comparison (post vaccination versus baseline) where STIMULATION is a binary variable indicating the response variable concerned (STIMULATION = 0 for Y_i^{NS} and STIMULATION = 1 for Y_i^S); ID_PATIENT a single identification number for each patient; Y_d_NS with Y_d_NS = 0 when STIMULATION = 0 and the value of the differential (post-vaccination / baseline) of the non-stimulated response when STIMULATION = 1.

```

proc mixed data = DATATABLE;

class ID_PATIENT STIMULATION ;

model Y_d = STIMULATION STIMULATION*Y_d_NS/ cl noint;

repeated /type=VC grp=stimul sub= ID_PATIENT ;

run ;

```

R code

Below an example of R code for a bivariate model for an inter-arm comparison at one time point post vaccination where STIMULATION is a binary variable indicating the response variable concerned (STIMULATION = 0 for Y_i^{NS} and STIMULATION = 1 for Y_i^S); ID_PATIENT a single identification number for each patient, VACCINE a qualitative variable for the treatment group; Y_NS with $Y_{NS} = 0$ when STIMULATION = 0 and the value of the non-stimulated response when STIMULATION = 1.

Model (1)

```

nlme::gls(Y ~ -1 + STIMULATION*ARM + Y_NS,

          data = DATATABLE,

          weights = nlme::varIdent(value = c("1" = 1), form = ~ 1 |

STIMULATION),

          method="REML)

```

Model (2)

```

nlme::gls(Y_d ~ -1 + STIMULATION + Y_d_NS,

          data = DATATABLE,

          weights = nlme::varIdent(value = c("1" = 1), form = ~ 1 |

STIMULATION),

          method="REML"

)

```


Data set

Example of dataset for running model (1)

| USUBJID | Y | ARM | Y_NS | STIMULATION |
|----------------|----------|------------|-------------|--------------------|
| 1 | 0.00339 | 0 | 0 | 0 |
| 1 | 0.0569 | 0 | 0.00339 | 1 |
| 2 | 0.0093 | 0 | 0 | 0 |
| 2 | 0.285 | 0 | 0.0093 | 1 |
| 3 | 0.0128 | 1 | 0 | 0 |
| 3 | 0.0517 | 1 | 0.0128 | 1 |
| 4 | 0.0118 | 1 | 0 | 0 |
| 4 | 0.135 | 1 | 0.0118 | 1 |
| 5 | 0.00444 | 1 | 0 | 0 |
| 5 | 0.27 | 1 | 0.00444 | 1 |
| 6 | 0.00488 | 1 | 0 | 0 |
| 6 | 0.0399 | 1 | 0.00488 | 1 |
| 7 | 0.00229 | 1 | 0 | 0 |
| 7 | 0.0743 | 1 | 0.00229 | 1 |
| 8 | 0.00256 | 0 | 0 | 0 |
| 8 | 0.0282 | 0 | 0.00256 | 1 |
| 9 | 0.00481 | 0 | 0 | 0 |
| 9 | 0.119 | 0 | 0.00481 | 1 |
| 10 | 0.00662 | 0 | 0 | 0 |
| 10 | 0.0416 | 0 | 0.00662 | 1 |

Appendix B – Simulations

Scenarios

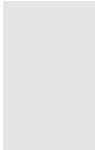
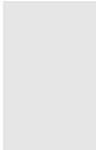
Appendix B – Table 1. Description of the different scenarios of simulation for Model 1 and Model 2 and parameters used for each scenario

| Scenarios of simulation | Hypothesis | Y_i^{NS} | | | Y_i^S | | | | |
|--|---|----------------|----------------|-------------------|-------------|-------------|-------------|-------------------|--|
| | | β_0^{NS} | β_1^{NS} | ϵ_i^{NS} | β_0^S | β_1^S | β_2^S | ϵ_i^S | |
| Model (1), two arms comparison at one time point | | | | | | | | | |
| Similar vaccine effect between arms on stimulated response | Vaccine effect on stimulated response: H0 ($\beta_1^S = 0$) Vaccine effect on non-stimulated response: H0 ($\beta_1^{NS} = 0$) Non-stimulated response effect on stimulated response: H0 ($\beta_2^S = 0$) | 0.02 | 0 | $\sim N(0, 0.01)$ | 0.04 | 0 | 0 | $\sim N(0, 0.02)$ | |
| Vaccine effect on stimulated response, no correlation | Vaccine effect on stimulated response: H1 ($\beta_1^S \neq 0$) | 0.02 | 0 | $\sim N(0, 0.01)$ | 0.04 | 0.01 | 0 | $\sim N(0, 0.02)$ | |

| | | | | | | | | | |
|--|---|------|---|-------------------|------|------|------|-------------------|--|
| between stimulated and non-stimulated response | Vaccine effect on non-stimulated response: H0 ($\beta_1^{NS} = 0$) Non-stimulated response effect on stimulated response: H0 ($\beta_2^S = 0$) | | | | | | | | |
| Similar vaccine effect between arms on stimulated response, correlation between stimulated and non-stimulated response | Vaccine effect on stimulated response: H0 ($\beta_1^S = 0$) Vaccine effect on non-stimulated response: H0 ($\beta_1^{NS} = 0$) Non-stimulated response effect on stimulated response: H1 ($\beta_2^S \neq 0$) | 0.02 | 0 | $\sim N(0, 0.01)$ | 0.04 | 0 | 0.01 | $\sim N(0, 0.02)$ | |
| Vaccine effect on stimulated response, no correlation | Vaccine effect on stimulated response: H1($\beta_1^S \neq 0$) | 0.02 | 0 | $\sim N(0, 0.01)$ | 0.04 | 0.01 | 0.01 | $\sim N(0, 0.02)$ | |
| between stimulated and non-stimulated response, correlation between stimulated and non-stimulated response | Vaccine effect on non-stimulated response: H0 ($\beta_1^{NS} = 0$) Non-stimulated response effect on stimulated response: H1 ($\beta_2^S \neq 0$) | | | | | | | | |

| | | | | | | | | |
|--|---|------|------|-------------------|------|------|------|-------------------|
| Similar vaccine effect between arms on stimulated response, | Vaccine effect on stimulated response: H0 ($\beta_1^S = 0$) | 0.02 | 0.01 | $\sim N(0, 0.01)$ | 0.04 | 0 | 0.01 | $\sim N(0, 0.02)$ |
| Vaccine effect on non-stimulated response | Vaccine effect on non-stimulated response: H1 ($\beta_1^{NS} \neq 0$) | | | | | | | |
| | Non-stimulated response effect on stimulated response: H0 ($\beta_2^S = 0$) | | | | | | | |
| Vaccine effect on stimulated response, Vaccine effect on non-stimulated response | Vaccine effect on stimulated response: H1 ($\beta_1^S \neq 0$) | 0.02 | 0.01 | $\sim N(0, 0.01)$ | 0.04 | 0.01 | 0.01 | $\sim N(0, 0.02)$ |
| | Vaccine effect on non-stimulated response: H1 ($\beta_1^{NS} \neq 0$) | | | | | | | |
| | Non-stimulated response effect on stimulated response: H0 ($\beta_2^S = 0$) | | | | | | | |

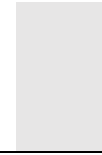
Model (2), one arm comparison (post-vaccination versus baseline)

| | | | | | | | | |
|-------------------|---|---|---|-------------------|---|---|---|-------------------|
| No vaccine effect | Vaccine effect on stimulated response: H0 ($\beta_0^S = 0$) | 0 |  | $\sim N(0, 0.01)$ | 0 | 0 |  | $\sim N(0, 0.02)$ |
|-------------------|---|---|---|-------------------|---|---|---|-------------------|

| | | | | | | |
|--|---|---|-------------------|------|------|-------------------|
| | Vaccine effect on non-stimulated response: H0 ($\beta_0^{NS} = 0$) | | | | | |
| | Non-stimulated response effect on stimulated response: H0 ($\beta_1^S = 0$) | | | | | |
| Vaccine effect on stimulated response, no correlation between stimulated and non- stimulated response | Vaccine effect on stimulated response: H1($\beta_0^S \neq 0$) | 0 | $\sim N(0, 0.01)$ | 0.01 | 0 | $\sim N(0, 0.02)$ |
| | Vaccine effect on non-stimulated response: H0 ($\beta_0^{NS} = 0$) | | | | | |
| | Non-stimulated response effect on stimulated response: H0 ($\beta_1^S = 0$) | | | | | |
| No vaccine effect between arms on stimulated response, correlation between stimulated and non-stimulated response | Vaccine effect on stimulated response: H0 ($\beta_0^S = 0$) | 0 | $\sim N(0, 0.01)$ | 0 | 0.01 | $\sim N(0, 0.02)$ |
| | Vaccine effect on non-stimulated response: H0 ($\beta_0^{NS} = 0$) | | | | | |
| | Non-stimulated response effect on stimulated response: H1 ($\beta_1^S \neq 0$) | | | | | |

| | | | | | | |
|--|--|------|-------------------|------|------|-------------------|
| Vaccine effect on stimulated response, no correlation between stimulated and non-stimulated response, correlation between stimulated and non-stimulated response | Vaccine effect on stimulated response: H1 ($\beta_0^S \neq 0$) Vaccine effect on non-stimulated response: H0 ($\beta_0^{NS} = 0$) Non-stimulated response effect on stimulated response: H1 ($\beta_1^S \neq 0$) | 0 | $\sim N(0, 0.01)$ | 0.01 | 0.01 | $\sim N(0, 0.02)$ |
| Similar vaccine effect between arms on stimulated response, Vaccine effect on non-stimulated response | Vaccine effect on stimulated response: H0 ($\beta_0^S = 0$) Vaccine effect on non-stimulated response: H1 ($\beta_0^{NS} \neq 0$) Non-stimulated response effect on stimulated response: H0 ($\beta_1^S = 0$) | 0.01 | $\sim N(0, 0.01)$ | 0 | 0 | $\sim N(0, 0.02)$ |
| Vaccine effect on stimulated response, Vaccine effect on non-stimulated response | Vaccine effect on stimulated response: H1 ($\beta_0^S \neq 0$) Vaccine effect on non-stimulated response: H1 ($\beta_0^{NS} \neq 0$) | 0.01 | $\sim N(0, 0.01)$ | 0.01 | 0 | $\sim N(0, 0.02)$ |

Non-stimulated response effect on
stimulated response: H0 ($\beta_1^S = 0$)



Appendix B – Table 2. Description of the coverage rate of the 95% confidence interval of the parameter of interest for the evaluation of the vaccine effect among the different scenarios of simulation for Model 1 (30 participants per arm) and Model 2 (30 participants in the arm).

| Scenarios of simulation | Hypothesis | Theoretical vaccine effect on stimulated response | Coverage rate (%) | | |
|--|---|--|--------------------------|--|----------|
| | | | t-test (raw response) | t-test (subtracted non- stimulated response) | Modeling |
| Model (1), two arms comparison at one time point | | | | | |
| Similar vaccine effect between arms on stimulated response | Vaccine effect on stimulated response: H0 ($\beta_1^S = 0$) | 0 | 95.4 | 95.7 | 95.5 |
| Vaccine effect on stimulated response, no correlation between stimulated and non-stimulated response | Vaccine effect on stimulated response: H1 ($\beta_1^S \neq 0$) | 0.01 | 95.4 | 95.7 | 95.5 |

| | | | | | |
|--|---|------|------|------|------|
| Similar vaccine effect between arms on stimulated response, correlation between stimulated and non-stimulated response | Vaccine effect on stimulated response: H_0 $(\beta_1^S = 0)$ | 0 | 95.4 | 95.8 | 95.5 |
| Vaccine effect on stimulated response, no correlation between stimulated and non-stimulated response, correlation between stimulated and non-stimulated response | Vaccine effect on stimulated response: $H_1(\beta_1^S \neq 0)$ | 0.01 | 95.4 | 95.8 | 95.5 |
| Similar vaccine effect between arms on stimulated response, | Vaccine effect on stimulated response: H_0 $(\beta_1^S = 0)$ | 0 | 95.4 | 60.2 | 95.5 |

Vaccine effect on non-stimulated response

| | | | | | |
|--|---|------|------|------|------|
| Vaccine effect on stimulated response, Vaccine effect on non-stimulated response | Vaccine effect on stimulated response: H1 $(\beta_1^S \neq 0)$ | 0.01 | 95.4 | 60.2 | 95.5 |
|--|---|------|------|------|------|

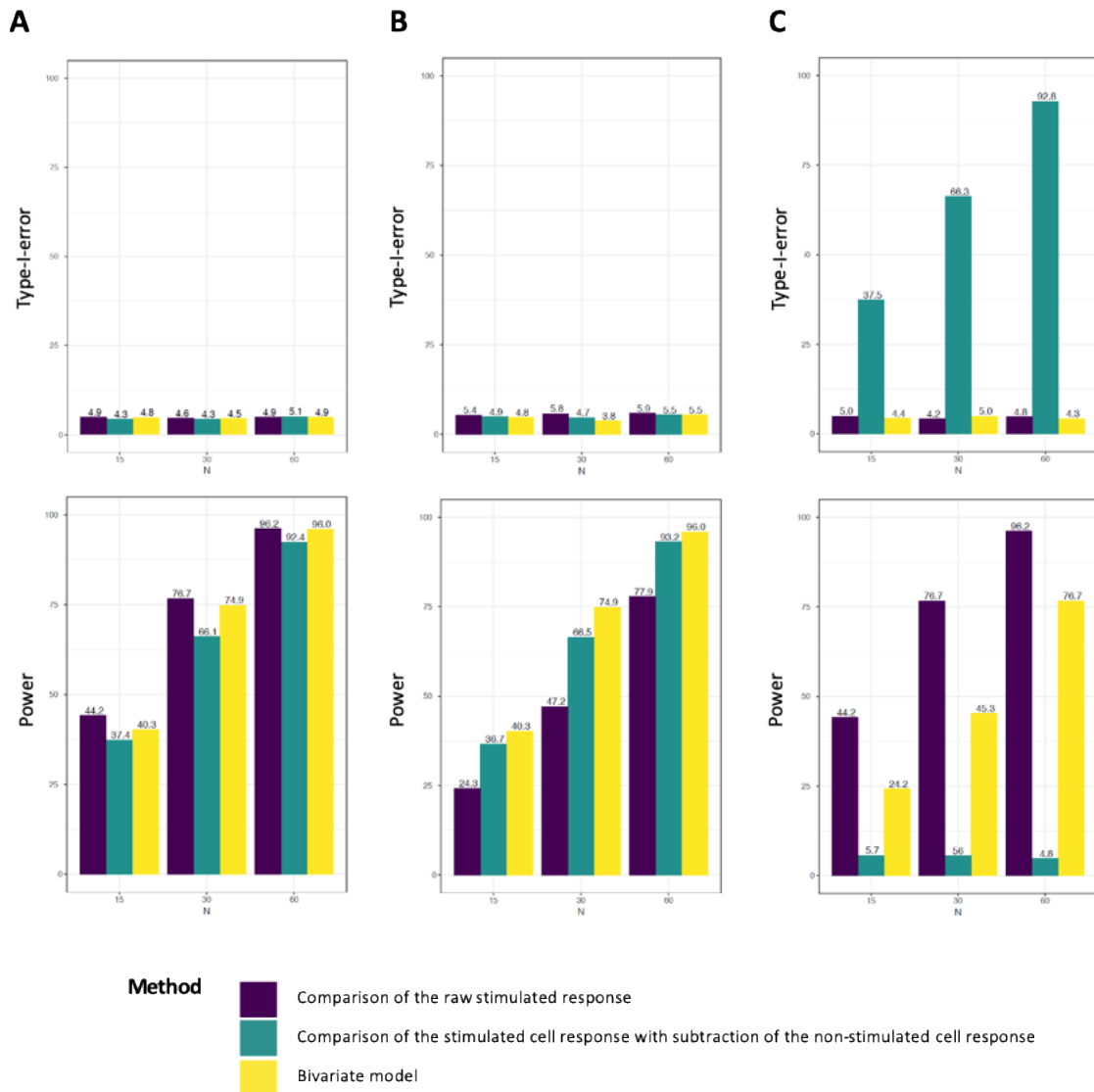
Model (2), one arm comparison (post-vaccination versus baseline)

| | | | | | |
|-------------------|--|---|------|------|------|
| No vaccine effect | Vaccine effect on stimulated response: H0 $(\beta_0^S = 0)$ | 0 | 95.8 | 94.4 | 96.2 |
|-------------------|--|---|------|------|------|

| | | | | | |
|--|--|------|------|------|------|
| Vaccine effect on stimulated response, no correlation between stimulated and non-stimulated response | Vaccine effect on stimulated response: H1($\beta_0^S \neq 0$) | 0.01 | 95.8 | 94.4 | 96.2 |
|--|--|------|------|------|------|

| | | | | | |
|--|---|------|------|------|------|
| No vaccine effect between arms on stimulated response, correlation between stimulated and non-stimulated response | Vaccine effect on stimulated response: H0 ($\beta_0^S = 0$) | 0 | 95.2 | 95.5 | 94.2 |
| Vaccine effect on stimulated response, no correlation between stimulated and non-stimulated response, correlation between stimulated and non-stimulated response | Vaccine effect on stimulated response: H1 ($\beta_0^S \neq 0$) | 0.01 | 95.2 | 95.5 | 94.2 |
| Similar vaccine effect between arms on stimulated response, Vaccine effect on non-stimulated response | Vaccine effect on stimulated response: H0 ($\beta_0^S = 0$) | 0 | 94.7 | 34.8 | 95.9 |

| | | | | | |
|--|---|------|------|------|------|
| Vaccine effect on stimulated response, Vaccine effect on non-stimulated response | Vaccine effect on stimulated response: H1 ($\beta_0^S \neq 0$) | 0.01 | 94.7 | 34.8 | 95.9 |
|--|---|------|------|------|------|



Appendix B – Figure 1: Evaluation of the performance of Model 2 in terms of control of type 1 error and statistical power of the bivariate model for evaluating the effect of the vaccine (arm-vaccine versus placebo) compared to conventional approaches (with or without subtraction of the non-stimulated response) via simulations (1000 simulations per scenario).

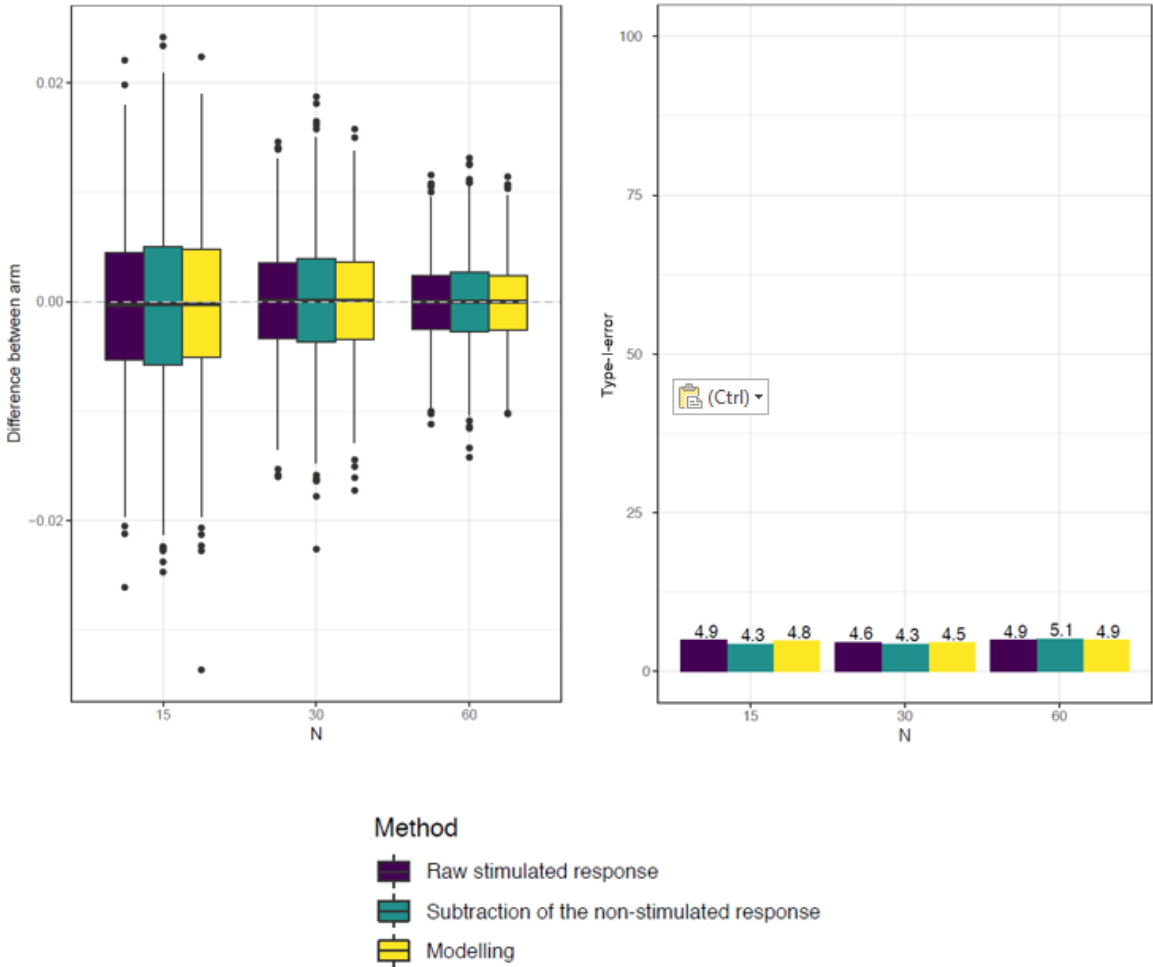
To have good statistical performance, a model must control the type 1 error at 5% and the power must be as high as possible (generally 80%).

A: Scenarios with no correlation between stimulated and non-stimulated response and no vaccine effect on the non-stimulated response

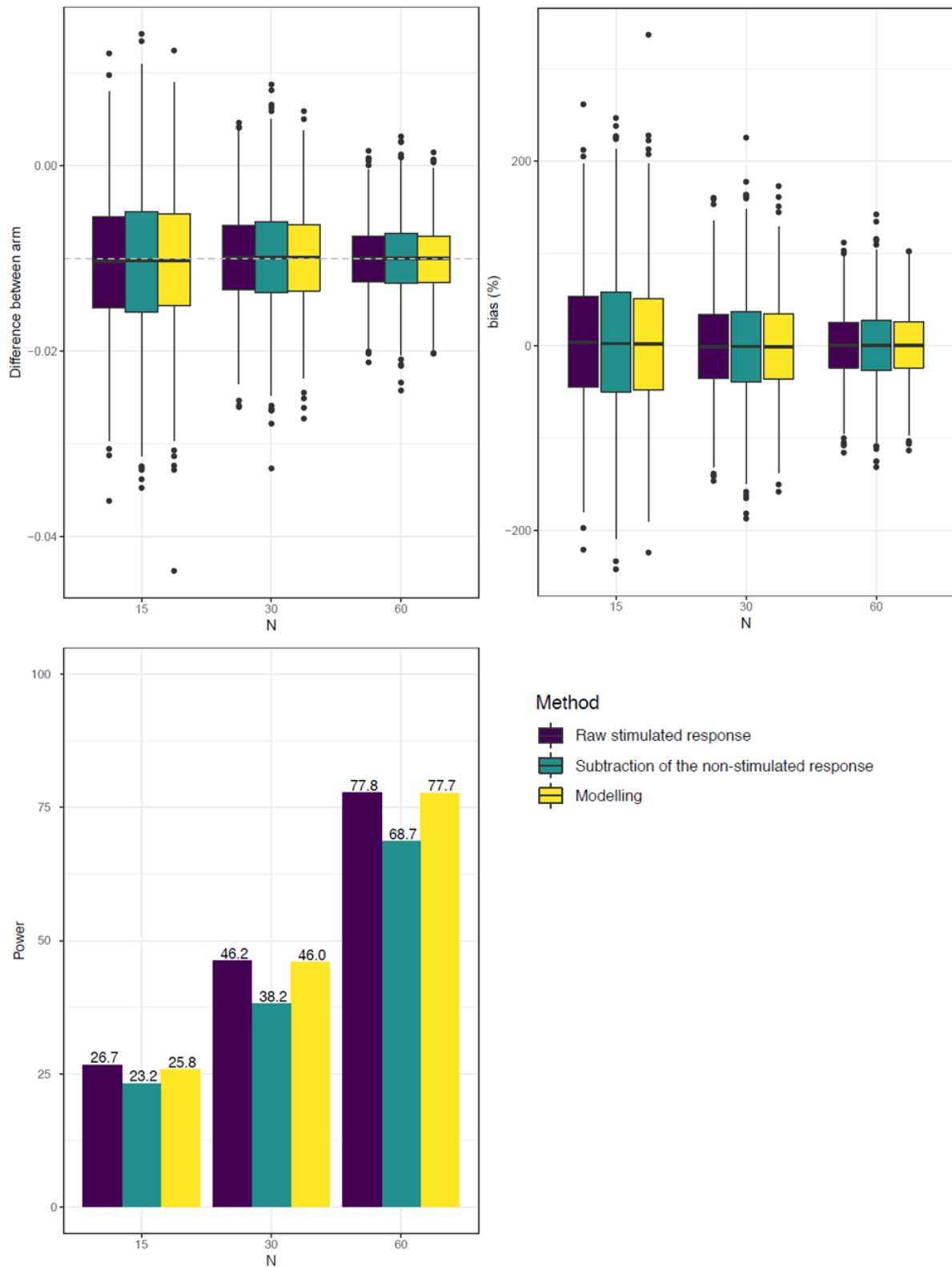
B: Scenarios with correlation ($p=0.70$) between stimulated and non-stimulated response

C: Scenarios with vaccine effect on the non-stimulated response

Simulation results – Model 1

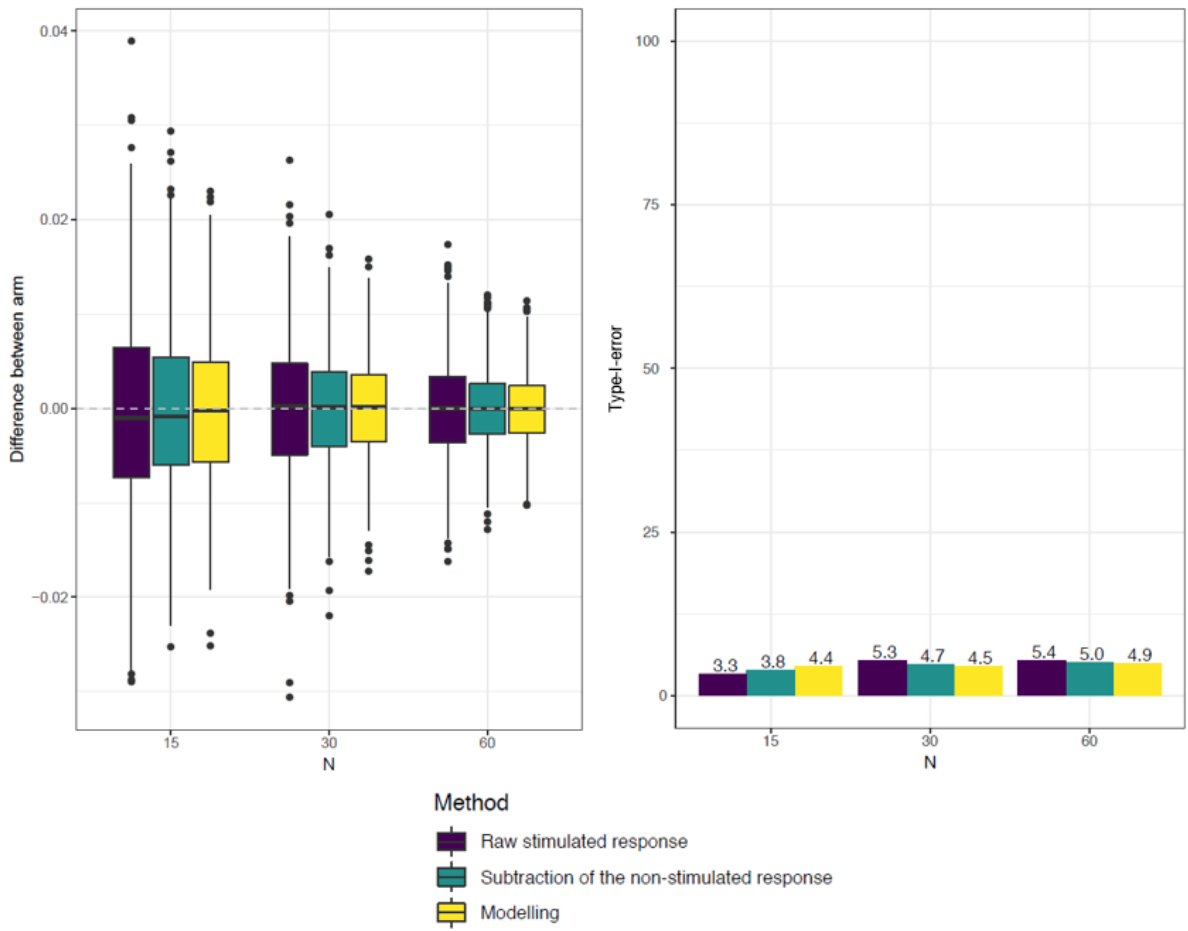


Appendix B – Figure 1. Scenario simulating a similar vaccine effect between arms on stimulated response. Comparison of the difference between arm (left) and the type I error risk (right) between the modelling approach (model 2) and two conventional approaches (comparing respectively the raw stimulated response or the stimulated response after subtraction of the non-stimulated response)

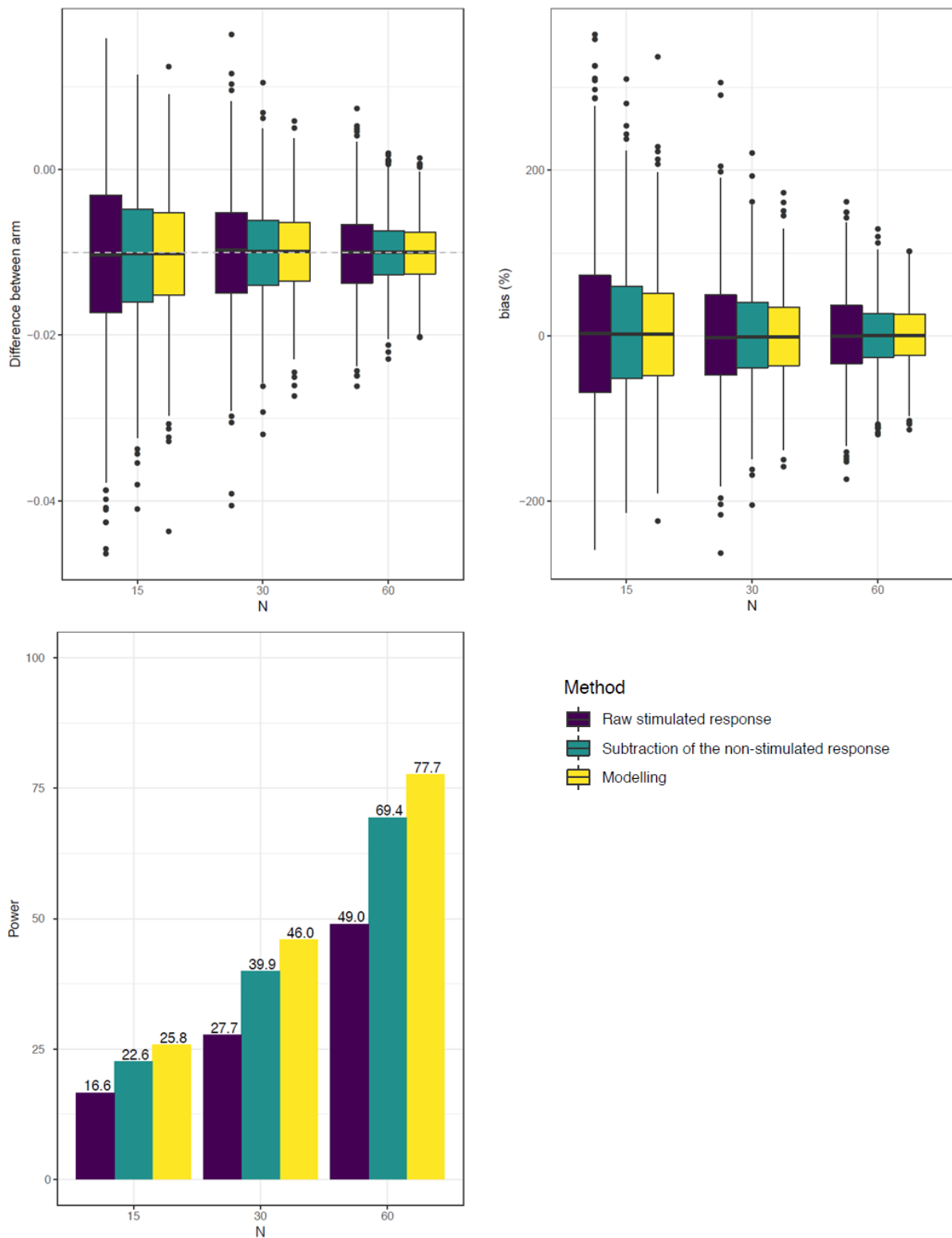


Appendix B – Figure 2. Scenario simulating a vaccine effect on stimulated response, no correlation between stimulated and non-stimulated response. Comparison of the difference between arm, the percentage of bias and the power between the modelling approach (model 2)

and two conventional approaches (comparing respectively the raw stimulated response or the stimulated response after subtraction of the non-stimulated response)

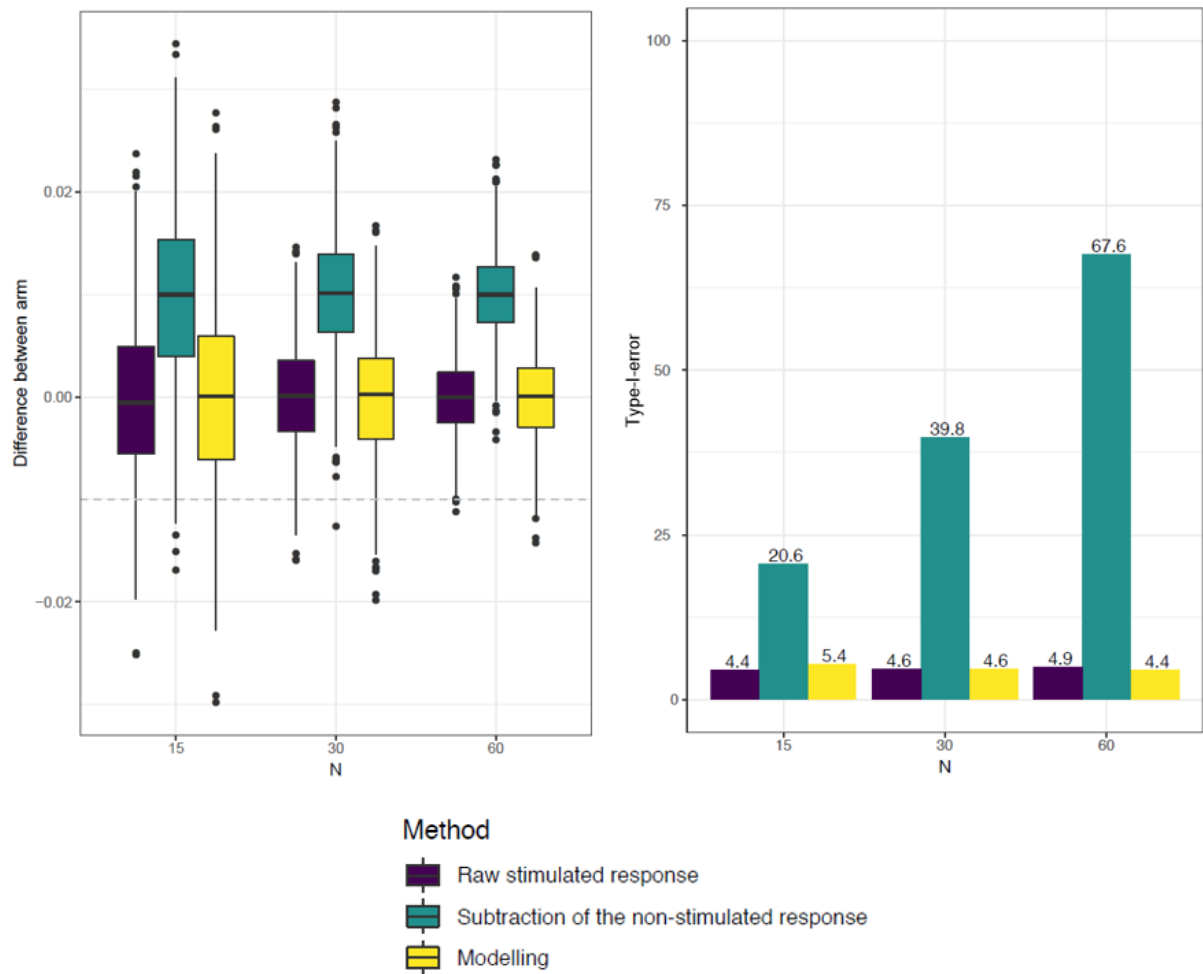


Appendix B – Figure 3. Scenario simulating a similar vaccine effect between arms on stimulated response, correlation between stimulated and non-stimulated response. Comparison of the difference between arm (left) and the type I error risk (right) between the modelling approach (model 2) and two conventional approaches (comparing respectively the raw stimulated response or the stimulated response after subtraction of the non-stimulated response)

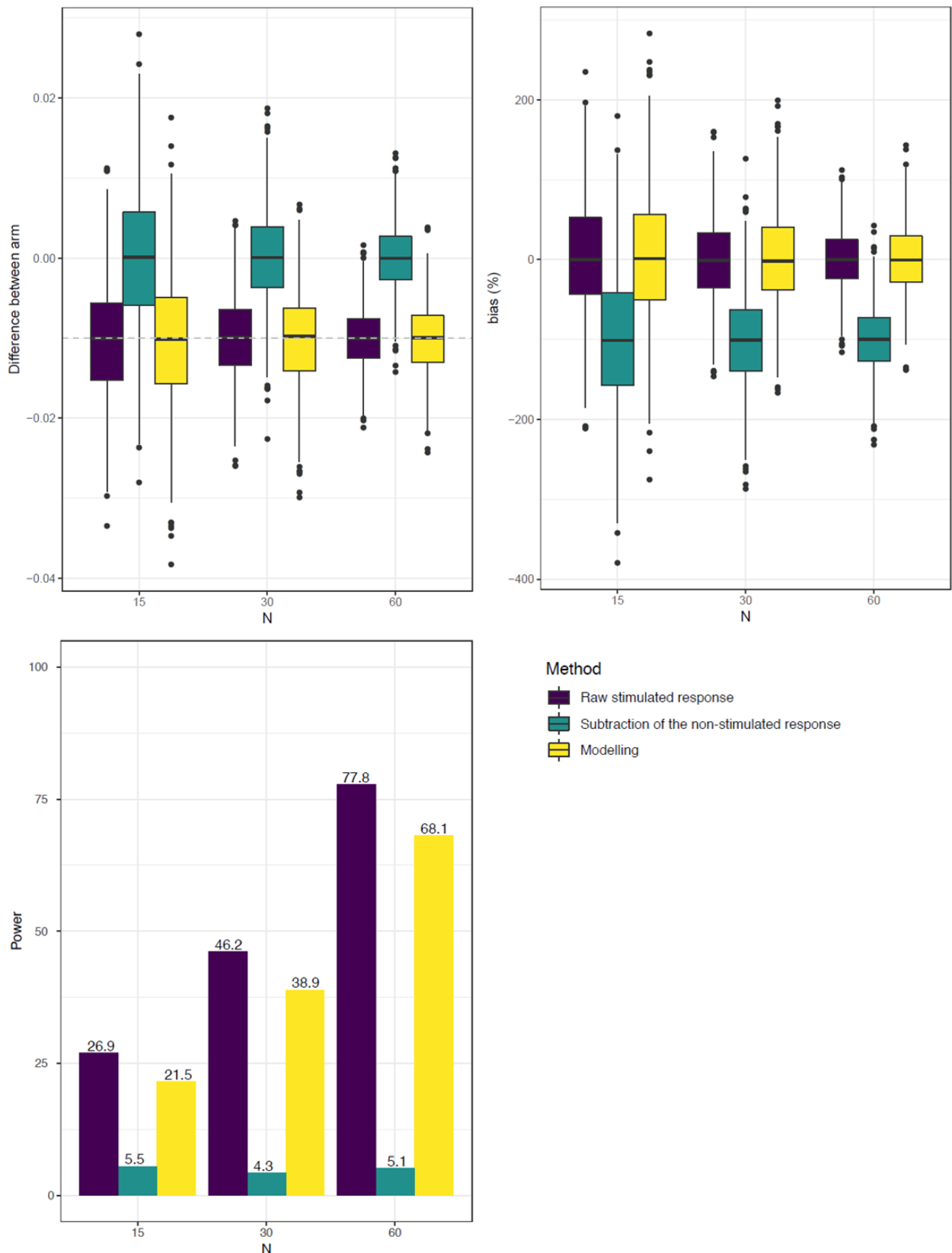


Appendix B – Figure 4. Scenario simulating a vaccine effect on stimulated response, correlation between stimulated and non-stimulated response. Comparison of the difference between arm, the percentage of bias and the power between the modelling approach (model 2) and two

conventional approaches (comparing respectively the raw stimulated response or the stimulated response after subtraction of the non-stimulated response)



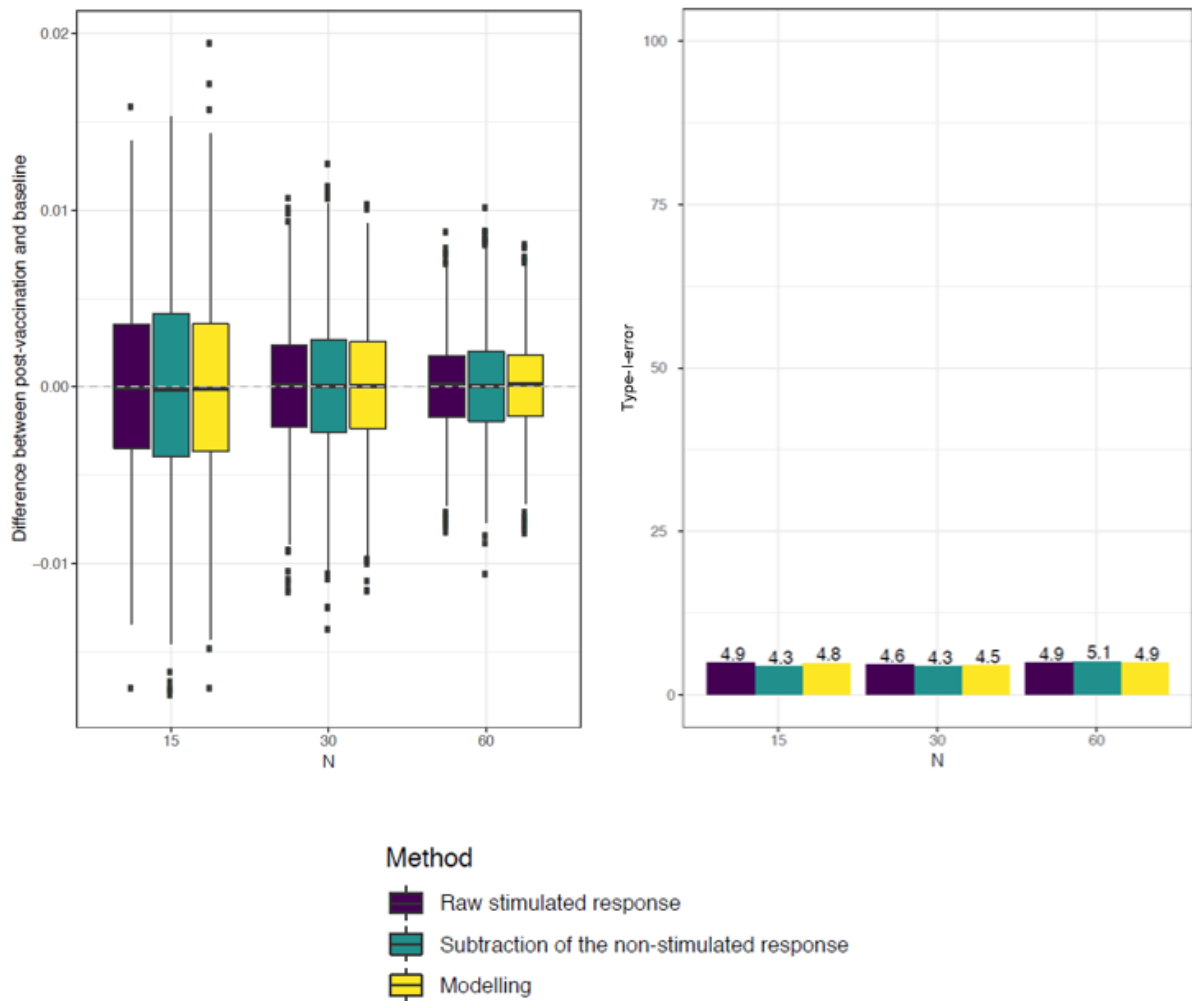
Appendix B – Figure 5. Scenario simulating a similar vaccine effect between arms on stimulated response, Vaccine effect on non-stimulated response. Comparison of the difference between arm (left) and the type I error risk (right) between the modelling approach (model 2) and two conventional approaches (comparing respectively the raw stimulated response or the stimulated response after subtraction of the non-stimulated response)



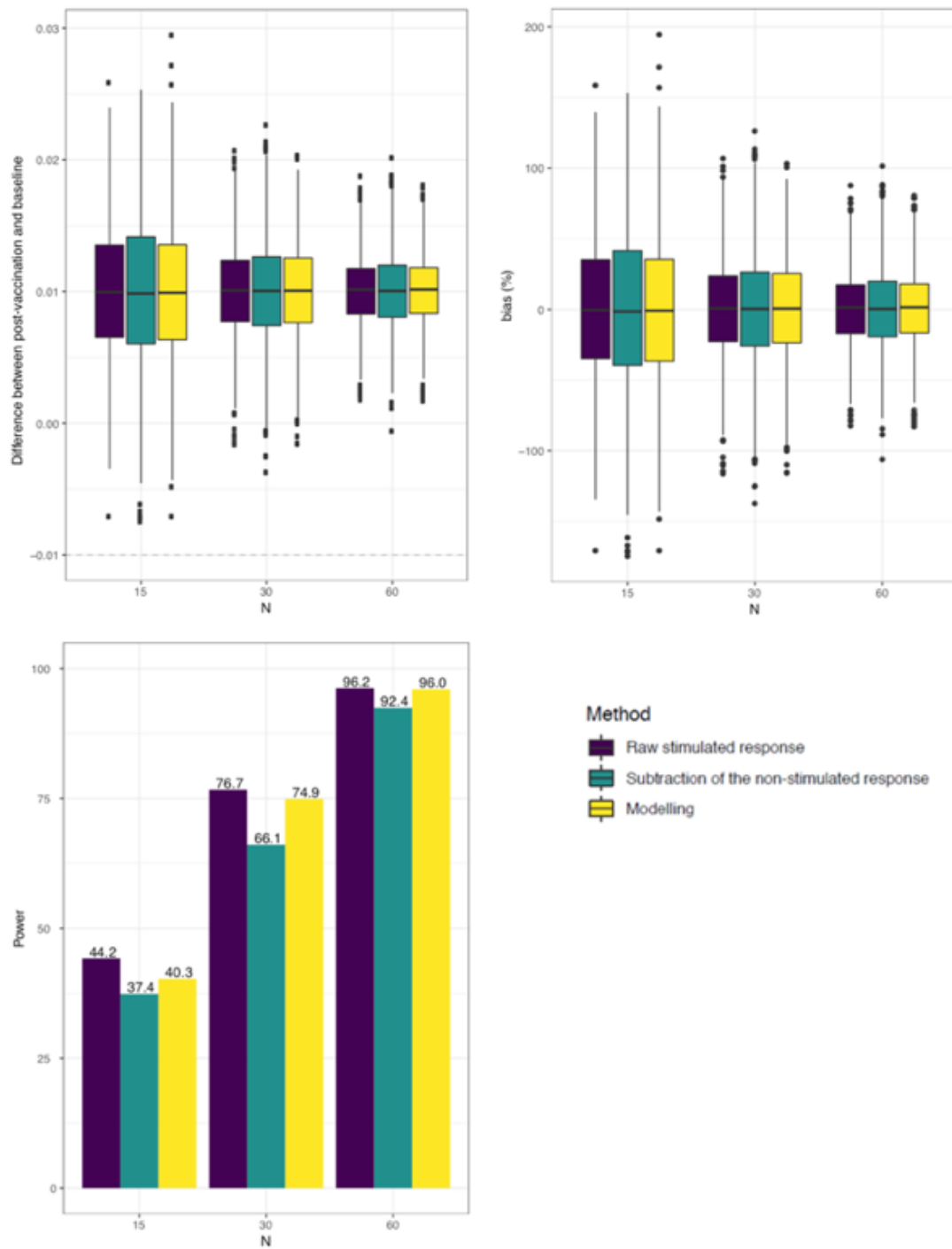
Appendix B – Figure 6. Scenario simulating a vaccine effect on stimulated response, Vaccine effect on non-stimulated response. Comparison of the difference between arm, the percentage of bias and the power between the modelling approach (model 2) and two conventional

approaches (comparing respectively the raw stimulated response or the stimulated response after subtraction of the non-stimulated response)

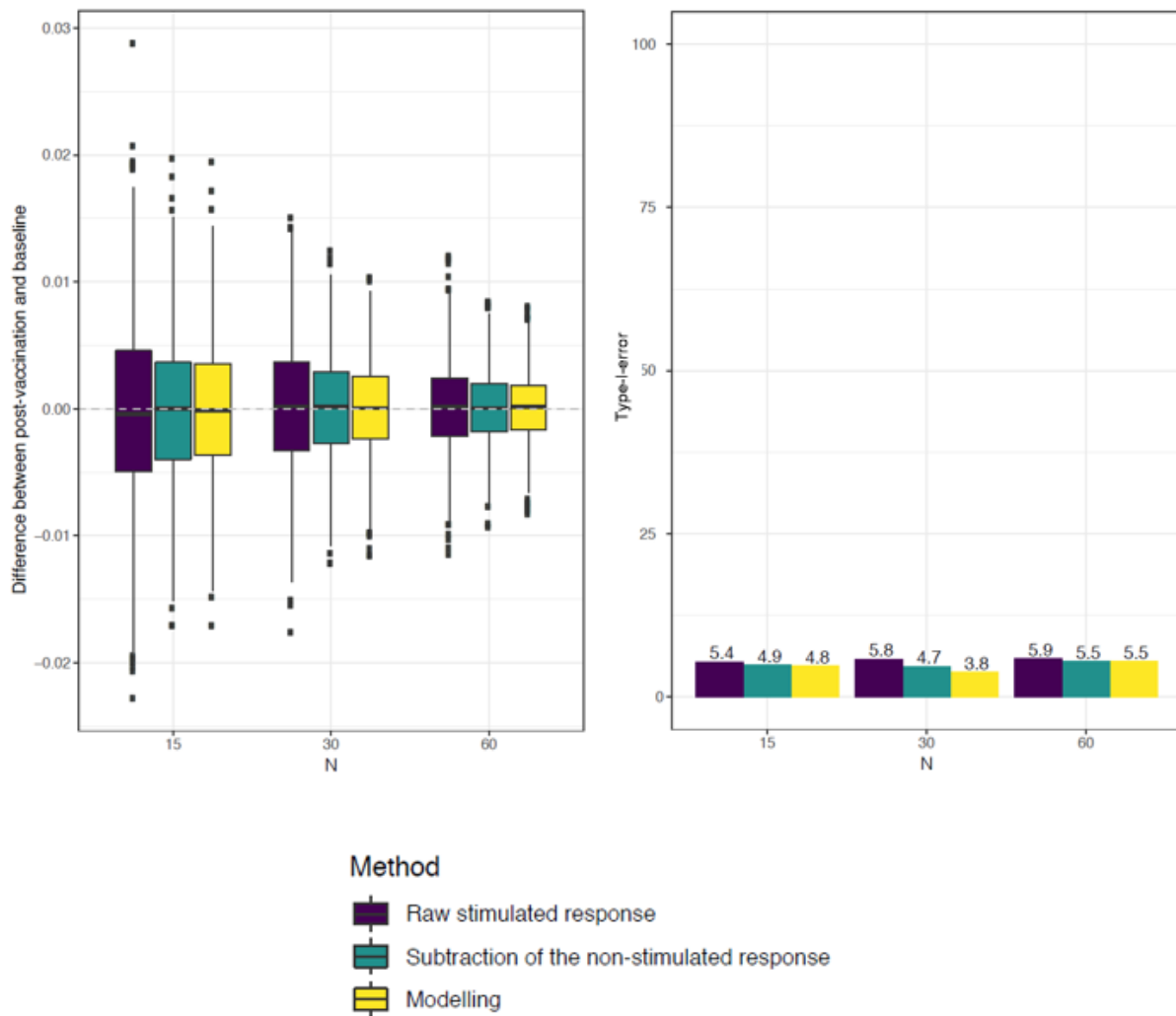
Simulation results – Model 2



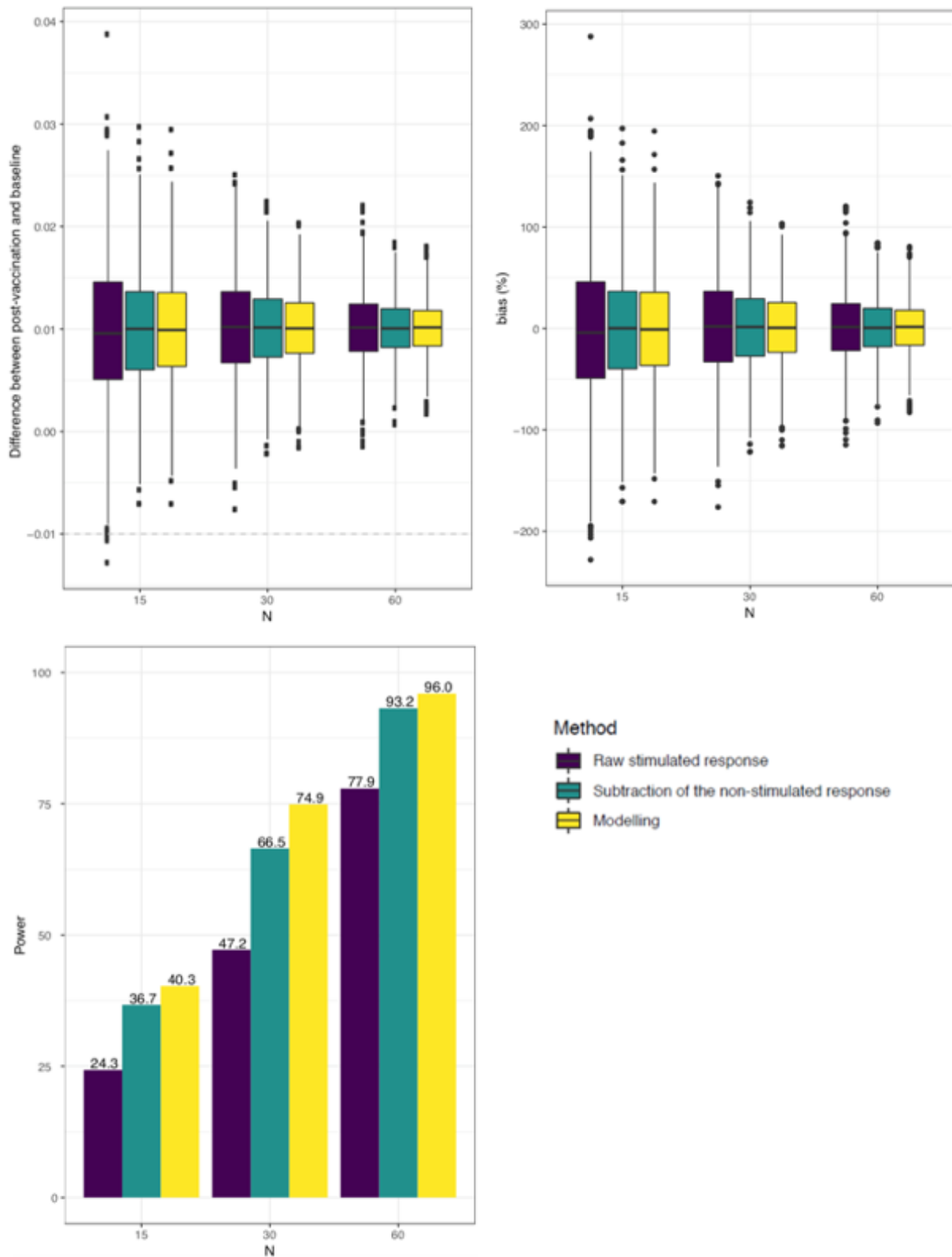
Appendix B – Figure 1. Scenario simulating a similar vaccine effect between arms on stimulated response. Comparison of the difference between arm (left) and the type I error risk (right) between the modelling approach (model 1) and two conventional approaches (comparing respectively the raw stimulated response or the stimulated response after subtraction of the non-stimulated response)



Appendix B – Figure 2. Scenario simulating a vaccine effect on stimulated response, no correlation between stimulated and non-stimulated response. Comparison of the difference between arm, the percentage of bias and the power between the modelling approach (model 1) and two conventional approaches (comparing respectively the raw stimulated response or the stimulated response after subtraction of the non-stimulated response)

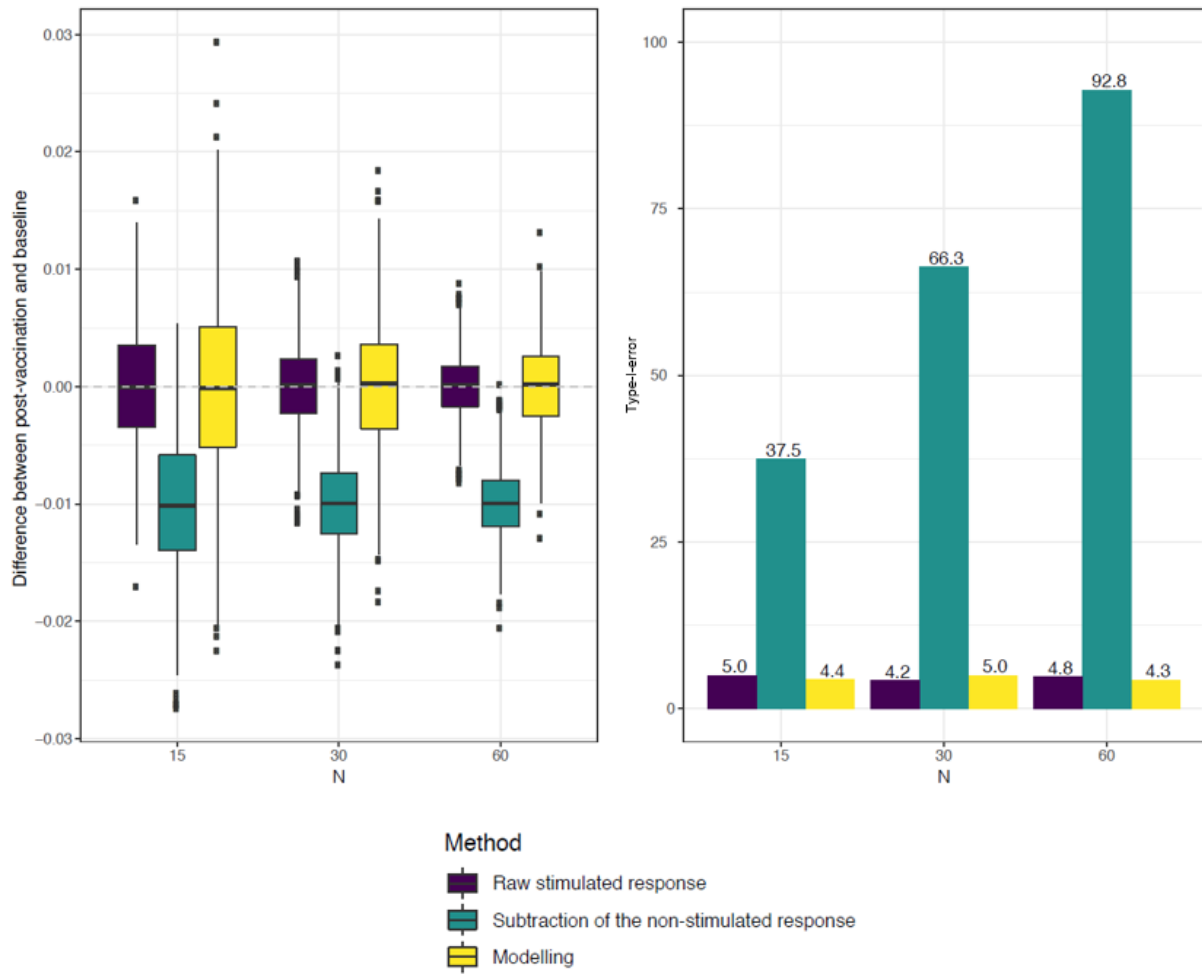


Appendix B – Figure 3. Scenario simulating a similar vaccine effect between arms on stimulated response, correlation between stimulated and non-stimulated response. Comparison of the difference between arm (left) and the type I error risk (right) between the modelling approach (model 1) and two conventional approaches (comparing respectively the raw stimulated response or the stimulated response after subtraction of the non-stimulated response)

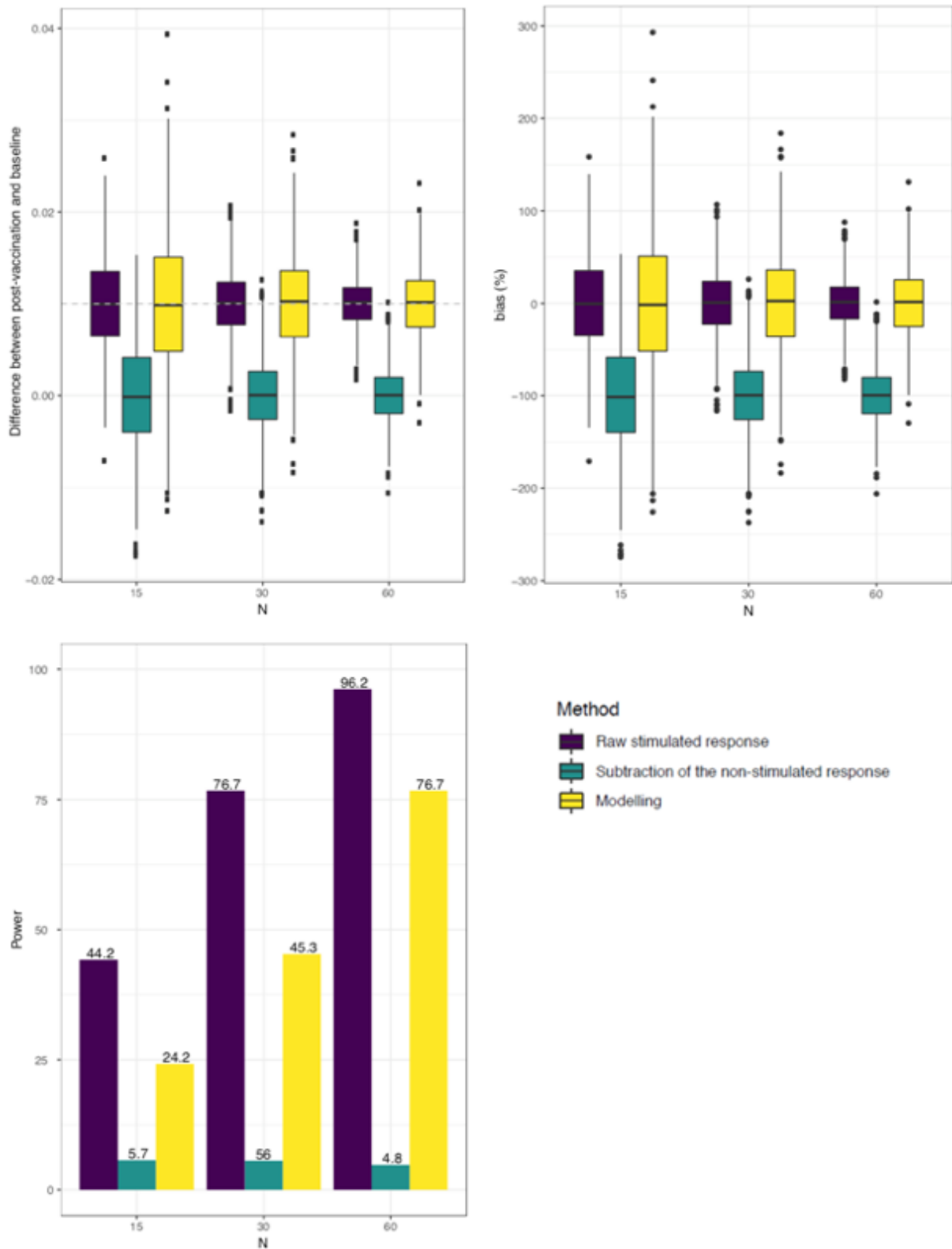


Appendix B – Figure 4. Scenario simulating a vaccine effect on stimulated response, no correlation between stimulated and non-stimulated response, correlation between stimulated and non-stimulated response. Comparison of the difference between arm, the percentage of bias

and the power between the modelling approach (model 1) and two conventional approaches (comparing respectively the raw stimulated response or the stimulated response after subtraction of the non-stimulated response)



Appendix B – Figure 5. Scenario simulating a similar vaccine effect between arms on stimulated response, Vaccine effect on non-stimulated response. Comparison of the difference between arm (left) and the type I error risk (right) between the modelling approach (model 1) and two conventional approaches (comparing respectively the raw stimulated response or the stimulated response after subtraction of the non-stimulated response)



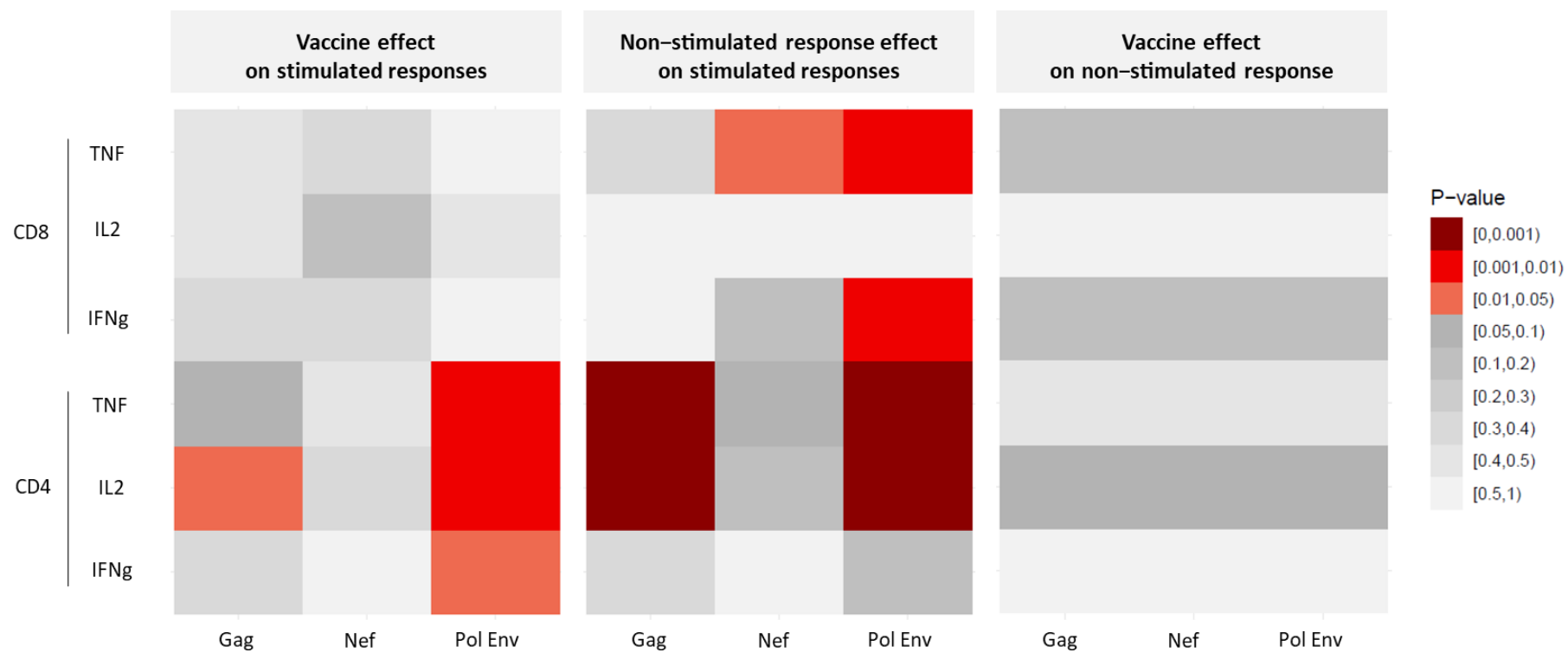
Appendix B – Figure 6. Scenario simulating a vaccine effect on stimulated response, Vaccine effect on non-stimulated response. Comparison of the difference between arm, the percentage of bias and the power between the modelling approach (model 1) and two conventional

approaches (comparing respectively the raw stimulated response or the stimulated response after subtraction of the non-stimulated response)

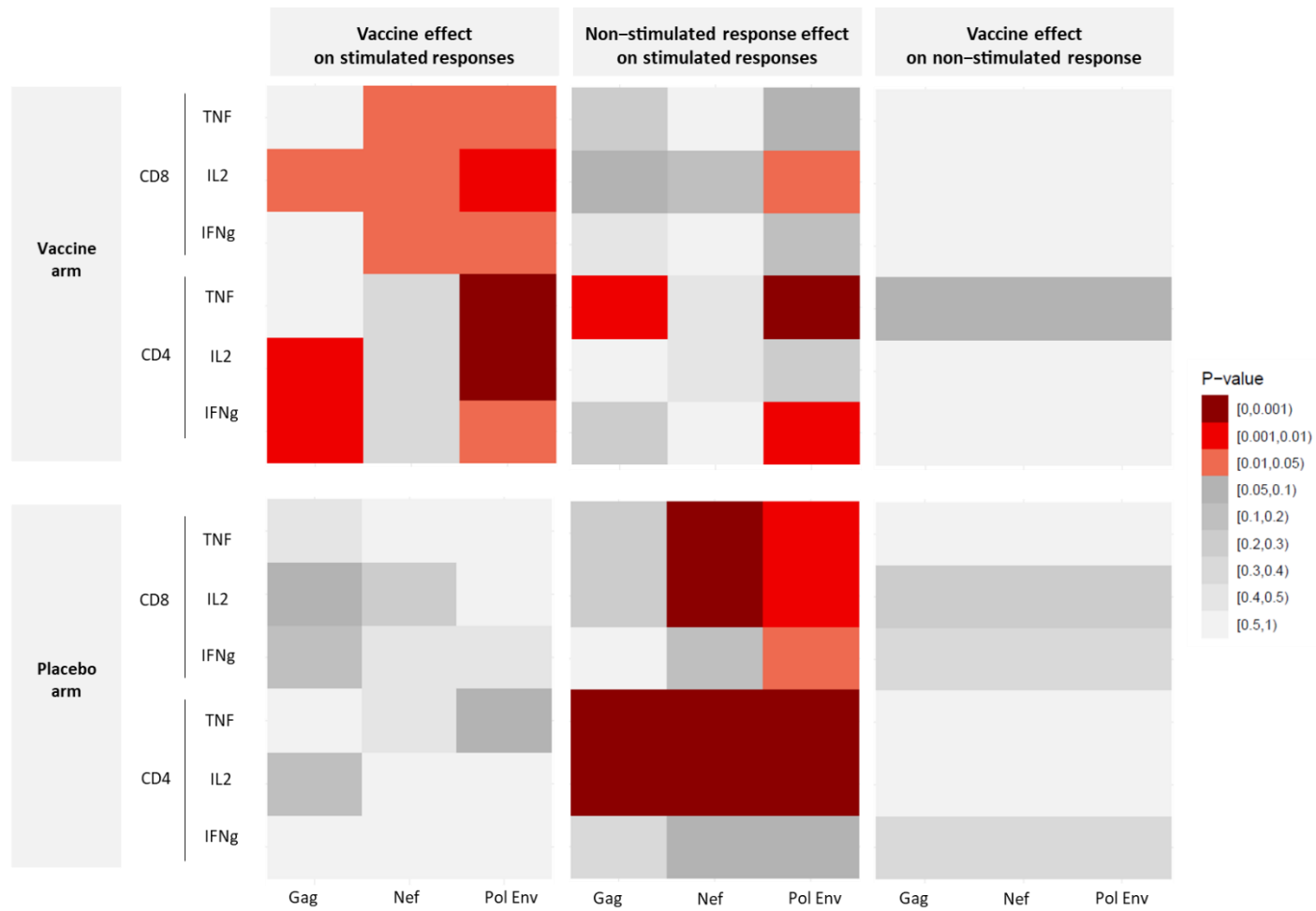
Appendix C – ICS analyzes

To assess antigen-specific T-cell responses, ICS assay was performed in a centralized laboratory (MIC-VRI, Creteil, France) on cryopreserved PBMC. PBMC were rested overnight and then stimulated (6h, 37°C, 5% CO₂) with HIV peptide pools (1µg/ml) in the presence of co-stimulatory molecules (anti-CD28 and anti-CD49d antibodies (1µg/ml)) and a protein transport inhibitor (Golgi Plug (1 µl/ml)) (BD Biosciences, Le Pont de Claix, France). SEB stimulation (100 ng/ml Staphylococcus Enterotoxin B; Sigma Aldrich, Saint Quentin Fallavier, France) served as positive control. After stimulation, cells were stained for dead cells with an amine-reactive dye (LIVE/DEAD Aqua, Invitrogen, Life Technologies, Saint Aubin, France) and with fluorochrome-conjugated monoclonal antibodies (anti-CD3 Alexa700, anti-CD4 PE, and anti-CD8 efluor 780; all from BD Biosciences) for 15 min at room temperature. After fixation and permeabilization using Cytotfix/Cytoperm kit (BD Biosciences) for 20 min and staining with anti-IFN- γ PerCpCy5.5, -TNF- α PE-Cy7 and -IL-2 APC (all BD Biosciences) for 20 minutes at room temperature, PBMCs were re-suspended in Paraformaldéhyde 1% (BD Biosciences) and stored at 4°C until analysis. Data were acquired on a LSRII Fortessa 4-laser (488, 640, 561 and 405 nm) cytometer (BD Biosciences), analyzed using FlowJo software version 9.9.4 (Tree Star inc.). At least 250,000 events gated on CD3⁺ were collected and analyzed using Boolean gating.

Appendix D – VRI02 Light trial

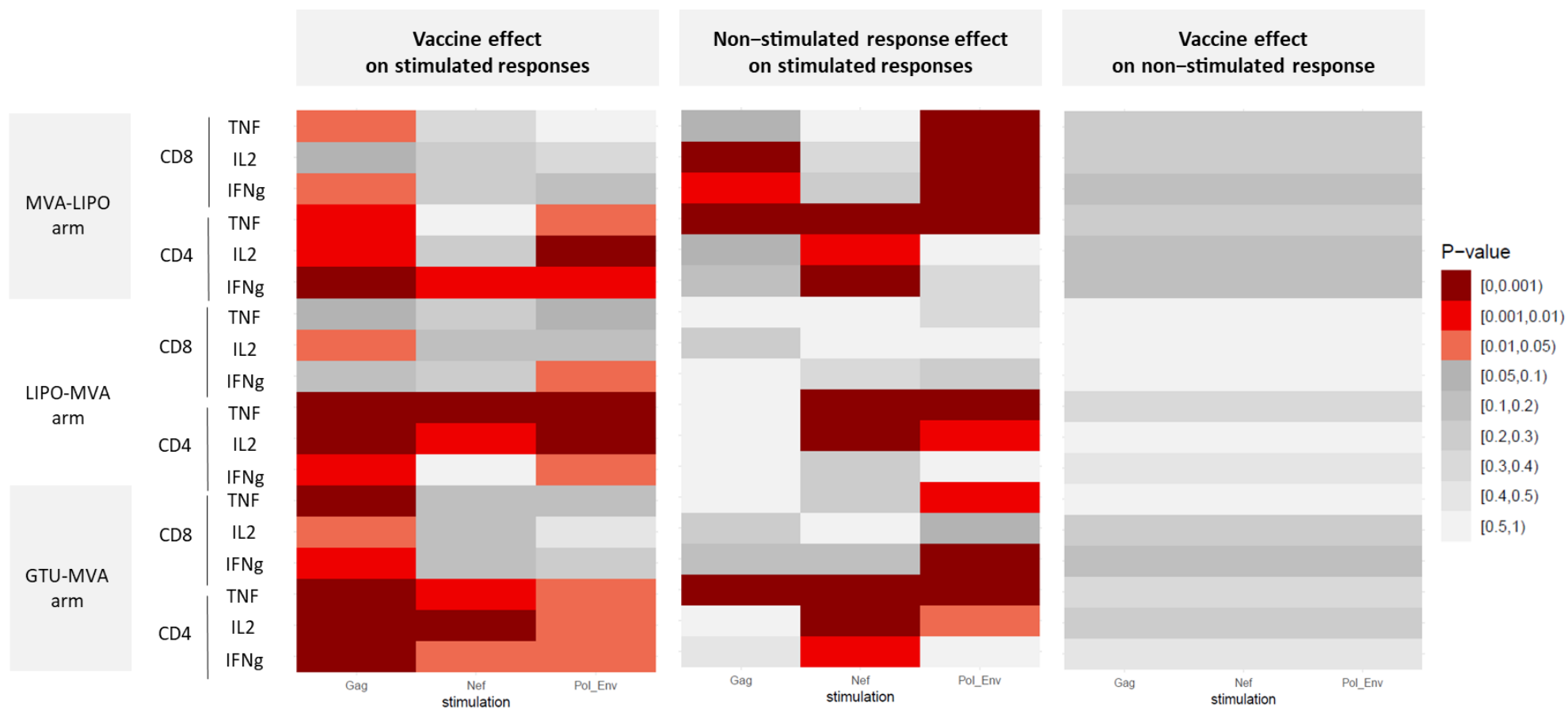


Appendix D – Figure 1. Description of the estimations obtained for inter-arm (vaccine versus placebo) comparison at W28 of the Light trial with modelling approach (Model 1)



Appendix D – Figure 2. Description of the estimations obtained for intra-arm (W0 versus W28) comparison of the Light trial with modelling approach (Model 2)

Appendix E – VRI01 trial



Appendix E – Figure 1. Description of the estimations obtained for intra-arm (W0 versus W30) comparison of the VRI01 trial with modelling approach (Model 2)

Appendix F – ANCOVA

ANCOVA has been described by Gilbert and al (Vaccine 2009). In their paper, the author showed that ANCOVA is an interesting method for analyzing immunogenicity of candidate vaccines in randomized clinical trials. ANCOVA was motivated in this context in order to account for baseline participant characteristics predictive of the response variable because this can improve statistical power. In the paper by Gilbert et al, the ANCOVA regression models the stimulated response - at the time point of interest – (independent variable) as a function of the variable defining the treatment arm (variable of interest), adjusted on the non-stimulated variable at baseline and the interaction between the two variables. The authors mentioned that this method is relevant in the case of a positive correlation between baseline non-stimulated response and the post-vaccination stimulated response at the time point of interest. Although this was not suggested by Gilbert et al, it is straightforward to use this model with adjustment for the non-stimulated variable at the same time point of interest.

Direct comparisons with ANCOVA have been performed with the others approaches in different scenarios (inter-arm comparison) with a vaccine effect on stimulated response ($\beta_1^S \neq 0$) with and without correlation (ρ) between the non-stimulated response at baseline, you can find the results below. ANCOVA showed inferior statistical performances in terms of statistical power (critically so in scenario A with no correlation) and an increase of the type-I-error in case of correlation (scenarios B and C):

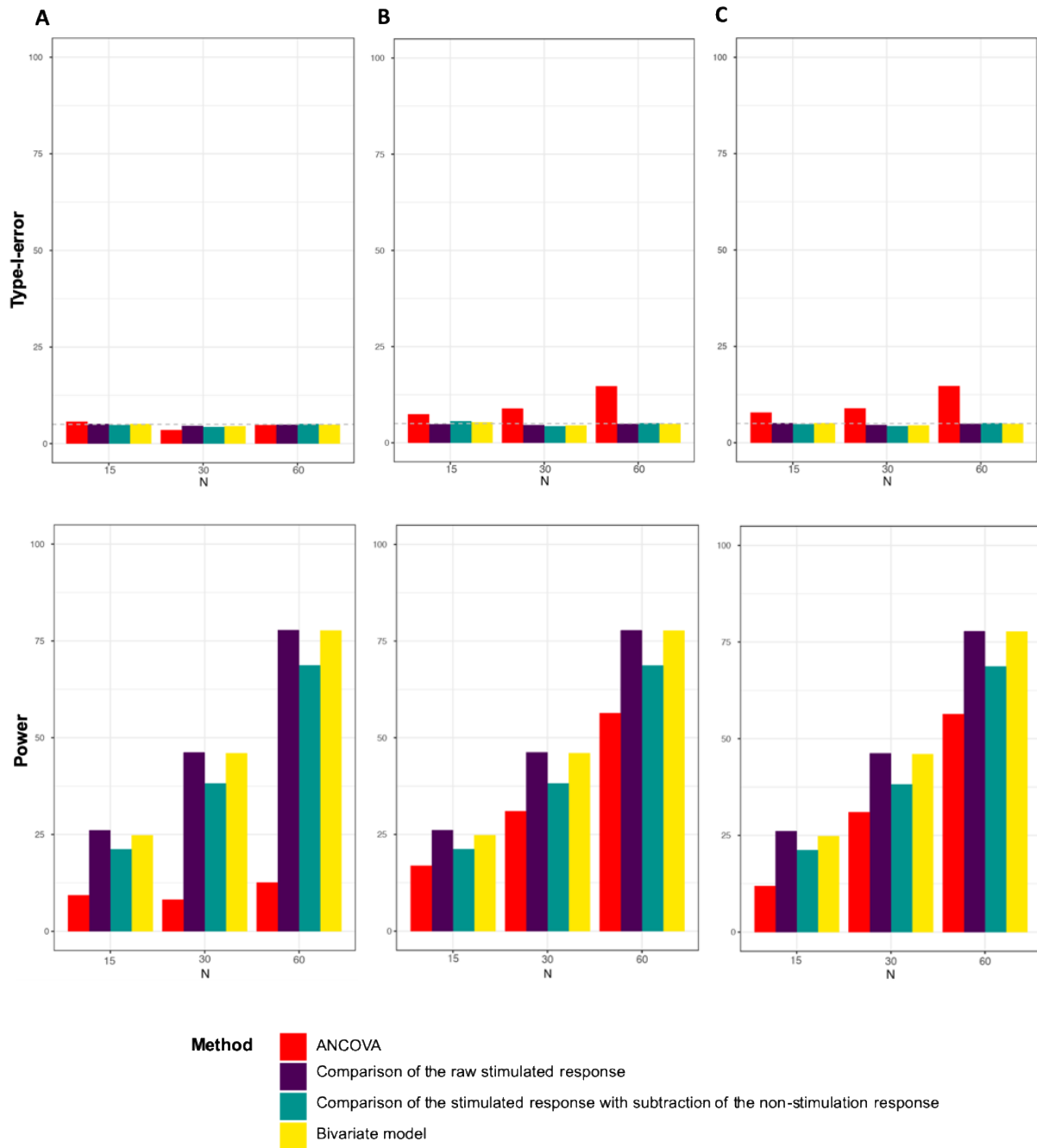


Figure1. Evaluation of the performance of model 1 in terms of control of type 1 error and statistical power of the bivariate model for evaluating the effect of the vaccine (arm-vaccine versus placebo) compared to conventional approaches (with or without subtraction of the non-stimulated cell response) and ANCOVA via simulations (1000 simulations per scenario).

A. Vaccine effect on stimulated response ($\beta_1^S \neq 0$), no correlation between stimulated response post vaccination and non-stimulated response at baseline

B. Vaccine effect on stimulated response ($\beta_1^S \neq 0$), correlation between stimulated response post vaccination and non-stimulated response at baseline (correlation coefficient $\rho=0.60$)

C. Vaccine effect on stimulated response ($\beta_1^S \neq 0$), correlation between stimulated response post vaccination and non-stimulated response at baseline (correlation coefficient $\rho=0.80$)

Appendix G – Additional simulations with non-Gaussian distribution

To check the method robustness, we have performed an additional simulation analysis using a non-Gaussian distribution. The data used for this are based on real data (CD8+ IFN γ + Tcell response from Light trial), on which we have added a random exponential noise (standard deviation = 0.5). The results of this simulation under H0 showed a good control of the type-I error at 5% for the bivariate model approach, as well as for the conventional approaches (see Figure 1 below).

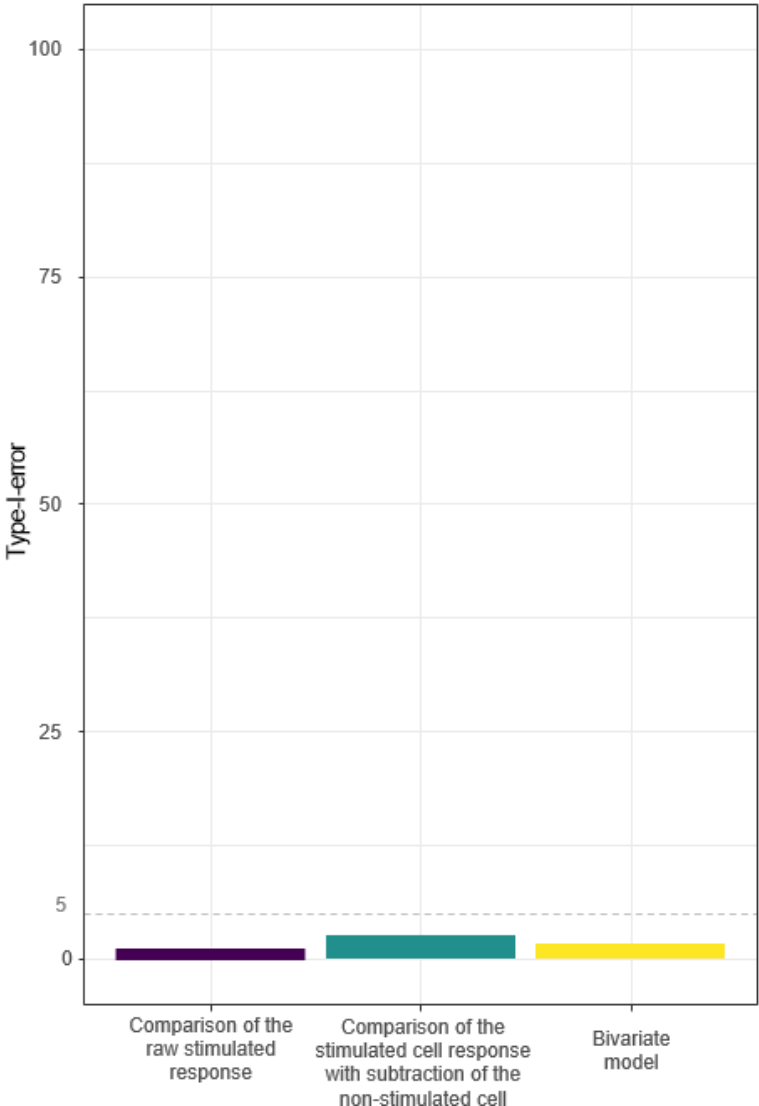


Figure 1. Evaluation of the performance of model 1 in terms of control of type-I error of the bivariate model for evaluating the effect of the vaccine (arm-vaccine versus placebo) compared to conventional approaches (with or without subtraction of the non-stimulated cell response) via non-Gaussian simulations (1000 simulations per scenario).

VICI: accurate estimation of Vaccine Induced Cellular Immunogenicity with bivariate modeling

The bivariate model has been implemented in both SAS and R statistical software. The SAS and R code are available in the manuscript, so that the model can be easily used for instance by biostatisticians from a clinical trials unit. However, as we would like to make our method available for a broader spectrum of users, for instance also for immunologists that are not trained for using code-based statistical software, we also developed a user friendly online interface called “VICI”. There, the user can upload their ICS data and directly analyze them with the modelling approach, with numerical and visual results provided on the online interface. We do believe that this will help immunologists to adopt this new statistical approach.

We choose to develop the online application using R Shiny, which is an R package that makes it easy to build interactive web apps straight from R. VICI is relying on the *nmle* package from which we have implemented the modelling approach with R and is already available online on the following URL: <https://shiny-vici.apps.math.cnrs.fr/> . My team colleague Boris Hejblum has developed the code of R Shiny application. Immunologists from Mondor Immunotoring Platform have performed user tests during the development of the interface to make it intuitive and understandable for this targeted audience.

The screen of the interface is divided in two parts. The left side is dedicated to the data-management steps (figure 6): first, the user can import a ICS dataset (CSV or TXT file), specify the type of analysis (inter-arm or intra-arm comparison) and the different variables (subject ID, ICS response, stimulation, arm). If several time-points, the user is also invited to select the column that identifies the observation's time-point.

After click on “run”, the user can see the results of the analysis in a first tab with a box plot of the different stimulations and a heatmap of the p-values. These figures can be directly use in a statistical report or a manuscript. In a third (figure 7). On a second tab, visualize the dataset and in a third tab, the statistical model fitted for each ICS response and additional estimates (number of estimated model parameters, Akaike information criterion, loglikelihood, estimated variance of the parameters).

VICI: accurate estimation of Vaccine Induced Cellular Immunogenicity with bivariate modeling

v0.5.0

Data input

Choose a CSV/TXT file to import

Browse... No file selected

Header

Separator

Comma

Semicolon

Tab

Input parameters

Model choice

inter-arm

Variable specification

Select the column that identifies the subject ID

Please select a column name below

Select the column(s) that identify ICS response

Please select a column name below

Select the column that identifies the stimulation

Please select a column name below

Select the column that identifies the arm

Please select a column name below

If several time-points (optional), please select the column that identifies the observation's time-point

Please select a column name below

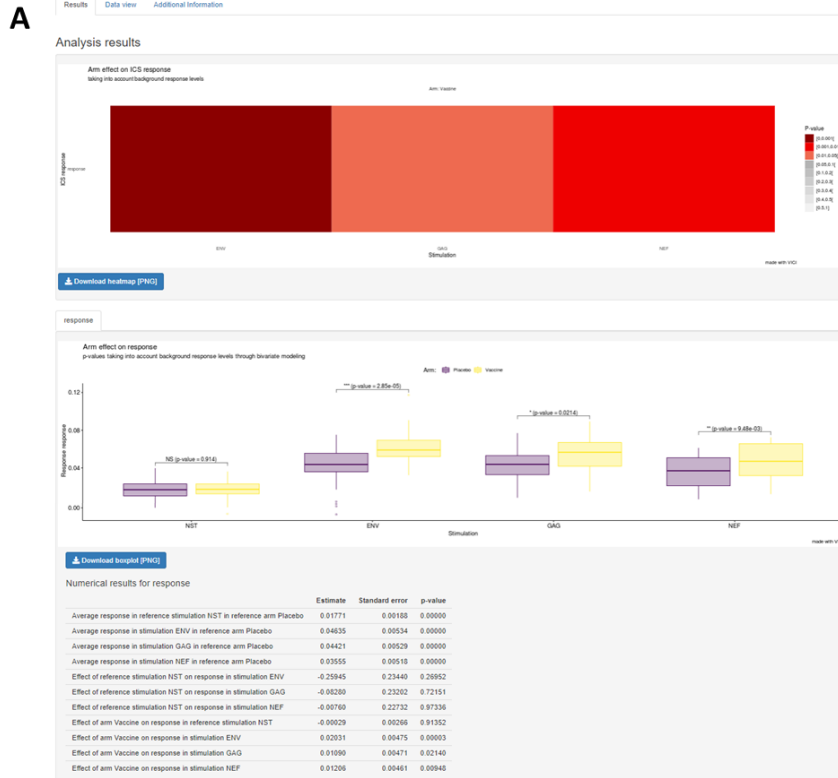
Run analysis

Fit model

Results Data view Additional information

Figure 6. Screenshot of the welcome screen of “VICI” R shiny application.

Concerning intellectual property, a software deposit has been completed at the Agency for the Protection of Programs (APP), which is a European organization for the defense of authors and publishers of digital works.



B

Results Data view Additional information

Show 1 to 10 of 240 entries

| | subject_ID | response | arm | stimulation |
|----|------------|--------------|---------|-------------|
| 1 | 1 | 0.010282467 | Placebo | NST |
| 2 | 2 | 0.009886814 | Placebo | NST |
| 3 | 3 | 0.0185279383 | Placebo | NST |
| 4 | 4 | 0.0102190004 | Placebo | NST |
| 5 | 5 | 0.0311600206 | Placebo | NST |
| 6 | 6 | 0.0166700359 | Placebo | NST |
| 7 | 7 | 0.010266492 | Placebo | NST |
| 8 | 8 | 0.0276842208 | Placebo | NST |
| 9 | 9 | 0.0001987258 | Placebo | NST |
| 10 | 10 | 0.0381495325 | Placebo | NST |

Showing 1 to 10 of 240 entries

C

Results Data view Additional information

Statistical model fitted for each ICS response:

$$y^{NST} = \beta_0^{NST} + \beta_{Vaccine}^{NST} \cdot Vaccine_i + \epsilon_i^{NST}$$

$$y^{ENV} = \beta_0^{ENV} + \beta_{Vaccine}^{ENV} \cdot Vaccine_i + \beta_{NST}^{ENV} \cdot y^{NST} + \epsilon_i^{ENV}$$

$$y^{GAG} = \beta_0^{GAG} + \beta_{Vaccine}^{GAG} \cdot Vaccine_i + \beta_{NST}^{GAG} \cdot y^{NST} + \epsilon_i^{GAG}$$

$$y^{NEF} = \beta_0^{NEF} + \beta_{Vaccine}^{NEF} \cdot Vaccine_i + \beta_{NST}^{NEF} \cdot y^{NST} + \epsilon_i^{NEF}$$

Additional estimates:

Number of estimated model parameters: 15

| | AIC | -2 Res. logLikelihood |
|----------|------------|-----------------------|
| response | -1209.7372 | -1239.7372 |

Estimated variances

| response | variance |
|----------|----------|
| NST | 0.000339 |
| ENV | 0.000332 |
| GAG | 0.000319 |
| NEF | 0.000106 |

Figure 7. Screenshot of the “VICI” application with an example of analysis of an ICS dataset with three stimulation. A. Results tab: presentation of the Heatmap of p-values for the vaccine effect on the stimulated response; boxplots of the non-stimulated and stimulated responses stratified by vaccine arm; Table of the different parameter estimates and their respective standard deviation and associated p-values.

B. Data view tab: visualization of the dataset uploaded on the application. C. Additional information tab: specification of the bivariate modelling used; additional estimates.

Discussion

In this chapter, we focused on the cellular responses, with the evaluation of HIV vaccine candidates as example. We addressed two methodological points linked on one hand to the dynamics of immune responses over time and the choice of time points to measure immunogenicity and on the other hand to the improvement of the statistical analysis methods of ICS response in early clinical trials. The work on the dynamics of the immune response in the HVTN 068 trial was the first to find an association between the measurement of CD4+ IL-2+ responses 2 weeks following the prime and subsequent CD8+ IFN-g+ T cell responses, emphasizing the role of early CD4+ helper cells in stimulating the response of CD8+ cytotoxic T cells. The second work on the development of the bivariate modelling approach for the analysis of the ICS response found that the new approach that we propose had good statistical performances and should be the new statistical standard method for ICS analyses.

These two works showed that modelling immune responses to vaccines requires complex models. The HVTN 068 modelling has identified a suitable method for modelling the immune response using mixed models and splines. Several methods have been tested during the development of the modelling of the HVTN 068 data before splines, for instance different slopes or fractional polynomials but were not adapted and led to convergence issues. The splines by their flexibility allowed to take into account the particular effect of time in vaccine clinical trials, especially when there is a complex prime boost strategy. The disadvantage of this method is that it does not allow direct interpretations of time coefficients in these models. In our case, in addition to our interest in prediction, the interpretation of the effect of time was limited to whether or not there was an overall effect and whether the effect of the CD4+ IL2+ T cell persisted with an adjustment over time. The modeling including splines for the time effect was therefore well adapted. The method with splines has since then already been used in other more recent work in progress in our team on the modelling of the immune response to identify clinical and sociodemographic factors that would explain the variability of the immune response. An alternative approach to modeling immunological responses to the vaccine would be the use of mechanistic models that are built from biological knowledge. In these models which constitute an axis of the SISTM team, biological knowledge is translated into mathematical equations using differential equations.

Regarding the development of the bivariate modelling approach, this method could be used for analyzing the T cell response with other immunological assays (eg. EliSpot assay) and more broadly other immune responses where there is a background response measurement. For instance, the model is currently tested in the team for the analysis of

proliferative response in Ebola vaccine trials. It could have an application beyond immunology in other fields where this type of data exists. The R package and R shiny app developed during this work are currently adapted to the ICS dataset but extension could be developed to take into account other type of data sets. This research results will have an impact on future vaccine clinical trials. First, regarding improvement of vaccine trial design, our finding of a specific association with a measure at week 2 indicates that adding this early sampling time point to future trials could be of interest at least for the measure of CD4+ T cell responses. Early sampling time points should be considered in future clinical trials to better understand the role of the early CD4 helper T cells and to evaluate their predictive role in the immune response to vaccines. Validation in external datasets other than HVTN 068 would be of interest. We have so far not had the opportunity to perform an external validation of our hypothesis because existing HIV vaccine clinical trial datasets with CD4+ cell response measurements early after the prime are scarce. However, given the implication of the SISTM research team, including myself, in the design of the new HIV vaccine trials, we are in the position to suggest adding this time point in future trial protocols we are involved in with the collaboration with the VRI.

Regarding the bivariate modelling, this work will have a direct impact on the assessment on the ICS response in vaccine clinical trials. We now do not recommend the use of the conventional approach with or without background subtraction. So far in our team, the analysis using the conventional approach with the subtraction of the non-stimulated response was realized as the principal analysis, and the conventional approach without subtraction as a robustness analysis, leading sometimes to discrepancies between the two approaches and long, difficult to digest statistical analysis reports. This work will be reflected in the future analysis plans of vaccine clinical trials where the bivariate modelling approach will be the primary analysis performed. We also hope for a broader impact in the scientific and immunological community thanks to VICI. The model has been developed for both intra-arm and inter-arm comparison to fit with the different clinical trial designs. In both cases, only one time point of interest post vaccination is currently taken into account in the bivariate model specification. The development of an extension of the bivariate model with the consideration of several time points post vaccination would be interesting to implement, and be highly relevant when longitudinal data are measured in vaccine clinical trials. To do this, the current model would be completed with random effects to take into account inter-individual variability (random effect intercept), a function of time and an interaction between the time and the vaccine arm that will allow to evaluate the vaccine effect.

The mathematical equations specifying each of the two longitudinal models are described below.

Let $Y_{ij} = \begin{bmatrix} Y_{ij}^{NS} \\ Y_{ij}^{Sk} \end{bmatrix}$, be the response vector for subject i at a time j , with Y_{ij}^{NS} the non-stimulated cell response, and Y_{ij}^{Sk} the n^k vector of the stimulated cell response. We define the two bivariate linear models presented above as:

Longitudinal model (1) for inter-arm comparison

$$\begin{cases} Y_{ij}^{NS} = \beta_0^{NS} + \gamma_0^{NS} + \beta_1^{NS} V_{ij} + \beta_2^{NS} f(t)_{ij} + \beta_3^{NS} (f(t) \times V)_{ij} + \varepsilon_{ij}^{NS} \\ Y_{ij}^{Sk} = \beta_0^S + \gamma_0^S + \beta_1^S V_{ij} + \beta_2^S Y_{ij}^{NS} + \beta_3^S f(t)_{ij} + \beta_4^S (f(t) \times V)_{ij} + \varepsilon_{ij}^S \end{cases}$$

where V_{ij} is the variable for the vaccine arm, $f(t)$ a function of the time, and β , γ and ε , respectively, are the parameters, random effect and the errors of the model. This formulation implies the following probability distribution for the responses and errors:

$$Y_{ij}^{NS} \sim N(\beta_0^{NS} + \gamma_0^{NS} + \beta_1^{NS} V_{ij} + \beta_2^{NS} f(t)_{ij} + \beta_3^{NS} (f(t) \times V)_{ij}, \sigma^{NS}) \text{ and } \varepsilon_{ij}^{NS} \sim N(0, \sigma^{NS})$$

$$Y_{ij}^S \sim N(\beta_0^S + \gamma_0^S + \beta_1^S V_{ij} + \beta_2^S Y_{ij}^{NS} + \beta_3^S f(t)_{ij} + \beta_4^S (f(t) \times V)_{ij}, \sigma^S) \text{ and } \varepsilon_{ij}^S \sim N(0, \sigma^S)$$

Longitudinal model (2) for intra-arm comparison

$$\begin{cases} Y_{ij}^{NS} = \beta_0^{NS} + \gamma_0^{NS} + \beta_1^{NS} f(t)_{ij} + \varepsilon_{ij}^{NS} \\ Y_{ij}^{Sk} = \beta_0^S + \gamma_0^S + \beta_2^S f(t)_{ij} + \beta_3^S Y_{ij}^{NS} + \varepsilon_{ij}^S \end{cases}$$

where $f(t)$ a function of the time, and β , γ and ε , respectively, are the parameters, random effect and the errors of the model. This formulation implies the following probability distribution for the responses and errors:

$$Y_{ij}^{NS} \sim N(\beta_0^{NS} + \gamma_0^{NS} + \beta_1^{NS} f(t)_{ij}, \sigma^{NS}) \text{ and } \varepsilon_{ij}^{NS} \sim N(0, \sigma^{NS})$$

$$Y_{ij}^S \sim N(\beta_0^S + \gamma_0^S + \beta_2^S f(t)_{ij} + \beta_3^S Y_{ij}^{NS}, \sigma^S) \text{ and } \varepsilon_{ij}^S \sim N(0, \sigma^S)$$

The splines used for the modelling of time in the chapter 3 would be to consider for function of the time $f(t)$ in the model. The limit is the increase of the number of the parameter that can lead to convergence issues.

In conclusion, modelling and analyzing T cell responses to vaccines requires complex models. This work will allow a better consideration of early time measurements in future vaccine trials and a better analysis of the ICS data for a better estimation of the T cell responses.

References

1. Querec TD, Akondy RS, Lee EK, Cao W, Nakaya HI, Teuwen D. Systems biology approach predicts immunogenicity of the yellow fever vaccine in humans. *Nat Immunol.* 2009;10(1): 116–125.
2. Rechten A, Richert L, Lorenzo H, Martrus G, Hejblum B, Dahlke C, et al. Systems Vaccinology Identifies an Early Innate Immune Signature as a Correlate of Antibody Responses to the Ebola Vaccine rVSV-ZEBOV. *Cell Rep.* 2017;20(9):2251-61.
3. De Rosa SC, Thomas EP, Bui J, Huang Y, deCamp A, Morgan C, et al. HIV-DNA priming alters T-cell responses to HIV-adenovirus vaccine even when responses to DNA are undetectable. *J Immunol.* 2011;187(6):3391-401.
4. Horton H, Thomas E, Stucky J, Frank I, Moodie Z, Huang Y, et al. Optimization and Validation of an 8-Color Intracellular Cytokine Staining (ICS) Assay to Quantify Antigen-Specific T Cells Induced by Vaccination. *J Immunol Methods.* 2007;323(1):39-54.
5. De Rosa SC. Vaccine applications of flow cytometry. *Methods.* 2012;57(3):383-91.
6. Lin L, Finak G, Ushey K, Seshadri C, Hawn TR, Frahm N, et al. COMPASS identifies T-cell subsets correlated with clinical outcomes. *Nat Biotechnol.* 2015;33(6):610-6.
7. Churchyard GJ, Morgan C, Adams E, Hural J, Graham BS, Moodie Z, et al. A phase IIA randomized clinical trial of a multiclade HIV-1 DNA prime followed by a multiclade rAd5 HIV-1 vaccine boost in healthy adults (HVTN204). *PLoS ONE.* 2011;6(8):e21225.
8. Tung FY, Tung JK, Pallikkuth S, Pahwa S, Fischl MA. A therapeutic HIV-1 vaccine enhances anti-HIV-1 immune responses in patients under highly active antiretroviral therapy. *Vaccine.* 2016;34(19):2225-32.
9. Janes HE, Cohen KW, Frahm N, De Rosa SC, Sanchez B, Hural J, et al. Higher T-Cell Responses Induced by DNA/rAd5 HIV-1 Preventive Vaccine Are Associated With Lower HIV-1 Infection Risk in an Efficacy Trial. *J Infect Dis.* 2017;215(9):1376-85.
10. Kalams SA, Parker SD, Elizaga M, Metch B, Edupuganti S, Hural J, et al. Safety and Comparative Immunogenicity of an HIV-1 DNA Vaccine in Combination with Plasmid Interleukin 12 and Impact of Intramuscular Electroporation for Delivery. *J Infect Dis.* 2013;208(5):818-29.
11. Horton H, Thomas E, Stucky J, Frank I, Moodie Z, Huang Y, et al. Optimization and Validation of an 8-Color Intracellular Cytokine Staining (ICS) Assay to Quantify Antigen-Specific T Cells Induced by Vaccination. *J Immunol Methods.* 2007;323(1):39-54.
12. De Rosa SC. Vaccine applications of flow cytometry. *Methods* 2012;57(3):383-91.
13. Richert L, Thiebaut R, Biostatistics/Bioinformatics Division of the ANRS Vaccine Research Institute. Is the current use of « positivity » thresholds meaningful for evaluating HIV-vaccine immunogenicity endpoints? *AIDS.* 2013;27(8):1362-5.
14. Liu AY, De Rosa SC, Guthrie BL, Choi RY, Kerubo-Bosire R, Richardson BA, Kiarie J, Farquhar C, Lohman-Payne B. High background in ELISpot assays is associated with elevated levels of immune activation in HIV-1-seronegative individuals in Nairobi. *Immun Inflamm Dis.* 2018;6(3):392-401.
15. Su LF, Kidd BA, Han A, Kotzin JJ, Davis MM. Virus-specific CD4+ memory phenotype T cells are abundant in unexposed adults. *Immunity.* 2013;38(2):373-83.
16. Gilbert PB, Sato A, Sun X, Mehrotra DV. Efficient and robust method for comparing the immunogenicity of candidate vaccines in randomized clinical trials. *Vaccine.* 2009;27(3):396-401.

IV. Modelling antibody responses

In this chapter, we will focus on the antibody response. For many vaccines (e.g. pneumococcus, hepatitis A virus, hepatitis B virus), induction of specific antibodies by the vaccine is thought to correlate with prevention from infection. In Ebola vaccine trials, the immunogenicity is also mainly measured by the antibody response (immunoglobulin G against EBOV glycoprotein) due to previous result for correlate of protection in NPH, pig and mice [1-4]. Here, we address the evaluation of factors influencing the measure of the antibody response for a better evaluation of the vaccine efficacy in Ebola vaccine trials. This work is an essential step towards a better understanding of the determinants of the immune response and so towards a standardization of them in future vaccine clinical trials. The determinants can be related to the vaccination strategy, the characteristics of the study population, but also to the technical specifications of the measurement technique.

Measure of the antibody response

The extent of the antibody response after vaccination is generally measured by the Enzyme-Linked Immunosorbent Assay (ELISA) method. This method, first described by Engvall and Perlmann in 1972 [5] - is a commonly used analytical biochemistry assay based on the principle of detecting an antigen-antibody complex via a colored solution that makes it possible to estimate the quantity of antibodies present in the serum of the vaccinated individual via a measurement of the intensity of staining by spectrophotometer (Figure 8). This immunological assay is used for the evaluation of the efficacy of several infectious diseases where the specific antibody response is identified as the correlate of protection (eg. Hepatitis B virus, Hepatitis A virus, human papillomavirus, Lyme disease, Pneumococcus). In Ebola vaccine trials, this test uses wells, at the bottom of which are located components of the Ebola virus (antigens), and into which the serum of the vaccinated individual is introduced. If the individual's serum contains antibodies specific to the Ebola virus, they bind to the antigens in wells. After washing, to remove products that have not attached to the antigens, a second solution containing antibodies bound to a recognition molecule whose staining is detectable after reaction with a substrate is added. The more Ebola antibodies there are in the serum, the more intense the staining read with the photometer. To quantify the antibodies present in the serum, the measurement is repeated with serial dilutions of the serum, and the antibody titer corresponding to the maximum dilution of the serum for which antibodies are still detected by the ELISA method can be used as a measure. It is also possible to use the "effective concentration X" which corresponds to the dilution for which there is an X% decrease in

antibody binding compared with the non-diluted serum. The viral components used for antibody detection may come from different species and strains of Ebola virus. It may be the same species/strain as the viral insert of the vaccine, but it is also possible to use another species/strain of Ebola virus.

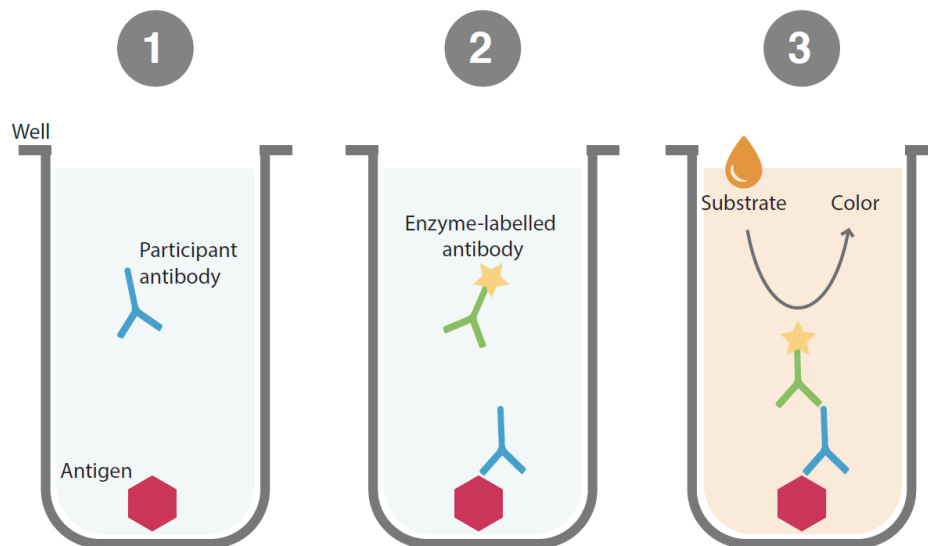


Figure 8. Overview of the Enzyme-Linked Immunosorbent Assay (ELISA) method. 1. The antigen is fixed on the bottom of the wells and the participant sample is added. If the sample contains specific antibodies they will bind to the antigen. 2. An enzyme labeled antibody is added and can only bind if an antibody against the antigen is present in the participant sample. 3. A substrate is added to the enzyme, a substance that changes colour when it is chemically converted by the enzyme. The colour can be quantitated in a spectrophotometer and is a measure of the level of antibodies in the participant.

Factors influencing the immune response

The quantification of the antibody response by ELISA in Ebola preclinical and clinical vaccine trials is critical to determine whether an Ebola candidate vaccine under development could constitute a relevant preventive strategy in a future Ebola epidemic. However, the factors that influence this response are still very little known and may have an impact in the vaccine development. From one vaccine trial to another, many factors can vary. These may include vaccine-related factors, such as the vaccine platform, viral insert type, vaccine dose, and administration schedule, including the number of injections, time interval between injections, and the use of autologous or heterologous prime boost strategy. These factors may also be related to the study population, since the study may be conducted in humans or animals, particularly in NPH, in individuals with or without co-morbidities. The location of the study site

may also vary according to the trials (Europe, North America, Africa, China). Finally, these factors may be of a technical nature with regard to the measurement of antibody levels, such as the type of strain used as an antigen to measure the level of anti-Ebola antibodies (Zaire, Kikwit, Mayinga, Makona), the measurement method used (maximum dilution, "effective concentration 90"), but also the period of the study that could influence the type of technique used. These many factors could have an impact on the level of antibodies measured in a study, and could explain differences in results between studies. It would therefore be interesting to know which factors explain the observed variability of the antibody response, and if so, to obtain a quantitative estimate of their influence. The knowledge of these factors will allow a better interpretation of clinical trials results and to improve future clinical trial designs with a standardization of the consideration of these factors.

The research question is then the following: what are the factors associated with the variability of antibody response in published studies of Ebola vaccine trials and which of these factors are most important? We hypothesize that vaccine dose may be the most influential factor, beyond the vaccine type, population and type of quantification technique. We performed a meta-analysis of published Ebola vaccine trials to address this question. Our meta-analysis does not aim to compare antibody responses strictly speaking according to the type of vaccine used: indeed, the number of expected variation factors is too high compared to the number of published vaccine trials to ensure that all factors can be taken into account simultaneously and to have sufficient power to calculate an adjusted measure of the vaccine type effect.

The main objective of this study was to identify factors associated with the variability of antibody response in published studies of Ebola vaccine trials for vaccination against Ebola. The secondary objective was to quantify the proportion of variability in the antibody response that is explained by these factors.

To meet the previously formulated objectives, a systematic literature review and meta-analysis were carried out. This work was conducted in collaboration with Lise Gross during her Master Degree in Epidemiology in the SISTM team. I was closely involved in the reflection during the project, participated in the independent double review of articles, the statistical analyses and the writing of the manuscript which was done in tandem with Lise.



Ebola vaccine development: Systematic review of pre-clinical and clinical studies, and meta-analysis of determinants of antibody response variability after vaccination

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ABSTRACT

Objectives: For Ebola vaccine development, antibody response is a major endpoint although its determinants are not well known. We aimed to review Ebola vaccine studies and to assess factors associated with antibody response variability in humans.

Methods: We searched PubMed and Scopus for preventive Ebola vaccine studies in humans or non-human primates (NHP), published up to February 2018. For each vaccination group with Ebola Zaire antibody titre measurements after vaccination, data about antibody response and its potential determinants were extracted. A random-effects meta-regression was conducted including human groups with at least 8 individuals.

Results: We reviewed 49 studies (202 vaccination groups including 74 human groups) with various vaccine platforms and antigen inserts. Mean antibody titre was slightly higher in NHP (3.10, 95% confidence interval [293; 327]) than in humans (2.75 [257; 293]). Vaccine platform ($p < 0.001$) and viral strain used for antibody detection ($p < 0.001$) were associated with antibody response in humans, but adjusted heterogeneity remained at 95%.

Conclusions: Various platforms have been evaluated in humans, including Ad26, Ad5, ChimpAd3, DNA, MVA, and VSV. In addition to platforms, viral strain used for antibody detection influences antibody response. However, variability remained mostly unexplained. Therefore, comparison of vaccine immunogenicity needs randomised controlled trials.

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Introduction

Following the deadly 2013–2016 epidemic in West Africa, there has been an accelerated development of several candidates for an Ebola preventive vaccine. Outbreaks of Ebola virus disease (EVD) have occurred recurrently and unpredictably for the past 40 years with a high lethality rate (Liu et al., 2015). The 2013–2015 outbreak was unprecedented in scale, with over 28,000 cases and more than 11,000 deaths (Ebola Situation Report, 2016). Incidental cases are still reported as recently in the Democratic Republic of Congo in May 2017 (Dhama et al., 2015). In the absence of any specific

treatment, EVD prevention and control measures are primarily based on case identification and isolation, early non-specific medical care, surveillance of suspect cases, and safe burial practices (Henaou-Restrepo et al., 2017). These measures are now sometimes complemented by ring vaccination of contacts of cases, based on the promising results of a phase III cluster-randomized ring vaccination efficacy trial conducted in Guinea in 2015 (Ohimain, 2016). However, the vaccine used for ring vaccination (rVSV ZEBOV vaccine) is not yet licenced and conducting new efficacy trials for licencing is not feasible in the absence of a large outbreak. Nevertheless, preparation for future outbreaks is required and the licencing of one or several preventive vaccines for stockpiling is a priority.

Several candidate vaccines strategies have been investigated since the first reported EVD outbreak in 1976. During and following

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the 2013–2015 epidemic, the process of vaccine development has been substantially accelerated, and several strategies have been moved into clinical phases. Despite the promising results of the ring vaccination trial in Guinea (Ohimain, 2016), many questions, such as durability of immune responses, and immune responses and protection in specific sub-groups such as young children, remain to be addressed and Ebola vaccine development continues to be very active. Based on their delivery technologies, several candidate vaccine platforms can be distinguished: whole-virus vaccines, DNA vaccines, virus-like particles vaccines, and recombinant vaccines with different viral vectors (vesicular stomatitis virus or VSV, modified vaccinia Ankara or MVA, human adenovirus or Ad, and chimpanzee adenovirus or ChAd) (World Health Organisation, 2013). Each platform may use specific dose levels and Ebola antigen inserts.

Vaccine trials aim to assess vaccine safety and immunogenicity in phase I and II trials in humans prior to testing for a protective effect in phase III. Assessment of vaccine efficacy during pre-clinical and clinical studies is required to go through the vaccine license steps. Clinical protection from EVD in human populations is impossible to observe outside an epidemic period. In the non-epidemic context, Ebola vaccines are thus currently evaluated by using a main immunogenicity endpoint: the antibody response after vaccination. There is no definite evidence that antibody response is the correlate of protection or surrogate endpoint for efficacy in humans, that is a specific immune response to vaccine associated with vaccine-induced protection (Sullivan et al., 2009) and it may vary according to the vaccine platforms (Sullivan et al., 2000a,b). However, we know that antibody response is correlated with survival after challenge in nonhuman primate models, which is the nearest model to humans for EVD and hence the animal gold standard to test candidate Ebola vaccines; this association is found consistently for different Ebola candidate vaccines (Wong et al., 2012; Food and Drug Administration, 2015; Sridhar, 2015).

For these reasons, antibody response is used as the main criterion to assess the Ebola candidate vaccines in phase I/II trials. In the absence of the possibility to conduct additional phase III trials, regulatory pathways not requiring such efficacy results are also under discussion (Food and Drug Administration, 2015). Significant variations in antibody responses are observable across studies, which could be due to the different types of vaccines evaluated, or not. Various factors are suspected to influence the level of antibody response beyond the vaccine features (vaccine platform, Ebola viral insert, dosage, single injection or boost, . . .) such as the measurement techniques (time of measurement, antigen used to detect antibody response, . . .) or the population type (human or nonhuman primates, age, sex, study site, . . .). There is a lack of quantification of the contribution of each factor in the observed variation of the reported antibody responses.

Although previous reviews exist on Ebola vaccines (Ohimain, 2016; Sridhar, 2015; Wu et al., 2015), the specific topic of antibody response determinants has not yet been addressed by a systematic review or meta-analysis. Yet, the identification of factors potentially associated with antibody response after Ebola vaccination could provide relevant information for further vaccine trials and for regulatory decision making.

By conducting this systematic review with a meta-analysis, we aimed to determine whether the reported antibody response variability in Ebola vaccine trials is not only determined by the vaccine platform but also by other characteristics of vaccine and by population and measurement characteristics and to quantify these factors.

Methods

Search strategy and selection criteria

Studies were identified by searching electronic databases PubMed and Scopus. PubMed was searched using the following terms: (« hemorrhagic fever, ebola » [MeSH Terms] OR « ebola » [All fields] OR « ebolavirus » [MeSH Terms] OR « ebolavirus » [All fields]) AND (« vaccines » [MeSH Terms] OR « vaccines » [All fields] OR « vaccine » [All Fields]). Scopus was searched using the following terms TITLE-ABS-KEY (ebola) AND TITLE-ABS-KEY (vaccine). Additionally, the Clinicaltrials.gov website was searched to identify unpublished and ongoing studies. Several experts in the field were contacted to find papers which could be not indexed in databases. Reference lists of relevant papers and reviews were examined to identify further articles.

The search was performed on March 23, 2016 and updated as of February 24, 2018 with a publication date limit of the same date in order to identify all published studies which met the inclusion criteria and without restriction on language. All preventive Ebola vaccine clinical trials conducted in humans or in nonhuman primates and with a measure of Ebola Zaire antibody titre after vaccination were included in our systematic review. Studies were excluded in case of duplicate study, studies without original data, preclinical studies conducted in animals other than nonhuman primates or in vitro experimentation.

Data extraction

A first step of selection was performed on the title and abstract, and then a second step was performed after reading the full article. Two authors independently assessed each full article to include papers matching the review's inclusion criteria. Disagreements between reviewers were resolved by consensus.

Data were extracted by two independent reviewers, with differences reconciled by consensus. The following variables were extracted: paper identification (title, first author, publication year), study design, inclusion and exclusion criteria, characteristics of the population (number of subjects; human or nonhuman primates; proportion of women, average age and study site for clinical trials; and animal species for pre-clinical studies using nonhuman primates), characteristics of vaccine (vaccine platform in terms of delivery technology used, specific vector for recombinant vaccines, Ebola viral insert, dosage, route of administration, vaccination schedule), characteristics of measurement techniques (time interval between last injection and measure, strain and nature of antigen used to detect antibody response, measurement method), antibody response after vaccination (geometric mean titre and its variance). Regarding the antibody response after vaccination, geometric mean titre was extracted from the text or estimated from figures. If a single vaccination group had more than one measure of antibody response, data from measurement after each injection were extracted. Therefore, if available, measurement post-prime and measurement post-boost from a same vaccination group were both included in our meta-analysis. If several measurements post-prime or if several measurements post-boost were available, for each injection we extracted the one closest to 28 days after injection, which is a standard time point in Ebola vaccine trials. Variance of titre (within-group variance) was extracted directly from the text or calculated from confidence interval or from individual values. The present study was registered in PROSPERO (no. 54303).

Data analysis

For all analyses, the statistical unit used was the vaccination group (one or several groups for a single study), i.e. a protocol-defined group undergoing the same intervention and follow-up procedures (such as a randomized arm of a clinical trial or an animal group in NHP studies).

First descriptive analyses were performed among all groups, separately for nonhuman primates and for humans. Then, a random-effect meta-regression analysis was performed including only human groups with 8 individuals or more. This threshold allowed both to have sufficient inter-individual variability in each group and to avoid excluding too many groups. Thus, it was not possible to perform the regression analysis with NHP studies because of the usual small sample size of the groups. The effect of every potential determinant of antibody response was assessed through fixed effects. A random intercept was allowed to capture between-group variability not explained by the fixed effects. The residual variance (within-group variance) was fixed in the model according to the values resulting from data extraction as described by Van Houwelingen (Van Houwelingen et al., 2002).

Each potential determinant associated in unadjusted analyses with a p -value <0.25 was included in the multivariable model using forward step-wise selection. The heterogeneity was checked visually with forest plots and quantified by using the Q test. The proportion of total variation across groups due to heterogeneity (I^2) and the amount of variability explained by the factors included in the random-effect model (R^2) were estimated. Antibody titres after vaccination were log transformed in the model.

For the meta-regression analysis, the dosage variable was categorized into "low dose" or "high dose" per vaccine platform, since units of measurement for dose level were platform-dependent. For each unit of dose measurement and each vaccine platform, the average dose level among the human groups included in the meta-regression model was used as a classification

threshold for this variable; if only one dose level was assessed for a vaccine platform, the dosage variable was defined as undifferentiated. The absence of interaction between vaccine platform and dosage was checked (likelihood ratio test: $p=0.223$).

All analyses were performed using the metafor package of R (i386 3.2.2 version, the R Foundation, Vienna, Austria).

Results

Study selection

The selection process of the studies and vaccination groups is described in Figure 1.

The search yielded a total of 2166 studies. Of these, 49 met the inclusion criteria to the research question corresponding to 202 vaccination groups. Unpublished clinical trials and one trial found by contact with an expert were excluded since no results were available. Studies not reporting any antibody measurements were also excluded. This led to the exclusion of the "Ebola ça suffit" ring vaccination trial conducted in Guinea, the only trial that was able to assess clinical efficacy in humans so far (Ohimain et al., 2016). This trial was conducted under emergency conditions and did not collect blood samples for immunogenicity measurements.

Table 1 shows details of all trials included in the systematic review: 32 studies were conducted in NHP, 13 trials in humans were phase 1, two trials phase 1/2, and two phase 2. The number of trials has increased significantly since the last outbreak of EVD. Clinical trials were conducted mostly in Europe and North America (Figure 2).

Description of included vaccination groups

Among the 202 vaccination groups included in our systematic review, 74 were human groups and 128 were non-human primate groups. The distribution of the number of individuals by groups is

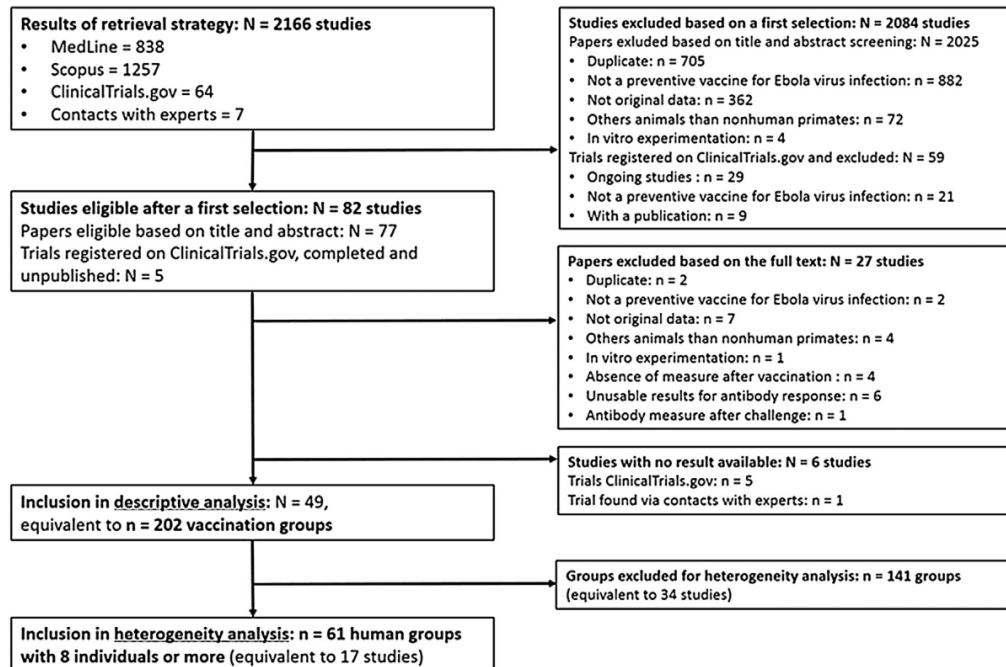


Figure 1. Flow chart for study/vaccination group selection.

Table 1
Main characteristics of the preclinical studies and clinical trials included in the systematic review.

| Title | First author | Year of publication | Population and study features | Vaccine(s) | Measurement of antibody response |
|--|--------------|---------------------|---|--|--|
| Phase 1 Trials of rVSV Ebola Vaccine in Africa and Europe (Agnandji et al., 2016) | Agnandji | 2016 | Humans (Germany, Switzerland, Gabon, Kenya), phase 1, randomization and placebo | Recombinant VSV-GP(Zaire), single injection, IM, 300 000 to 50 million PFU | Antibodies anti GP (Kikwit), D28 or D180 |
| Successful topical respiratory tract immunization of primates against Ebola virus (Bukreyev et al., 2007) | Bukreyev | 2007 | NHP: rhesus monkeys; placebo | Recombinant HPIV3(+/- modified)-GP +/- NP (Zaire Mayinga), single injection +/- boost D28, IN+IT, 4 to 20 million TCID50 | Antibodies anti virion, D28 (or D39 after boost) |
| Mucosal parainfluenza virus-vectored vaccine against Ebola virus replicates in the respiratory tract of vector-immune monkeys and is immunogenic (Bukreyev et al., 2010) | Bukreyev | 2010 | NHP: rhesus monkeys (+/- HPIV3 seropositive); placebo | Recombinant HPIV3-GP(Zaire Mayinga), boost D28, IN+IT, 20 million PFU | Antibodies anti virion, D28 |
| Safety and immunogenicity of a chimpanzee adenovirus-vectored Ebola vaccine in healthy adults: a randomised, double-blind, placebo-controlled, dose-finding, phase 1/2a study (De Santis et al., 2016) | De Santis | 2016 | Humans (Switzerland), phase 1/2, randomization and placebo | Recombinant ChAd3-GP(Zaire Mayinga), single injection, IM, 25 to 50 billion VP | Antibodies anti GP (Mayinga), D28, results in EC90 |
| Respiratory tract immunization of non-human primates with a Newcastle disease virus-vectored vaccine candidate against Ebola virus elicits a neutralizing antibody response (DiNapoli et al., 2010) | DiNapoli | 2010 | NHP: rhesus monkeys; no placebo | Recombinant NDV-GP(Zaire Mayinga) or HPIV3-GP(Zaire Mayinga), boost D28, IN+IT, 20 million PFU | Antibodies anti virion (Mayinga), D28 |
| A Monovalent Chimpanzee Adenovirus Ebola Vaccine Boosted with MVA (Ewer et al., 2016) | Ewer | 2016 | Humans (United Kingdom), phase 1, no randomization, no placebo | Recombinant ChAd3-GP(Zaire Mayinga) 10 to 50 billion VP, boost between D7 and D46 with recombinant MVA-GP(Zaire Mayinga + Sudan Gulu)/NP(Tai Forest) 150 to 300 millions PFU, IM | Antibodies anti GP (Mayinga) or anti virion (Makona) |
| Vesicular stomatitis virus-based vaccines protect nonhuman primates against aerosol challenge with Ebola and Marburg viruses (Geisbert et al., 2008a,b) | Geisbert | 2008 | NHP: cynomolgus macaques; placebo | Recombinant VSV-GP(Zaire Kikwit), single injection, 20 million PFU | Antibodies anti virion (Kikwit), D14 or D27 |
| Vesicular stomatitis virus-based ebola vaccine is well-tolerated and protects immunocompromised nonhuman primates (Geisbert et al., 2008a,b) | Geisbert | 2008 | NHP: rhesus monkeys (SHIV infected); placebo | Recombinant VSV-GP(Zaire Mayinga), single injection, IM, 10 million PFU | Antibodies anti virion (Mayinga), D14 |
| Single-injection vaccine protects nonhuman primates against infection with marburg virus and three species of ebola virus (Geisbert et al., 2009) | Geisbert | 2009 | NHP: cynomolgus macaques and rhesus monkeys; placebo | Recombinant VSV-GP(Zaire Mayinga and/or Sudan Boniface +/- Marburg), single injection +/- boost D14, IM, 10 to 20 million PFU | Antibodies anti virion, between D14 and D28 |
| Recombinant adenovirus serotype 26 (Ad26) and Ad35 vaccine vectors bypass immunity to Ad5 and protect nonhuman primates against ebolavirus challenge (Geisbert et al., 2011) | Geisbert | 2011 | NHP: cynomolgus macaques (+/- Ad5 seropositive); placebo | Recombinant Ad5, Ad26, or Ad35, or prime Ad26 + boost Ad35 D28 - GP (Zaire + Sudan Gulu), IM, 20 to 200 billion VP | Antibodies anti GP, D21, results in EC90 |
| Codon-optimized filovirus DNA vaccines delivered by intramuscular electroporation protect cynomolgus macaques from lethal Ebola and Marburg virus challenges (Grant-Klein et al., 2015) | Grant-Klein | 2015 | NHP: cynomolgus macaques; placebo | Vaccin ADN-GP(Zaire +/- Sudan, Reston et Marburg), 3 injections (28 jours apart), electroporation IM, 500 µg to 2 mg | Antibodies anti GP (Mayinga) ΔTM or ΔMuc, D28 |
| Demonstration of cross-protective vaccine immunity against an emerging pathogenic Ebolavirus Species (Hensley et al., 2010) | Hensley | 2010 | NHP: cynomolgus macaques; placebo | Vaccin ADN-GP(Zaire Mayinga + Sudan Gulu), 4 injections IM, 4 mg (28 to 42 days apart +/- boost D371 recombinant Ad5-GP (Zaire Mayinga) IM 100 billion VP | Antibodies anti GP, D21 or D371, results in EC90 |
| Venezuelan equine encephalitis virus replicon particle vaccine protects nonhuman primates from intramuscular and aerosol challenge with ebolavirus (Herbert et al., 2013) | Herbert | 2013 | NHP: cynomolgus macaques; placebo | VRP GP(Zaire Kikwit +/- Sudan Boniface), single injection, IM, 10 to 20 billion FFU | Antibodies anti GP, D28 |
| The effect of dose on the safety and immunogenicity of the VSV Ebola candidate vaccine: a randomised double-blind, placebo-controlled phase 1/2 trial (Huttner et al., 2015) | Huttner | 2015 | Humans (Switzerland), phase 1/2, randomization and placebo | Recombinant VSV-GP(Zaire), single injection, IM, 300 000 PFU | Antibodies anti GP, D28 |
| Live attenuated recombinant vaccine protects nonhuman primates against Ebola and Marburg viruses (Jones et al., 2005) | Jones | 2005 | NHP: cynomolgus macaques; placebo | Recombinant VSV-GP(Zaire Mayinga), single injection, IM, 10 million PFU | Antibodies anti virion, D28 |
| Phase 2 Placebo-Controlled Trial of Two Vaccines to Prevent Ebola in Liberia (Kennedy et al., 2017) | Kennedy | 2017 | Humans (Liberia), phase 2, randomization and placebo | Recombinant ChAd3-GP(Saire) 100 billion VP or VSV-GP(Zaire Kikwit) 20 million PFU, single injection, IM | Antibodies anti GP (Kikwit), D28 |
| Safety and immunogenicity of Ebola virus and Marburg virus glycoprotein DNA vaccines assessed separately and concomitantly in healthy Ugandan adults: a phase 1b, | Kibuuka | 2015 | Humans (Uganda), phase 1b; randomization and placebo | Vaccin ADN GP(Zaire + Sudan +/- Marburg), 3 injections, IM, 4 mg | Antibodies anti GP, D28 |

Table 1 (Continued)

| Title | First author | Year of publication | Population and study features | Vaccine(s) | Measurement of antibody response |
|---|--------------|---------------------|--|--|--|
| randomised, double-blind, placebo-controlled clinical trial (Kibuuka et al., 2015) | | | | | |
| A replication defective recombinant Ad5 vaccine expressing Ebola virus GP is safe and immunogenic in healthy adults (Ledgerwood et al., 2010) | Ledgerwood | 2010 | Humans (USA), phase 1; randomization and placebo | Recombinant Ad5-GP(Zaire Mayinga + Sudan Gulu), single injection, IM, 2 to 20 billion VP | Antibodies anti GP (Mayinga), D28 |
| Chimpanzee Adenovirus Vector Ebola Vaccine – Preliminary Report (Ledgerwood et al., 2015) | Ledgerwood | 2015 | Humans (USA), phase 1, no randomization and no placebo | Recombinant ChAd3-GP(Zaire Mayinga + Sudan), single injection, IM, 20 or 200 billion VP | Antibodies anti GP (Mayinga or Zaire-Guinea), D28, results in EC90 |
| Immunity duration of a recombinant adenovirus type-5 vector-based Ebola vaccine and a homologous prime-boost immunisation in healthy adults in China: final report of a randomised, double-blind, placebo-controlled, phase 1 trial (Li et al., 2017) | Li | 2017 | Humans (China), phase 1; randomization and placebo | Recombinant Ad5-GP(Zaire Makona), 2 injections (168 days apart), IM, 40 or 160 billion VP | Antibodies anti GP, D28, results in EC90 |
| A DNA vaccine for Ebola virus is safe and immunogenic in a phase I clinical trial (Martin et al., 2006) | Martin | 2006 | Humans (USA), phase 1, randomization and placebo | Vaccin ADN GP/NP(Zaire Mayinga) + GP (Sudan Gulu), 3 injections (28 days apart), IM, 2 to 8 mg | Antibodies anti GP or NP (Mayinga), D28 |
| Antibodies are necessary for rVSV/ZEBOV-GP-mediated protection against lethal Ebola virus challenge in nonhuman primates (Marzi et al., 2013) | Marzi | 2013 | NHP: cynomolgus macaques (with depletion CD4+ or CD8+ or CD20+); placebo | Recombinant VSV-GP(Zaire Mayinga), single injection, IM, 10 million PFU | Antibodies anti GP, D28 |
| Vesicular stomatitis virus-based vaccines against Lassa and Ebola viruses (Marzi et al., 2015a,b,c) | Marzi | 2015 | NHP: cynomolgus macaques (vaccinated with VSV-Lassa); placebo | Recombinant VSV-GP(Zaire Mayinga), single injection, IM, 10 million PFU | Antibodies anti GP, day of measurement non specified |
| Vaccines. An Ebola whole-virus vaccine is protective in nonhuman primates (Marzi et al., 2015a,b,c) | Marzi | 2015 | NHP: cynomolgus macaques; placebo | Attenuated whole-virus Zaire Mayinga, single injection, IM, 10 to 20 million FFU | Antibodies anti GP, D28 |
| EBOLA VACCINE. VSV-EBOV rapidly protects macaques against infection with the 2014/15 Ebola virus outbreak strain (Marzi et al., 2015a,b,c) | Marzi | 2015 | NHP: cynomolgus macaques; placebo | Recombinant VSV-GP(Zaire Kikwit), single injection, unique, 50 million PFU | Antibodies anti GP, between D3 and D28 |
| Cytomegalovirus-based vaccine expressing Ebola virus glycoprotein protects nonhuman primates from Ebola virus infection (Marzi et al., 2016) | Marzi | 2016 | NHP: rhesus monkeys (CMV seropositive); placebo | Recombinant RhCMV-GP(Zaire Mayinga), boost D84, SC, 10 million PFU | Antibodies anti GP, D28 |
| Vaccination With a Highly Attenuated Recombinant Vesicular Stomatitis Virus Vector Protects Against Challenge With a Lethal Dose of Ebola Virus (Matassov et al., 2015) | Matassov | 2015 | NHP: rhesus monkeys; placebo | Recombinant VSV-GP(Zaire Mayinga), single injection, IM, 10 million PFU | Antibodies anti GP, D21 |
| Aerosolized Ebola vaccine protects primates and elicits lung-resident T cell responses (Meyer et al., 2015) | Meyer | 2015 | NHP: rhesus monkeys; placebo | Recombinant HPIV3-GP(Zaire Mayinga) 40 to 400 million PFU or VRP(Zaire Mayinga) 10 billion PFU, boost D28, IM or aerosol or IN + IT | Antibodies anti virion (Mayinga), D23 or D28 |
| Safety and immunogenicity of novel adenovirus type 26-and modified vaccinia ankara-vectored ebola vaccines: A randomized clinical trial (Milligan et al., 2016) | Milligan | 2016 | Humans (United Kingdom), phase 1, randomization and placebo | Recombinant Ad26-GP(Zaire Mayinga) 50 billion VP or recombinant MVA-GP(Zaire Mayinga + Sudan Gulu)/NP(Tai Forest) 100 millions TCID50, boost between D15 and D56, IM | Antibodies anti GP Kikwit), D28 after prime and D21 after boost |
| Vesicular stomatitis virus-based vaccines protect nonhuman primates against Bundibugyo ebolavirus (Mire et al., 2013) | Mire | 2013 | NHP: cynomolgus macaques; placebo | Recombinant VSV-GP(Zaire Mayinga and/or Sudan Boniface or Bundibugyo) +/- boost VSV-GP(Zaire Mayinga) D14, IM, 20 million PFU | Antibodies anti GP, between D22 and D29 |
| Single-dose attenuated Vesiculovax vaccines protect primates against Ebola Makona virus (Mire et al., 2015) | Mire | 2015 | NHP: cynomolgus macaques; placebo | Recombinant VSV-GP(Zaire Mayinga), single injection, IM, 20 million PFU | Antibodies anti GP, D28 |
| Protection of nonhuman primates against two species of Ebola virus infection with a single complex adenovirus vector (Pratt et al., 2010) | Pratt | 2010 | NHP: cynomolgus macaques or rhesus monkeys (+/- Ad5 seropositive); placebo | Recombinant CAdivax-GP(Zaire Kikwit + Sudan Boniface +/- Marburg), boost between D65 and D238, IM, 100 million to 20 billion PFU | Antibodies anti virion, between D7 and D49 |
| A Kunjin Replicon Virus-like Particle Vaccine Provides Protection Against Ebola Virus Infection in Nonhuman Primates (Pyankov et al., 2015) | Pyankov | 2015 | NHP: African green monkeys; placebo | Recombinant VLP Kunjin-GP(Zaire Mayinga), boost D28, SC, 1 billion VLP | Antibodies anti virion, D21 or D28 |
| A Monovalent Chimpanzee Adenovirus Ebola Vaccine – Preliminary Report (Rampling et al., 2015) | Rampling | 2015 | Humans (United Kingdom), phase 1, no randomization, no placebo | Recombinant ChAd3-GP(Zaire), single injection, IM, 10 to 50 billions VP | Antibodies anti GP, D28, results in EC90 |
| A Recombinant Vesicular Stomatitis Virus Ebola Vaccine - Preliminary Report (Regules et al., 2015) | Regules | 2015 | Humans (USA), phase 1, randomization and placebo | Recombinant VSV-GP(Zaire Kikwit), single injection, IM, 3 to 20 million PFU | Antibodies anti GP (Kikwit or Mayinga), D28 |

Table 1 (Continued)

| Title | First author | Year of publication | Population and study features | Vaccine(s) | Measurement of antibody response |
|--|--------------|---------------------|---|--|---|
| Safety and immunogenicity of DNA vaccines encoding Ebola virus and Marburgvirus wild-type glycoproteins in a phase I clinical trial (Sarwar et al., 2015) | Sarwar | 2015 | Humans (USA), phase 1, no randomization and no placebo | Vaccin ADN GP(Zaire + Sudan), 3 injections (28 days apart) + boost D168, IM, 4 mg | Antibodies anti GP, D28 |
| Chimpanzee adenovirus vaccine generates acute and durable protective immunity against ebolavirus challenge (Stanley et al., 2014) | Stanley | 2014 | NHP: cynomolgus macaques; placebo | Recombinant ChAd3-GP(Zaire + Sudan), 1 to 10 billion VP or recombinant ChAd3-GP (Zaire + Sudan) or recombinant MVA-GP (Zaire + Sudan) 100 million VP, single injection, IM | Antibodies anti GP, D21, results in EC90 |
| Development of a preventive vaccine for Ebola virus infection in primates (Sullivan et al., 2000a,b) | Sullivan | 2000 | NHP: cynomolgus macaques; placebo | DNA vaccine GP/NP(Zaire) + GP(Sudan + Tai Forest), 3 injections, 4 mg (28 days apart), boost D84 recombinant Ad5-GP(Z) 10 billion PFU, IM | Nature of viral antigen non specified, results in EC90 |
| Accelerated vaccination for Ebola virus haemorrhagic fever in non-human primates (Sullivan et al., 2003) | Sullivan | 2003 | NHP: cynomolgus macaques; placebo | Recombinant Ad5-GP/NP(Zaire) +/- boost D63, IM, 2000 billion VP | Antibodies anti virion, between D7 and D63 |
| CD8+ cellular immunity mediates rAd5 vaccine protection against Ebola virus infection of nonhuman primates (Sullivan et al., 2011) | Sullivan | 2011 | NHP: cynomolgus macaques; placebo | Recombinant Ad5-GP(Zaire), single injection, IM, 10 billion VP | Antibodies anti GP, day of measurement non specified, results in EC90 |
| Vaccine to confer to nonhuman primates complete protection against multistrain Ebola and Marburg virus infections (Swenson et al., 2008) | Swenson | 2008 | NHP: cynomolgus macaques; placebo | Recombinant Ad5-GP/NP(Zaire) + GP(Sudan Boniface), boost D63, IM, 40 billion PFU | Antibodies anti virion, D14 after prime and D21 after boost |
| Use of ChAd3-EBO-Z Ebola virus vaccine in Malian and US adults, and boosting of Malian adults with MVA-BN-Filo: a phase 1, single-blind, randomised trial, a phase 1b, open-label and double-blind, dose-escalation trial, and a nested, randomised, double-blind, placebo-controlled trial (Tapia et al., 2016) | Tapia | 2016 | Humans (Mali), phase 1, randomization and placebo | Recombinant ChAd3-GP(Zaire), 10 to 100 billion VP, boost D97 recombinant MVA-GP (Zaire + Sudan + Marburg) + NP (Tai Forest) 200 millions PFU, IM | Antibodies anti GP, D28 |
| Ebola virus-like particle-based vaccine protects nonhuman primates against lethal Ebola virus challenge (Warfield et al., 2007) | Warfield | 2007 | NHP: cynomolgus macaques; placebo | VLP GP/VP40/NP(Zaire), 2 injections (42 days apart), boost D42, IM, 250 µg | Antibodies anti virion, D42 |
| Vaccinating captive chimpanzees to save wild chimpanzees (Warfield et al., 2014) | Warfield | 2014 | NHP: chimpanzee; no placebo | VLP (with adjuvant: IDC-1001 ou CpG) GP/VP40/NP(Zaire), 2 injections (29 days apart), boost D27, IM, 3 mg | Antibodies anti GPΔTM or VP40, between D27 and D29, results in EC50 |
| Homologous and heterologous protection of nonhuman primates by Ebola and Sudan virus-like particles (Warfield et al., 2015) | Warfield | 2015 | NHP: cynomolgus macaques; placebo | VLP GP/VP40/NP(Zaire et/ou Sudan), boost D42, IM, 3 mg | Antibodies anti GPΔTM or VP40, between D14 and D28 |
| Immune parameters correlate with protection against ebola virus infection in rodents and nonhuman primates (Wong et al., 2012) | Wong | 2012 | NHP: cynomolgus macaques; placebo | Recombinant VSV-GP(Zaire Mayinga), single injection, IM or IT or PO, 20 millions PFU | Antibodies anti GP, D28 |
| An Adenovirus Vaccine Expressing Ebola Virus Variant Makona Glycoprotein Is Efficacious in Guinea Pigs and Nonhuman Primates (Wu et al., 2016) | Wu | 2016 | NHP: cynomolgus macaques; placebo | Recombinant Ad5-GP(Zaire Makona), single injection, IM, 40 or 200 billion VP | Antibodies anti GP, D28 |
| Safety and immunogenicity of a novel recombinant adenovirus type-5 vector-based Ebola vaccine in healthy adults in China: preliminary report of a randomised, double-blind, placebo-controlled, phase 1 trial (Zhu et al., 2015) | Zhu | 2015 | Humans (China), phase 1, randomization and placebo | Recombinant Ad5-GP(Zaire Makona), single injection, IM, 40 to 160 billions VP | Antibodies anti GP (Makona), D28 |
| Safety and immunogenicity of a recombinant adenovirus type-5 vector-based Ebola vaccine in healthy adults in Sierra Leone: a single-centre, randomised, double-blind, placebo-controlled, phase 2 trial (Zhu et al., 2017) | Zhu | 2017 | Humans (Sierra Leone), phase 2; randomization and placebo | Recombinant Ad5-GP(Zaire Makona), single injection, IM, 40 or 160 billion VP | Antibodies anti GP, D28 |

presented in Figure 3. The vast majority (82.4%) of human groups included 8 or more individuals, while only 6 for non-human primate groups (range 2; 22 with an average of 4.1 individuals by group).

Characteristics of nonhuman primate and human groups are described in Tables 2 and 3, respectively.

There is a wide heterogeneity of features among studies included in the systematic review. Vaccine platforms varied between studies, especially in NHP (18 different vaccine platforms in NHP groups versus 8 in human groups). The strain of Ebola virus used as vaccine insert or for the antibody detection after vaccination was also variable. For almost a third of the human

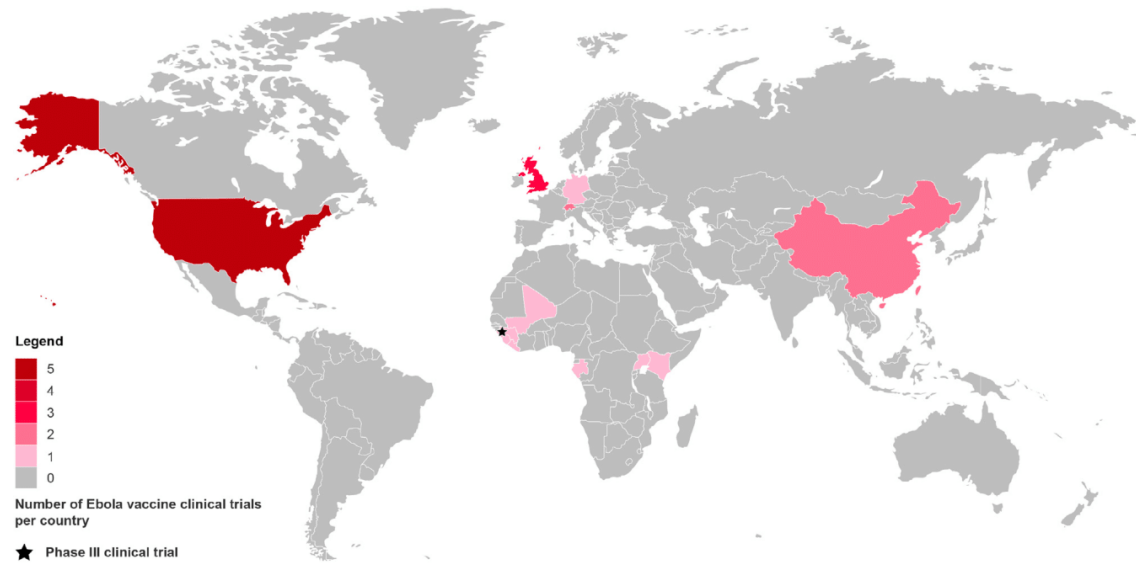


Figure 2. Description of the number of vaccine clinical trials against Ebola per country.

The Ring trial, single phase 3 trial (Guinea), has been excluded from the systematic review.

Several other vaccine clinical trials against Ebola are currently ongoing worldwide but only published trials are reported in the figure.

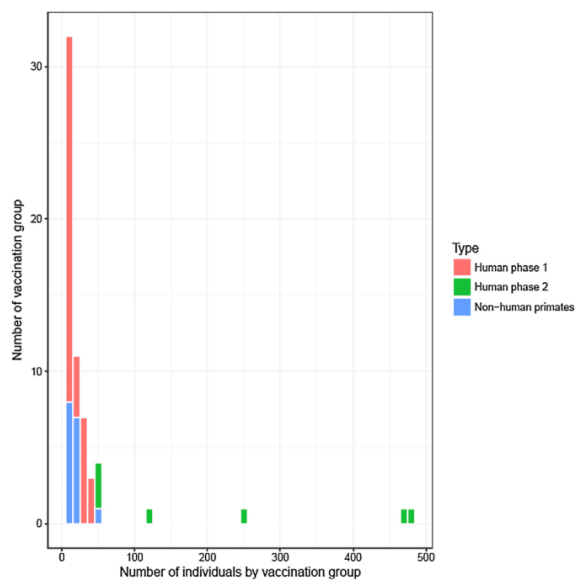


Figure 3. Number of vaccination groups of humans and of nonhuman primates, according to the number of individuals by group.

groups, the detection of antibody response was done with a heterologous strain. The time interval between the last vaccination and the antibody detection was also remarkably variable (range 3; 371 days).

Among all the 202 vaccination groups, the mean antibody titre ranged from 0 (for a group of NHP infected by the simian/human immunodeficiency virus prior to the Ebola vaccination) to 5.81 log₁₀, with an average of 2.97 (95% CI: [2.84; 3.10]).

The NHP groups had a crude antibody response level that was significantly higher than the human groups ($p=0.006$): in NHP groups the log₁₀ geometric mean titre ranged from 0 to 5.81 with an average of 3.10 (95% CI: [2.93; 3.27]), and in human groups the titre ranged from 0.90 to 4.60 with an average of 2.75 (95% CI: [2.57; 2.93]) Figure S1 (appendix) shows antibody responses in human groups and in NHP groups.

Meta-regression of factors associated with variability in antibody response levels in humans and evaluation of between-groups heterogeneity

Sixty-one human vaccination groups with 8 individuals or more were included in the meta-regression analysis.

Among these, 32 were vaccinated with a low dose of vaccine, 19 with a high dose (for 10 groups, the dose category was undeterminable as only one dose level was assessed for the given vaccine platform).

The distribution of the antibody titres after Ebola vaccination per vaccination group is shown by vaccine platform in Figure 4. The antibody response seems to be higher in groups with a prime-boost strategy (Ad26/MVA or ChAd3/MVA) than in the other groups. The distribution of the antibody titres by viral strain used for antibody detection is presented in Figure S2 (appendix).

In univariate meta-regression analyses (appendix: Table S1), the antibody response after Ebola vaccination was significantly associated with the vaccine platform ($p<0.001$), the viral strain used to detect the antibody response after vaccination ($p<0.001$), the year of publication (for publication in 2014 and after versus before 2014: +1.15, $p<0.001$), the mean age of vaccinated population (for ≥ 39 years versus <32 years: +0.90; $p<0.001$), the vaccine dosage (for high dose versus low dose: +0.57, $p=0.006$), the use of a vaccine boost (for boost versus no boost: +0.63, $p=0.009$), the similarity between the viral strain used as vaccine insert and the viral strain used to detect the antibody response (for identical strains versus different strains: -0.74 , $p=0.009$), the site of the study ($p=0.014$), the time interval between the last vaccine injection and the antibody measure (for <28 days versus ≥ 28 days:

Table 2
Main characteristics of included non-human primates (NHP) groups.

| Characteristic | Vaccination schedule | | | | All NHP groups | |
|--|----------------------|--------|----------------|--------|----------------|--------|
| | No boost (n = 98) | | Boost (n = 30) | | n = 128 | |
| Vaccine platform | | | | | | |
| DNA vaccine (plasmid) | 6 | 6.1% | 0 | 0.0% | 6 | 4.7% |
| Adenovirus 26 | 4 | 4.1% | 0 | 0.0% | 4 | 3.1% |
| Adenovirus 26 then adenovirus 35 | 0 | 0.0% | 1 | 3.3% | 1 | 0.8% |
| Adenovirus 35 | 4 | 4.1% | 0 | 0.0% | 4 | 3.1% |
| Adenovirus 5 | 8 | 8.2% | 3 | 10.0% | 11 | 8.6% |
| DNA vaccine (plasmid)/adenovirus 5 | 0 | 0.0% | 2 | 6.7% | 2 | 1.6% |
| CAdVax | 6 | 6.1% | 2 | 6.7% | 8 | 6.2% |
| Chimpanzee adenovirus 3 | 2 | 2.0% | 0 | 0.0% | 2 | 1.6% |
| Chimpanzee adenovirus 63 | 1 | 1.0% | 0 | 0.0% | 1 | 0.8% |
| HPIV3 | 12 | 12.2% | 7 | 23.3% | 19 | 14.8% |
| MVA | 1 | 1.0% | 0 | 0.0% | 1 | 0.8% |
| NDV | 1 | 1.0% | 1 | 3.3% | 2 | 1.6% |
| RhCMV | 1 | 1.0% | 0 | 0.0% | 1 | 0.8% |
| Whole-virus vaccine | 4 | 4.1% | 0 | 0.0% | 4 | 3.1% |
| VLP | 21 | 21.4% | 10 | 33.3% | 31 | 24.2% |
| VLP Kunjin | 1 | 1.0% | 1 | 3.3% | 2 | 1.6% |
| VRP VEEV | 3 | 3.1% | 1 | 3.3% | 4 | 3.1% |
| VSV | 23 | 23.5% | 2 | 6.7% | 25 | 19.5% |
| Route of administration | | | | | | |
| Intramuscular | 78 | 79.6% | 21 | 70.0% | 99 | 77.3% |
| Other routes | 20 | 20.4% | 9 | 30.0% | 29 | 22.7% |
| Vaccine insert: Ebola species | | | | | | |
| Monovalent Zaire | 63 | 64.3% | 17 | 56.7% | 80 | 62.5% |
| Monovalent no Zaire | 6 | 6.1% | 2 | 6.7% | 8 | 6.2% |
| Monovalent no Zaire + monovalent Zaire | 0 | 0.0% | 1 | 3.3% | 1 | 0.8% |
| Monovalent no Zaire + multivalent | 0 | 0.0% | 1 | 3.3% | 1 | 0.8% |
| Multivalent | 29 | 29.6% | 7 | 23.3% | 36 | 28.1% |
| Multivalent + monovalent Zaire | 0 | 0.0% | 2 | 6.7% | 2 | 1.6% |
| Vaccine insert: Ebola strain (only for Zaire species) | | | | | | |
| Mayinga | 34 | 64.2% | 13 | 76.5% | 47 | 67.1% |
| Kikwit | 16 | 30.2% | 4 | 23.5% | 20 | 28.6% |
| Makona | 3 | 5.7% | 0 | 0.0% | 3 | 4.3% |
| Missing data | 45 | – | 13 | – | 58 | – |
| Nonhuman primates species | | | | | | |
| Cynomolgus macaques | 65 | 66.3% | 17 | 56.7% | 82 | 64.1% |
| Chimpanzees | 10 | 10.2% | 2 | 6.7% | 12 | 9.4% |
| Rhesus macaques | 22 | 22.4% | 10 | 33.3% | 32 | 25.0% |
| African green monkeys | 1 | 1.0% | 1 | 3.3% | 2 | 1.6% |
| Year of publication | | | | | | |
| Publication < 2014 | 49 | 50.0% | 14 | 46.7% | 63 | 49.2% |
| Publication ≥ 2014 | 49 | 50.0% | 16 | 53.3% | 65 | 50.8% |
| Time interval between last injection and antibody measure | | | | | | |
| Mean [standard deviation] | 29.1 | [363] | 25.5 | [762] | 28.3 | [319] |
| Missing data | 3 | – | 0 | – | 3 | – |
| Antibody measurement method | | | | | | |
| Maximal dilution | 65 | 66.3% | 18 | 60.0% | 83 | 64.8% |
| Effective concentration 90 (EC90) | 15 | 15.3% | 2 | 6.7% | 17 | 13.3% |
| Effective concentration 50 (EC50) | 18 | 18.4% | 10 | 33.3% | 28 | 21.9% |
| Antigen used for antibody detection: nature | | | | | | |
| Glycoprotein (GP) | 55 | 56.7% | 7 | 24.1% | 62 | 49.2% |
| Other nature (virion, viral protein 40) | 42 | 43.3% | 22 | 75.9% | 64 | 50.8% |
| Missing data | 1 | – | 1 | – | 2 | – |
| Antigen used for antibody detection: Ebola strain | | | | | | |
| Mayinga | 14 | 87.5% | 8 | 100.0% | 22 | 91.7% |
| Kikwit | 2 | 12.5% | 0 | 0.0% | 2 | 8.3% |
| Missing data | 82 | – | 22 | – | 104 | – |
| Similarity between strain used as vaccine insert and strain used for antibody detection | | | | | | |
| Identical strains | 12 | 100.0% | 8 | 100.0% | 20 | 100.0% |
| Missing data | 86 | – | 22 | – | 108 | – |

CAdVax: complex adenovirus-based vector, DNA: deoxyribonucleic acid, GP: glycoprotein, HPIV3: human parainfluenza virus 3, MVA: modified vaccinia Ankara, NDV: Newcastle disease virus, RhCMV: rhesus cytomegalovirus cytomegalovirus, VLP: virus-like particles, VRP VEEV: Venezuelan equine encephalitis virus replicon particle, VSV: vesicular stomatitis virus.

Table 3
Main characteristics of included human groups.

| Characteristics | Vaccination schedule | | | | All human groups | |
|--|----------------------|--------|----------------|--------|------------------|--------|
| | No boost (n = 48) | | Boost (n = 26) | | (n = 74) | |
| Vaccine platform | | | | | | |
| DNA vaccine (plasmid) | 9 | 18.8% | 1 | 3.8% | 10 | 13.5% |
| Adenovirus 26 | 3 | 6.2% | 0 | 0.0% | 3 | 4.1% |
| Adenovirus 26/MVA or MVA/adenovirus 26 | 0 | 0.0% | 5 | 19.2% | 5 | 6.8% |
| Adenovirus 5 | 6 | 12.5% | 2 | 7.7% | 8 | 10.8% |
| Chimpanzee adenovirus 3 | 14 | 29.2% | 0 | 0.0% | 14 | 18.9% |
| Chimpanzee adenovirus 3/MVA | 0 | 0.0% | 18 | 69.2% | 18 | 24.3% |
| MVA | 2 | 4.2% | 0 | 0.0% | 2 | 2.7% |
| VSV | 14 | 29.2% | 0 | 0.0% | 14 | 18.9% |
| Route of administration | | | | | | |
| Intramuscular | 48 | 100.0% | 26 | 100.0% | 74 | 100.0% |
| Vaccine insert: species | | | | | | |
| Monovalent Zaire | 31 | 64.6% | 19 | 73.1% | 50 | 67.4% |
| Monovalent Zaire + multivalent | 0 | 0.0% | 4 | 15.4% | 4 | 5.4% |
| Multivalent | 17 | 35.4% | 1 | 3.8% | 18 | 24.3% |
| Multivalent + monovalent Zaire | 0 | 0.0% | 2 | 7.7% | 2 | 2.7% |
| Vaccine insert: strain (only for Zaire species) | | | | | | |
| Mayinga | 22 | 71.0% | 22 | 91.7% | 44 | 80.0% |
| Kikwit | 5 | 16.1% | 0 | 0.0% | 5 | 9.1% |
| Makona | 4 | 12.9% | 0 | 0.0% | 6 | 10.9% |
| Missing data | 17 | – | 4 | – | 19 | – |
| Proportion of women | | | | | | |
| Mean [standard deviation] | 40% | [18%] | 52% | [10%] | 44% | [17%] |
| Mean age (years) | | | | | | |
| Mean [standard deviation] | 34.8 | [45] | 34.6 | [54] | 34.7 | [48] |
| Geographic location of the study | | | | | | |
| Africa | 14 | 29.2% | 1 | 3.8% | 15 | 20.3% |
| China | 2 | 4.2% | 2 | 7.7% | 4 | 5.4% |
| Europe | 15 | 31.2% | 22 | 84.6% | 37 | 50.0% |
| USA | 17 | 35.4% | 1 | 3.8% | 18 | 24.3% |
| Year of publication | | | | | | |
| Publication < 2014 | 8 | 16.7% | 0 | 0.0% | 8 | 10.8% |
| Publication ≥ 2014 | 40 | 83.3% | 26 | 100.0% | 66 | 89.2% |
| Time interval between last injection and antibody measure (days) | | | | | | |
| Mean [standard deviation] | 31.2 | [220] | 26.1 | [32] | 29.3 | [179] |
| Antibody measurement method | | | | | | |
| Maximal dilution | 35 | 72.9% | 24 | 92.3% | 59 | 79.7% |
| Effective concentration 90 (EC90) | 13 | 27.1% | 2 | 7.7% | 15 | 20.3% |
| Antigen used for antibody detection: nature | | | | | | |
| Glycoprotein (GP) | 45 | 93.8% | 25 | 96.2% | 70 | 94.6% |
| Other nature (virion, nucleoprotein) | 3 | 6.2% | 1 | 3.8% | 4 | 5.4% |
| Antigen used for antibody detection: Ebola strain | | | | | | |
| Mayinga | 14 | 36.8% | 16 | 72.7% | 30 | 50.0% |
| Kikwit | 18 | 47.4% | 5 | 22.7% | 23 | 38.3% |
| Makona | 6 | 15.8% | 1 | 4.5% | 7 | 11.7% |
| Missing data | 10 | – | 4 | – | 14 | – |
| Similarity between strain used as vaccine insert and strain used for antibody detection | | | | | | |
| Different strains | 9 | 32.1% | 6 | 27.3% | 15 | 30.0% |
| Identical strains | 19 | 67.9% | 16 | 72.7% | 35 | 70.0% |
| Missing data | 20 | – | 4 | – | 24 | – |

CAVax: complex adenovirus-based vector, DNA: deoxyribonucleic acid, GP: glycoprotein, HPIV3: human parainfluenza virus 3, MVA: modified vaccinia Ankara, NDV: Newcastle disease virus, RhCMV: rhesus cytomegalovirus cytomegalovirus, VLP: virus-like particles, VRP VEEV: Venezuelan equine encephalitis virus replicon particle, VSV: vesicular stomatitis virus.

+0.70, $p=0.021$), and the Ebola species of vaccine insert (for multivalent and other species versus monovalent Zaire: -0.47 , $p=0.027$).

Alone, the vaccine platform was the factor which explained the largest part of heterogeneity among all the studied factors (R^2 for

vaccine platform = 55%). For all the univariate models, the heterogeneity was very high with I^2 ranging from 97% to 99%.

Results of the final multivariate meta-regression model are shown in Table 4. High heterogeneity was found with a I^2 of 95% and a R^2 of 68%, even after adjustment on the factors associated

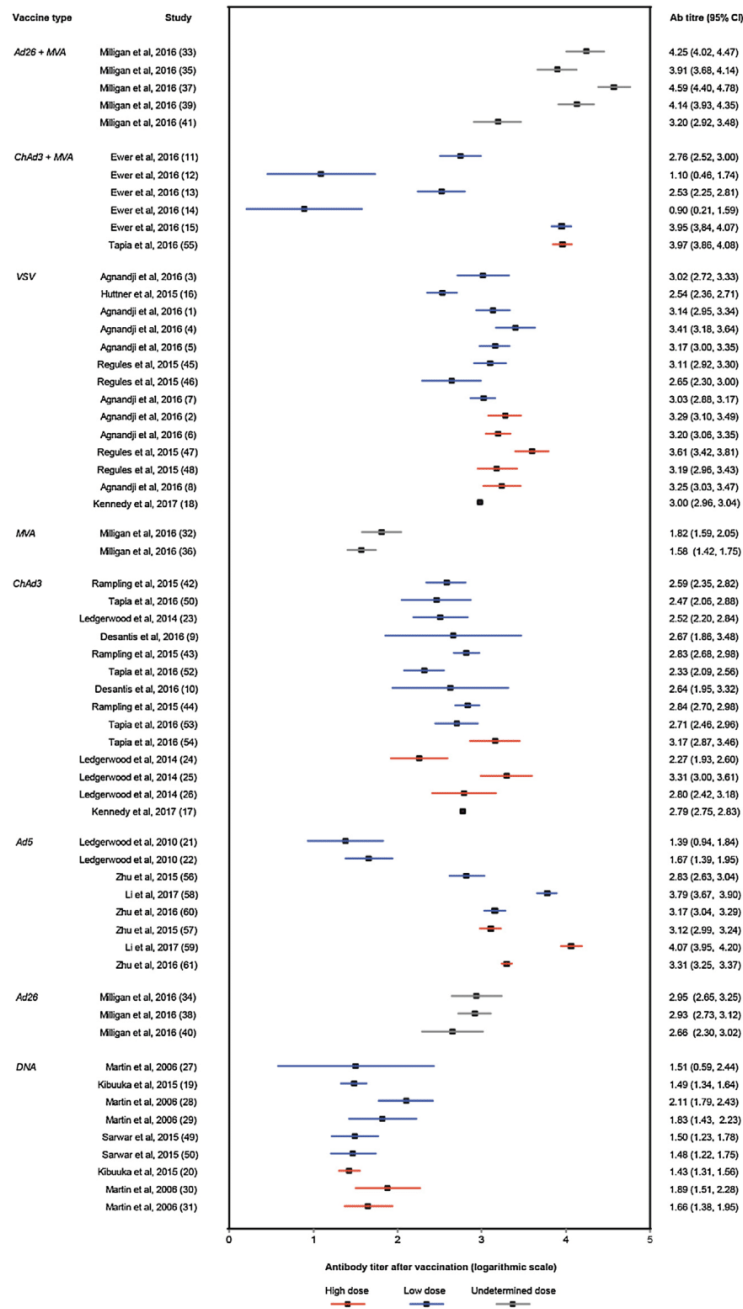


Figure 4. Forest plot of antibody titre after Ebola vaccination for each vaccination group by vaccine platform. Colour codes indicate dose levels within a given platform. GP: glycoprotein. PFU: plaque forming unit. VP: viral particle. TCID: tissue culture infectious dose.

References for Figure 4:

- 1: Agnandji 2016, VSV vaccine (3.10⁶ PFU) with Zaire insert, Germany, detection with Zaire Kikwit GP
- 2: Agnandji 2016, VSV vaccine (2.10⁷ PFU) with Zaire insert, Germany, detection with Zaire Kikwit GP
- 3: Agnandji 2016, VSV vaccine (3.10⁵ PFU) with Zaire insert, Gabon, detection with Zaire Kikwit GP
- 4: Agnandji 2016, VSV vaccine (3.10⁶ PFU) with Zaire insert, Gabon, detection with Zaire Kikwit GP
- 5: Agnandji 2016, VSV vaccine (3.10⁶ PFU) with Zaire insert, Kenya, detection with Zaire Kikwit GP
- 6: Agnandji 2016, VSV vaccine (2.10⁷ PFU) with Zaire insert, Kenya, detection with Zaire Kikwit GP
- 7: Agnandji 2016, VSV vaccine (1.10⁷ PFU) with Zaire insert, Switzerland, detection with Zaire Kikwit GP
- 8: Agnandji 2016, VSV vaccine (5.10⁷ PFU) with Zaire insert, Switzerland, detection with Zaire Kikwit GP
- 9: De Santis 2016, ChAd3 vaccine (2.5.10¹⁰ VP) with Zaire Mayinga insert, Switzerland, detection with Zaire Mayinga GP
- 10: De Santis 2016, ChAd3 vaccine (5.10¹⁰ VP) with Zaire Mayinga insert, Switzerland, detection with Zaire Mayinga GP

with antibody response in this final model. This emphasises the lack of factors that explained the antibody response among the variables included in the model.

Vaccine platform and viral strain used for detection were the two factors which were independently associated with antibody response after vaccination against Ebola. Compared to the MVA vaccine platform, the recombinant vaccines using DNA or Ad26 (associated or not with an injection of MVA vaccine), ChAd3or VSV vectors were significantly associated with a higher antibody response after vaccination (more than 1.2 log₁₀ units more compared to MVA alone). The statistical association between the vaccine platform and the antibody response was strong and consistent regardless of which other variables were included in the model (sensitivity analyses, data not shown). The antibody response using Makona strain for antibody detection was significantly higher than with use of Mayinga strain (1 log₁₀ unit more compared to the Mayinga strain). By contrast, the antibody

response with Kikwit detection strain was not significantly different from the ones with Mayinga strain.

The vaccine dosage, analysed as a binary variable of high versus low dose in the present analyses, was not found to be associated with antibody response variability. Different classifications were tested for this variable (same threshold across the different vaccine platforms corresponding to the mean dose level for categorizing into “low-dose” and “high-dose” groups, classification into three categories, classification of groups with undifferentiated dosages into “low-dose” or into “high-dose” groups), but the dosage was never significant in the multivariate models in these sensitivity analyses (data not shown), nor was the interaction between dose and vaccine platform.

In additional sensitivity analyses, a full model including all variables significantly associated with the antibody response in univariate models (i.e. with no forward selection procedure) did not modify heterogeneity ($I^2=92%$) compared to the model

- 11: Ewer 2016, ChAd3 vaccine (2.5.10¹⁰ VP) with Zaire Mayinga insert + boost MVA vaccine at D7 (1.5.10⁸ PFU) with multivalent insert, United Kingdom (UK), detection with Zaire Mayinga GP (Jenner method)
- 12: Ewer 2016, ChAd3 vaccine (2.5.10¹⁰ VP) with Zaire Mayinga insert + boost MVA vaccine at D7 (1.5.10⁸ PFU) with multivalent insert, UK, detection with Zaire Mayinga GP (ADI method)
- 13: Ewer 2016, ChAd3 vaccine (2.5.10¹⁰ VP) with Zaire Mayinga insert + boost MVA vaccine at D14 (1.5.10⁸ PFU) with multivalent insert, UK, detection with Zaire Mayinga GP (Jenner method)
- 14: Ewer 2016, ChAd3 vaccine (2.5.10¹⁰ VP) with Zaire Mayinga insert + boost MVA vaccine at D14 (1.5.10⁸ PFU) with multivalent insert, UK, detection with Zaire Mayinga GP (ADI method)
- 15: Ewer 2016, ChAd3 vaccine (1 to 5.10¹⁰ VP) with Zaire Mayinga insert + boost MVA vaccine (1.5.10⁸ PFU) with multivalent insert, UK, detection with Zaire Makona virion
- 16: Huttner 2015, VSV vaccine (3.10⁵ PFU) with Zaire insert, Switzerland, detection with Zaire Kikwit GP
- 17: Kennedy 2017, ChAd3 vaccine (2.10¹¹ PU) with Zaire insert, Liberia
- 18: Kennedy 2017, VSV vaccine (2.10⁷ PFU) with Zaire insert, Liberia
- 19: Kibuuka 2015, 3 injections of DNA vaccine (4 mg) with multivalent insert, Uganda, detection with Zaire GP
- 20: Kibuuka 2015, 3 injections of DNA vaccine (8 mg) with multivalent insert, Uganda, detection with Zaire GP
- 21: Ledgerwood 2010, Ad5 vaccine (2.10⁹ VP) with multivalent insert, USA, detection with Zaire GP
- 22: Ledgerwood 2010, Ad5 vaccine (2.10¹⁰ VP) with multivalent insert, USA, detection with Zaire GP
- 23: Ledgerwood 2014, ChAd3 vaccine (2.10¹⁰ PU) with multivalent insert, USA, detection with Zaire Mayinga GP
- 24: Ledgerwood 2014, ChAd3 vaccine (2.10¹⁰ PU) with multivalent insert, USA, detection with Zaire Makona GP
- 25: Ledgerwood 2014, ChAd3 vaccine (2.10¹¹ PU) with multivalent insert, USA, detection with Zaire Mayinga GP
- 26: Ledgerwood 2014, ChAd3 vaccine (2.10¹¹ PU) with multivalent insert, USA, detection with Zaire Makona GP
- 27: Li 2017, 2 injections of Ad5 vaccine (4.10¹⁰ VP) with Zaire Makona insert, China
- 28: Li 2017, 2 injections of Ad5 vaccine (1.6.10¹¹ VP) with Zaire Makona insert, China
- 29: Martin 2006, 3 injections of DNA vaccine (2 mg) with multivalent insert, USA, detection with Zaire NP
- 30: Martin 2006, 3 injections of DNA vaccine (4 mg) with multivalent insert, USA, detection with Zaire GP
- 31: Martin 2006, 3 injections of DNA vaccine (4 mg) with multivalent insert, USA, detection with Zaire NP
- 32: Martin 2006, 3 injections of DNA vaccine (8 mg) with multivalent insert, USA, detection with Zaire GP
- 33: Martin 2006, 3 injections of DNA vaccine (8 mg) with multivalent insert, USA, detection with Zaire NP
- 34: Milligan 2016, MVA vaccine (10⁸ TCID50) with multivalent insert, UK, detection with Zaire Kikwit GP
- 35: Milligan 2016, MVA vaccine (10⁸ TCID50) with multivalent insert + boost Ad26 vaccine at D28 (5.10¹⁰ VP) with Zaire Mayinga insert, UK, detection with Zaire Kikwit GP
- 36: Milligan 2016, Ad26 vaccine (5.10¹⁰ VP) with Zaire Mayinga insert, UK, detection with Zaire Kikwit GP
- 37: Milligan 2016, Ad26 vaccine (5.10¹⁰ VP) with Zaire Mayinga insert + boost MVA vaccine at D28 (10⁸ TCID50) with multivalent insert, UK, detection with Zaire Kikwit GP
- 38: Milligan 2016, MVA vaccine (10⁸ TCID50) with multivalent insert, UK, detection with Zaire Kikwit GP
- 39: Milligan 2016, MVA vaccine (10⁸ TCID50) with multivalent insert + boost Ad26 vaccine at D56 (5.10¹⁰ VP) with Zaire Mayinga insert, UK, detection with Zaire Kikwit GP
- 40: Milligan 2016, Ad26 vaccine (5.10¹⁰ VP) with Zaire Mayinga insert, UK, detection with Zaire Kikwit GP
- 41: Milligan 2016, Ad26 vaccine (5.10¹⁰ VP) with Zaire Mayinga insert + boost MVA vaccine at D56 (10⁸ TCID50) with multivalent insert, UK, detection with Zaire Kikwit GP
- 42: Milligan 2016, Ad26 vaccine (5.10¹⁰ VP) with Zaire Mayinga insert, UK, detection with Zaire Kikwit GP
- 43: Milligan 2016, Ad26 vaccine (5.10¹⁰ VP) with Zaire Mayinga insert + boost MVA vaccine at D14 (10⁸ TCID50) with multivalent insert, UK, detection with Zaire Kikwit GP
- 44: Rampling 2015, ChAd3 vaccine (10¹⁰ VP) with Zaire Mayinga insert, UK, detection with Zaire GP
- 45: Rampling 2015, ChAd3 vaccine (2.5.10¹⁰ VP) with Zaire Mayinga insert, UK, detection with Zaire GP
- 46: Rampling 2015, ChAd3 vaccine (5.10¹⁰ VP) with Zaire Mayinga insert, UK, detection with Zaire GP
- 47: Regules 2015, VSV vaccine (3.10⁵ PFU) with Zaire Kikwit insert, USA, detection with Zaire Kikwit GP
- 48: Regules 2015, VSV vaccine (3.10⁵ PFU) with Zaire Kikwit insert, USA, detection with Zaire Mayinga GP
- 49: Regules 2015, VSV vaccine (2.10⁷ PFU) with Zaire Kikwit insert, USA, detection with Zaire Kikwit GP
- 50: Regules 2015, VSV vaccine (2.10⁷ PFU) with Zaire Kikwit insert, USA, detection with Zaire Mayinga GP
- 51: Sarwar 2015, 3 injections of DNA vaccine (4 mg) with multivalent insert, USA, detection with Zaire GP
- 52: Sarwar 2015, 4 injections of DNA vaccine (4 mg) with multivalent insert, USA, detection with Zaire GP
- 53: Tapia 2016, ChAd3 vaccine (10¹⁰ VP) with Zaire insert, Mali, detection with Zaire GP
- 54: Tapia 2016, ChAd3 vaccine (2.5.10¹⁰ VP) with Zaire insert, Mali, detection with Zaire GP
- 55: Tapia 2016, ChAd3 vaccine (5.10¹¹ VP) with Zaire insert, Mali, detection with Zaire GP
- 56: Tapia 2016, ChAd3 vaccine (10¹² VP) with Zaire insert, Mali, detection with Zaire GP
- 57: Tapia 2016, ChAd3 vaccine (10¹⁰ to 10¹² VP) with Zaire insert + boost MVA vaccine at D97 (2.10⁸ PFU) with multivalent insert, Mali, detection with Zaire GP
- 58: Zhu 2015, Ad5 vaccine (4.10¹⁰ VP) with Zaire Makona insert, China, detection with Zaire Makona GP
- 59: Zhu 2015, Ad5 vaccine (1.6.10¹¹ VP) with Zaire Makona insert, China, detection with Zaire Makona GP
- 60: Zhu 2016, Ad5 vaccine (4.10¹⁰ VP) with Zaire Makona insert, Sierra Leone, detection with Zaire Makona GP
- 61: Zhu 2016, Ad5 vaccine (1.6.10¹¹ VP) with Zaire Makona insert, Sierra Leone, detection with Zaire Makona GP

Table 4

Results of a random-effect meta-regression model (with fixed intragroup variance) of determinants of antibody titre (log10) after Ebola vaccination according to characteristics of vaccine, population, and measurement techniques. Multivariate analysis. $I^2 = 95.31\%$, $R^2 = 68.45\%$.

| Determinants of antibody response | Estimated β [CI 95%] | p value |
|---|----------------------------|---------------|
| Vaccine platform (reference: MVA vaccine) | | |
| DNA | 0.43 | [-0.52; 1.37] |
| Ad26 | 1.15 | [033; 197] |
| Ad26/MVA or MVA/Ad26 | 2.32 | [158; 307] |
| Ad5 | 0.54 | [-0.42; 1.50] |
| ChAd3 | 0.97 | [010; 183] |
| ChAd3/MVA | 0.81 | [-0.13; 1.76] |
| VSV | 1.46 | [079; 213] |
| Viral strain used for antibody detection (reference: Mayinga strain) | | |
| Kikwit | 0.30 | [-0.27; 0.86] |
| Makona | 0.99 | [050; 148] |

presented above. In the full model, the vaccine platform was significantly associated with the antibody response ($p = 0.002$), but the viral strain used to detect the antibody response after vaccination was not ($p = 0.996$). The other variables were not associated with the antibody response.

Discussion

This systematic review on preventive Ebola vaccine trials has found 49 studies conducted in humans or in NHP. The meta-analysis, using a random-effect inverse variance meta-regression including 61 human vaccination groups, showed a major part of antibody response variability in humans that remained unexplained by the factors included in the model. Indeed, the between-group heterogeneity I^2 exceeded 90%, even after adjustment for the factors associated with antibody response. Two significant determinants were independently associated with antibody response after preventive vaccination against EVD: the Ebola vaccine platform and the Ebola strain used for antibody detection.

The use of a systematic review methodology, including solicitation of experts, allowed us to conduct exhaustive descriptive analyses on all Ebola vaccinated groups in NHP or humans published in the literature up to January 2017. Our descriptive results showed an extreme variability of study designs and features, especially in nonhuman primate trials. This variability is related to the recentness of the research topic. The higher variability within nonhuman primate studies compared to human trials is easily explained by the process of vaccine development, which selects for further clinical trials only the subset of candidate vaccines proven to be immunogenic in nonhuman primates. The comparison of antibody response levels between humans and nonhuman primate only had an indicative purpose. It is indeed difficult to compare these very different models, mostly because of potential multiple confounding factors.

Due to the low sample size of each group of nonhuman primates, we decided to restrict heterogeneity analyses to human groups. Human groups with small sample size were excluded, since their between-group variance would have been too low to contribute to the meta-regression model. It was not possible to pool small groups together because of high heterogeneity in the factors likely to influence the antibody response (vaccine and population characteristics, and measure of antibody response). The threshold of at least 8 individuals per group allowed us to include the majority of human groups in the meta-regression. Sensitivity analyses using a threshold of 10 individuals led to the same final results.

The very high heterogeneity between vaccination groups could be explained by various reasons. Firstly, some factors influencing the antibody response may be missing, for instance, genetic factors that are influencing the immunogenicity of the vaccines (Sridhar,

2015). Secondly, the analysis of grouped data, due to unavailability of individual data for the groups included in our meta-regression model, led to a lack of precision in the estimation of influence of factors on antibody response, and also in the evaluation of antibody response heterogeneity across vaccination groups. Thirdly, the enzyme-linked immunosorbent assay (ELISA) measuring relative antibody concentration of immunoglobulin G against EBOV glycoprotein used in the different trials could have a variation of its precision (Logue et al., 2018). Lastly, the extreme variability of study designs certainly explains parts of the high between-group variance for antibody response observed in our results.

Despite the major between-group heterogeneity in our meta-regression model, two factors significantly associated with antibody response variability could be identified. The Ebola strain used for antibody detection seems to influence the results of ELISA tests. This demonstrates the importance of harmonisation for the measurement methods used in vaccines evaluations, and highlights the difficulty in directly comparing published results across several trials. The Ebola vaccine platform was also strongly associated with antibody response.

For the other factors studied in our meta-analysis, no association was found with the antibody response variability. In particular, the vaccine dosage did not have any significant influence on the level of the antibody response in our results. We acknowledge that the use of a binary variable may have limited the ability to detect a dose-effect in the meta-regression. However, the regression result is consistent with the descriptive results that also did not suggest a clear dose-immunogenicity relationship within a given vaccine platform.

No population characteristic was independently associated with the antibody response after Ebola vaccination. It may be possible that the low diversity of the population, which is directly related to the strict criteria for selection of trial participants, prevented the identification of a potential impact of these population characteristics on the antibody response.

Conclusion

Our findings show that there are still significant uncertainties in the determinants of the antibody response after preventive vaccination against Ebola virus disease. This emphasises the interest of harmonizing measurement methods and study designs. Furthermore, it indicates the impossibility to directly compare results from one published study to another or to extrapolate results, due to considerable variations in studies features. Assessment of immunogenicity between Ebola vaccines needs randomised controlled multi-arm trials, as performed in PREVAIL study (NCT02344407) and PREVAC study (NCT02876328).

Conflict of interest

This project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No. 115861 This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation program and EFPIA.

Laura Richert and Rodolphe Thiebaut are also involved in the ongoing PREVAC (NCT02876328) and Ebovac2 (NCT02564523, NCT02416453) trials. Edouard Lhomme is involved in the PREVAC trial (NCT02876328).

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The funder of the study had no role in the study design, data collection, data analysis, data interpretation, or writing the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Ethical considerations

According to the National Public Health Code, review and meta-analysis do not require ethical approval.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.ijid.2018.06.022>.

References

- Agnandji ST, Huttner A, Zinser ME, Njuguna P, Dahlke C, Fernandes JF, et al. Phase 1 trials of rVSV Ebola vaccine in Africa and Europe. *N Engl J Med* 2016;374(17):1647–60.
- Bukreyev A, Rollin PE, Tate MK, Yang L, Zaki SR, Shieh W-J, et al. Successful topical respiratory tract immunization of primates against Ebola virus. *J Virol* 2007;81(June (12)):6379–88.
- Bukreyev AA, Dinapoli JM, Yang L, Murphy BR, Collins PL. Mucosal parainfluenza virus-vectored vaccine against Ebola virus replicates in the respiratory tract of vector-immune monkeys and is immunogenic. *Virology* 2010;399(2):290–8.
- De Santis O, Audran R, Pothin E, Warpelin-Decrausaz L, Vallotton L, Wuerzner G, et al. Safety and immunogenicity of a chimpanzee adenovirus-vectored Ebola vaccine in healthy adults: a randomised, double-blind, placebo-controlled, dose-finding, phase 1/2a study. *Lancet Infect Dis* 2016;16(3):311–20.
- Dhama K, Malik YS, Malik SVS, Singh RK. Ebola from emergence to epidemic: the virus and the disease, global preparedness and perspectives. *J Infect Dev Ctries* 2015;9(5):441–55.
- DiNapoli JM, Yang L, Samal SK, Murphy BR, Collins PL, Bukreyev A. Respiratory tract immunization of non-human primates with a Newcastle disease virus-vectored vaccine candidate against Ebola virus elicits a neutralizing antibody response. *Vaccine* 2010;29(1):17–25.
- Ebola Situation Report—30 March 2016 | Ebola [Internet]. [Cited 4 January 2017]. Available from: <http://apps.who.int/ebola/current-situation/ebola-situation-report-30-march-2016>.
- Ewer K, Rampling T, Venkatraman N, Bowyer G, Wright D, Lambe T, et al. A monovalent chimpanzee adenovirus ebola vaccine boosted with MVA. *N Engl J Med* 2016;374(17):1635–46.
- Food and Drug Administration. Licensure of Ebola vaccines: demonstration of effectiveness [Internet]. 2015 Available from: <https://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/BloodVaccinesandOtherBiologics/VaccinesandRelatedBiologicalProductsAdvisoryCommittee/UCM445819.pdf>.
- Geisbert TW, Daddario-Dicaprio KM, Geisbert JB, Reed DS, Feldmann F, Grolla A, et al. Vesicular stomatitis virus-based vaccines protect nonhuman primates against aerosol challenge with Ebola and Marburg viruses. *Vaccine* 2008a;26(52):6894–900.
- Geisbert TW, Daddario-Dicaprio KM, Lewis MG, Geisbert JB, Grolla A, Leung A, et al. Vesicular stomatitis virus-based ebola vaccine is well-tolerated and protects immunocompromised nonhuman primates. *PLoS Pathog* 2008b;4(11):e1000225.
- Geisbert TW, Geisbert JB, Leung A, Daddario-Dicaprio KM, Hensley LE, Grolla A, et al. Single-injection vaccine protects nonhuman primates against infection with marburg virus and three species of ebola virus. *J Virol* 2009;83(14):7296–304.
- Geisbert TW, Bailey M, Hensley L, Asiedu C, Geisbert J, Stanley D, et al. Recombinant adenovirus serotype 26 (Ad26) and Ad35 vaccine vectors bypass immunity to Ad5 and protect nonhuman primates against ebolavirus challenge. *J Virol* 2011;85(9):4222–33.
- Grant-Klein RJ, Altamura LA, Badger CV, Bounds CE, Van Deusen NM, Kwilas SA, et al. Codon-optimized filovirus DNA vaccines delivered by intramuscular electroporation protect cynomolgus macaques from lethal Ebola and Marburg virus challenges. *Hum Vaccines Immunother* 2015;11(8):1991–2004.
- Henao-Restrepo AM, Camacho A, Longini IM, Watson CH, Edmunds WJ, Egger M, et al. Efficacy and effectiveness of an rVSV-vectored vaccine in preventing Ebola virus disease: final results from the Guinea ring vaccination, open-label, cluster-randomised trial (Ebola Ça Suffit!). *Lancet* 2017;389(10068):505–18.
- Hensley LE, Mulangu S, Asiedu C, Johnson J, Honko AN, Stanley D, et al. Demonstration of cross-protective vaccine immunity against an emerging pathogenic Ebolavirus species. *PLoS Pathog* 2010;6(5):e1000904.
- Herbert AS, Kuehne AI, Barth JF, Ortiz RA, Nichols DK, Zak SE, et al. Venezuelan equine encephalitis virus replicon particle vaccine protects nonhuman primates from intramuscular and aerosol challenge with ebolavirus. *J Virol* 2013;87(9):4952–64.
- Huttner A, Dayer J-A, Yerly S, Combescurie C, Auderset F, Desmeules J, et al. The effect of dose on the safety and immunogenicity of the VSV Ebola candidate vaccine: a randomised double-blind, placebo-controlled phase 1/2 trial. *Lancet Infect Dis* 2015;15(10):1156–66.
- Jones SM, Feldmann H, Ströher U, Geisbert JB, Fernando L, Grolla A, et al. Live attenuated recombinant vaccine protects nonhuman primates against Ebola and Marburg viruses. *Nat Med* 2005;11(7):786–90.
- Kennedy SB, Bolay F, Kieh M, Grandits G, Badio M, Ballou R, et al. Phase 2 placebo-controlled trial of two vaccines to prevent ebola in Liberia. *N Engl J Med* 2017;377:1438–47.
- Kibuuka H, Berkowitz NM, Millard M, Enama ME, Tindikahwa A, Sekiziyivu AB, et al. Safety and immunogenicity of Ebola virus and Marburg virus glycoprotein DNA vaccines assessed separately and concomitantly in healthy Ugandan adults: a phase 1b, randomised, double-blind, placebo-controlled clinical trial. *Lancet* 2015;385(9977):1545–54.
- Ledgerwood JE, Costner P, Desai N, Holman L, Enama ME, Yamshchikov G, et al. A replication defective recombinant Ad5 vaccine expressing Ebola virus GP is safe and immunogenic in healthy adults. *Vaccine* 2010;29(2):304–13.
- Ledgerwood JE, Sullivan NJ, Graham BS. Chimpanzee adenovirus vector ebola vaccine—preliminary report. *N Engl J Med* 2015;373(8):776.
- Li J-X, Hou L-H, Meng F-Y, Wu S-P, Hu Y-M, Liang Q, et al. Immunity duration of a recombinant adenovirus type-5 vector-based Ebola vaccine and a homologous prime-boost immunisation in healthy adults in China: final report of a randomised, double-blind, placebo-controlled, phase 1 trial. *Lancet Glob Health* 2017;5(3):e324–34.
- Liu WB, Li ZX, Du Y, Cao GW. Ebola virus disease: from epidemiology to prophylaxis. *Mil Med Res* 2015;2:7.
- Logue J, Tuznik K, Follmann D, Grandits G, Marchand J, Reilly C, et al. Use of the Filovirus Animal Non-Clinical Group (FANG) Ebola virus Immuno-assay requires fewer study participants to power a study than the Alpha Diagnostic International assay. *J Virol Methods* 2018;255:84–90.
- Martin JE, Sullivan NJ, Enama ME, Gordon IJ, Roederer M, Koup RA, et al. A DNA vaccine for Ebola virus is safe and immunogenic in a phase I clinical trial. *Clin Vaccine Immunol* 2006;13(11):1267–77.
- Marzi A, Engelmann F, Feldmann F, Habberthür K, Shupert WL, Brining D, et al. Antibodies are necessary for rVSV/ZEBOV-GP-mediated protection against lethal Ebola virus challenge in nonhuman primates. *Proc Natl Acad Sci U S A* 2013;110(5):1893–8.
- Marzi A, Feldmann F, Geisbert TW, Feldmann H, Safronetz D. Vesicular stomatitis virus-based vaccines against lassa and ebola viruses. *Emerg Infect Dis* 2015a;21(2):305–7.
- Marzi A, Halfmann P, Hill-Batorski L, Feldmann F, Shupert WL, Neumann G, et al. Vaccines. An Ebola whole-virus vaccine is protective in nonhuman primates. *Science* 2015b;348(6233):439–42.
- Marzi A, Robertson SJ, Haddock E, Feldmann F, Hanley PW, Scott DP, et al. Ebola vaccine. VSV-EBOV rapidly protects macaques against infection with the 2014/15 Ebola virus outbreak strain. *Science* 2015c;349(6249):739–42.
- Marzi A, Murphy AA, Feldmann F, Parkins CJ, Haddock E, Hanley PW, et al. Cytomegalovirus-based vaccine expressing Ebola virus glycoprotein protects nonhuman primates from Ebola virus infection. *Sci Rep* 2016;6:21674.
- Matassov D, Marzi A, Latham T, Xu R, Ota-Setlik A, Feldmann F, et al. Vaccination with a highly attenuated recombinant vesicular stomatitis virus vector protects against challenge with a lethal dose of ebola virus. *J Infect Dis* 2015;212 Suppl 2: S443–51.
- Meyer M, Garron T, Lubaki NM, Mire CE, Fenton KA, Klages C, et al. Aerosolized Ebola vaccine protects primates and elicits lung-resident T cell responses. *J Clin Invest* 2015;125(8):3241–55.
- Milligan ID, Gibani MM, Sewell R, et al. Safety and immunogenicity of novel adenovirus type 26—and modified vaccinia ankara—vectored ebola vaccines: a randomized clinical trial. *JAMA* 2016;315(15):1610–23.

- Mire CE, Geisbert JB, Marzi A, Agans KN, Feldmann H, Geisbert TW. Vesicular stomatitis virus-based vaccines protect nonhuman primates against Bundibugyo ebolavirus. *PLoS Negl Trop Dis* 2013;7(12):e2600.
- Mire CE, Matasov D, Geisbert JB, Latham TE, Agans KN, Xu R, et al. Single-dose attenuated Vesiculovax vaccines protect primates against Ebola Makona virus. *Nature* 2015;520(7549):688–91.
- Ohimain EI. Recent advances in the development of vaccines for Ebola virus disease. *Virus Res* 2016;211:174–85.
- Pratt WD, Wang D, Nichols DK, Luo M, Woraratanadharm J, Dye JM, et al. Protection of nonhuman primates against two species of Ebola virus infection with a single complex adenovirus vector. *Clin Vaccine Immunol* 2010;17(4):572–81.
- Pyankov OV, Bodnev SA, Pyankova OG, Solodkiy VV, Pyankov SA, Setoh YX, et al. A Kunjin Replicon virus-like particle vaccine provides protection against Ebola virus infection in nonhuman primates. *J Infect Dis* 2015;212 Suppl 2:S368–71.
- Rampling T, Ewer K, Bowyer G, Wright D, Imoukhuede EB, Payne R, et al. A monovalent chimpanzee adenovirus Ebola vaccine—preliminary report. *N Engl J Med* 2015;374(17):1635–46.
- Regules JA, Beigel JH, Paolino KM, Voell J, Castellano AR, Muñoz P, et al. A recombinant vesicular stomatitis virus ebola vaccine—preliminary report. *N Engl J Med* 2015;376(4):330–41.
- Sarwar UN, Costner P, Enama ME, Berkowitz N, Hu Z, Hendel CS, et al. Safety and immunogenicity of DNA vaccines encoding Ebolavirus and Marburgvirus wild-type glycoproteins in a phase I clinical trial. *J Infect Dis* 2015;211(4):549–57.
- Sridhar S. Clinical development of Ebola vaccines. *Ther Adv Vaccines* 2015;3(5–6):125–38.
- Stanley DA, Honko AN, Asiedu C, Trefry JC, Lau-Kilby AW, Johnson JC, et al. Chimpanzee adenovirus vaccine generates acute and durable protective immunity against ebolavirus challenge. *Nat Med* 2014;20(10):1126–9.
- Sullivan NJ, Sanchez A, Rollin PE, Yang ZY, Nabel GJ. Development of a preventive vaccine for Ebola virus infection in primates. *Nature* 2000a;408(6812):605–9.
- Sullivan NJ, Sanchez A, Rollin PE, Yang ZY, Nabel GJ. Development of a preventive vaccine for Ebola virus infection in primates. *Nature* 2000b;408(6812):605–9.
- Sullivan NJ, Geisbert TW, Geisbert JB, Xu L, Yang Z-Y, Roederer M, et al. Accelerated vaccination for Ebola virus haemorrhagic fever in non-human primates. *Nature* 2003;424(6949):681–4.
- Sullivan NJ, Martin JE, Graham BS, Nabel GJ. Correlates of protective immunity for Ebola vaccines: implications for regulatory approval by the animal rule. *Nat Rev Microbiol* 2009;7(5):393–400.
- Sullivan NJ, Hensley L, Asiedu C, Geisbert TW, Stanley D, Johnson J, et al. CD8+ cellular immunity mediates rAd5 vaccine protection against Ebola virus infection of nonhuman primates. *Nat Med* 2011;17(9):1128–31.
- Swenson DL, Wang D, Luo M, Warfield KL, Woraratanadharm J, Holman DH, et al. Vaccine to confer to nonhuman primates complete protection against multi-strain Ebola and Marburg virus infections. *Clin Vaccine Immunol* 2008;15(3):460–7.
- Tapia MD, Sow SO, Lyke KE, Haidara FC, Diallo F, Doumbia M, et al. Use of ChAd3-EBO-Z Ebola virus vaccine in Malian and US adults, and boosting of Malian adults with MVA-BN-Filo: a phase 1, single-blind, randomised trial, a phase 1b, open-label and double-blind, dose-escalation trial, and a nested, randomised, double-blind, placebo-controlled trial. *Lancet Infect Dis* 2016;16(1):31–42.
- Van Houwelingen HC, Arends LR, Stijnen T. Advanced methods in meta-analysis: multivariate approach and meta-regression. *Stat Med* 2002;21(4):589–624.
- Warfield KL, Swenson DL, Olinger GG, Kalina WV, Aman MJ, Bavari S. Ebola virus-like particle-based vaccine protects nonhuman primates against lethal Ebola virus challenge. *J Infect Dis* 2007;196 Suppl 2:S430–7.
- Warfield KL, Goetzmann JE, Biggins JE, Kasda MB, Unfer RC, Vu H, et al. Vaccinating captive chimpanzees to save wild chimpanzees. *Proc Natl Acad Sci U S A* 2014;111(24):8873–6.
- Warfield KL, Dye JM, Wells JB, Unfer RC, Holtsberg FW, Shulenin S, et al. Homologous and heterologous protection of nonhuman primates by Ebola and Sudan virus-like particles. *PLoS One* 2015;10(3):e0118881.
- Wong C, Richardson JS, Pillet S, Patel A, Qiu X, Alimonti J, et al. Immune parameters correlate with protection against ebola virus infection in rodents and nonhuman primates. *Sci Transl Med* 2012;4(158):158ra146.
- World Health Organisation. Correlates of vaccine-induced protection: methods and implications. 2013.
- Wu X-X, Yao H-P, Wu N-P, Gao H-N, Wu H-B, Jin C-Z, et al. Ebolavirus vaccines: progress in the fight against Ebola virus disease. *Cell Physiol Biochem* 2015;37(5):1641–58.
- Wu S, Kroeker A, Wong G, He S, Hou L, Audet J, et al. An adenovirus vaccine expressing Ebola virus variant makona glycoprotein is efficacious in guinea pigs and nonhuman primates. *J Infect Dis* 2016;214(Suppl. 3):S326–32.
- Zhu F-C, Hou L-H, Li J-X, Wu S-P, Liu P, Zhang G-R, et al. Safety and immunogenicity of a novel recombinant adenovirus type-5 vector-based Ebola vaccine in healthy adults in China: preliminary report of a randomised, double-blind, placebo-controlled, phase 1 trial. *Lancet* 2015;385(9984):2272–9.
- Zhu F-C, Wurie AH, Hou L-H, Liang Q, Li Y-H, Russell JBW, et al. Safety and immunogenicity of a recombinant adenovirus type-5 vector-based Ebola vaccine in healthy adults in Sierra Leone: a single-centre, randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet* 2017;389(10069):621–8.

Supplementary material

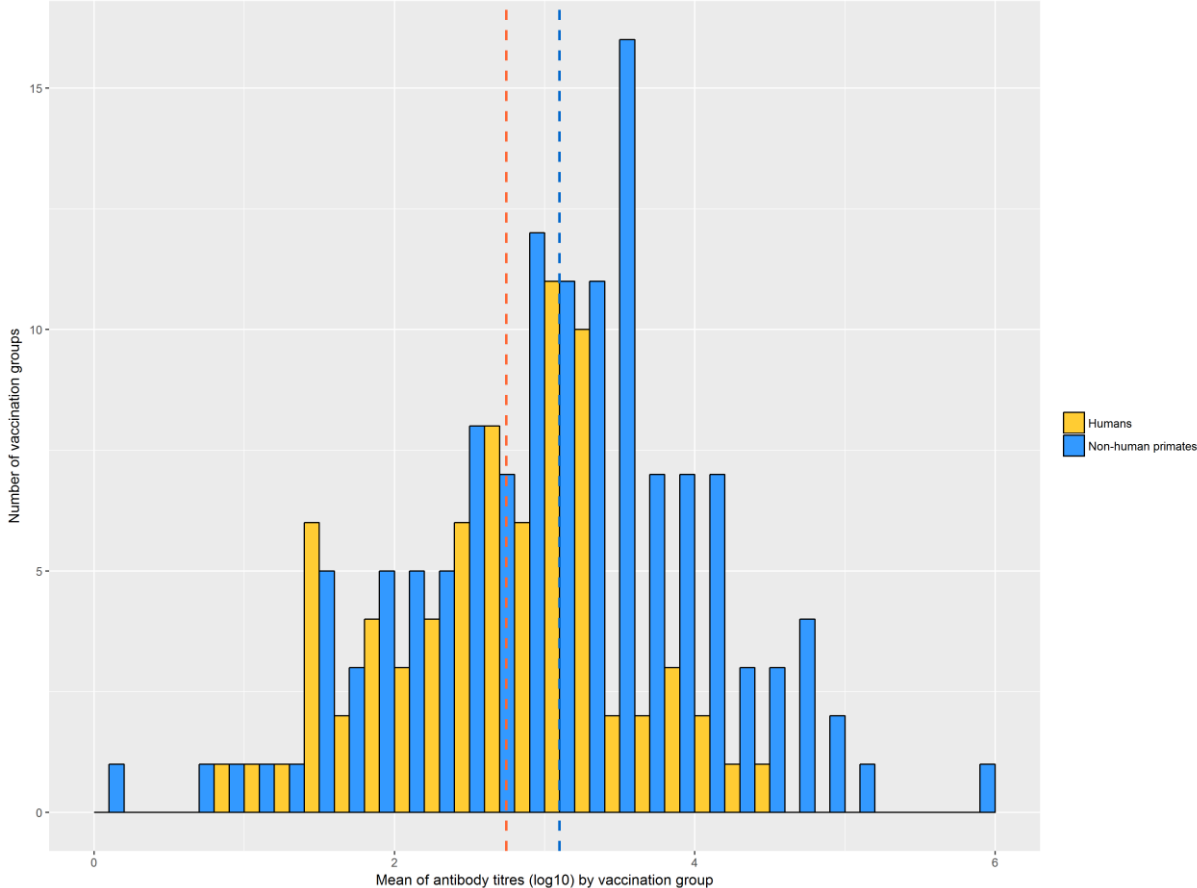


Figure S1: Antibody response after Ebola vaccination or human groups and non-human primates (NHP) groups. The AR is expressed as the mean by vaccination group of the log transformed titre after vaccination. Vertical dotted lines indicate the mean values: the red one for human groups, and the blue one for NHP groups.

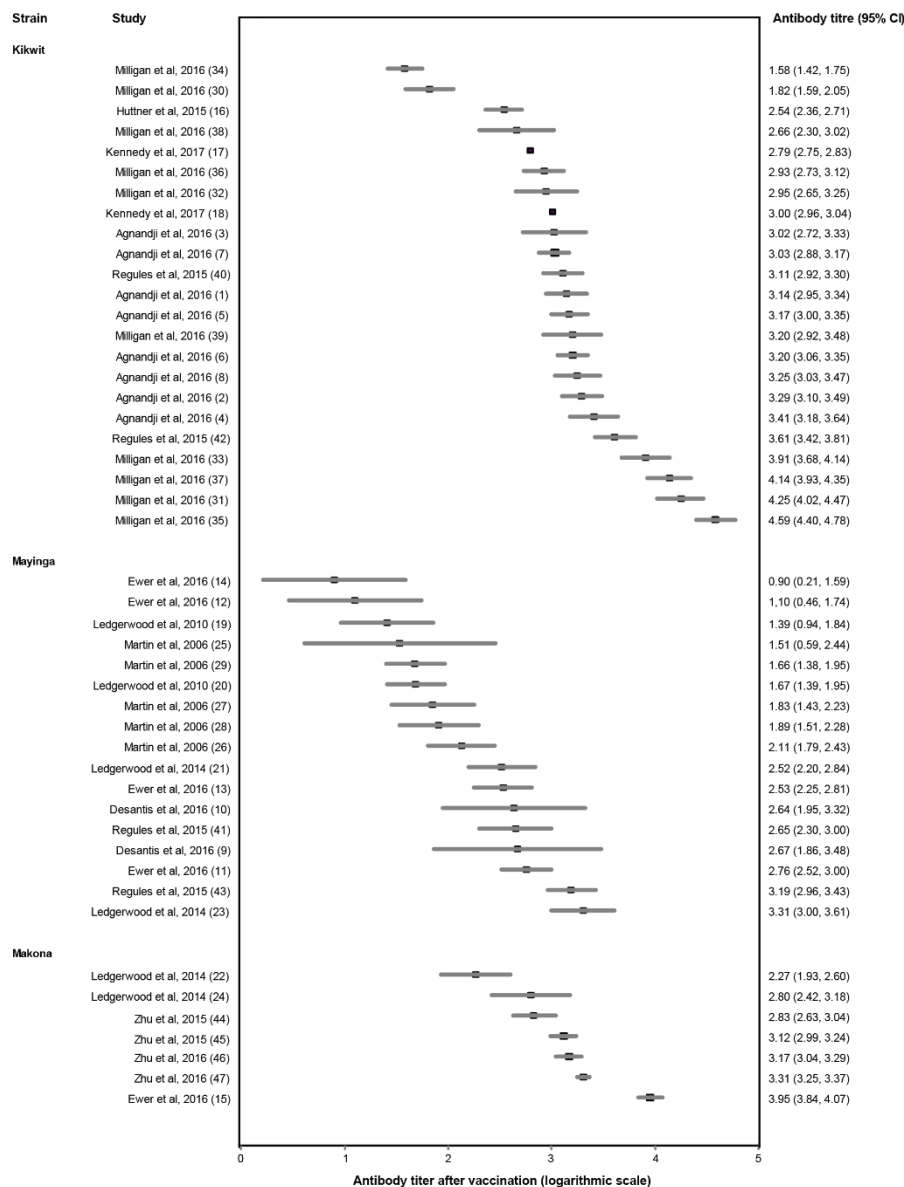


Figure S2: Forest plot of antibody titre after Ebola vaccination for each vaccination group by viral strain used for antibody detection. PFU: plaque forming unit. VP: viral particle. TCID: tissue culture infectious dose. GP: glycoprotein.

References for figure S2:

1: Agnandji 2016, VSV vaccine ($3 \cdot 10^6$ PFU) with Zaire insert, Germany, detection with Zaire Kikwit GP

2: Agnandji 2016, VSV vaccine ($2 \cdot 10^7$ PFU) with Zaire insert, Germany, detection with Zaire Kikwit GP

3: Agnandji 2016, VSV vaccine ($3 \cdot 10^5$ PFU) with Zaire insert, Gabon, detection with Zaire Kikwit GP

- 4: Agnandji 2016**, VSV vaccine ($3 \cdot 10^6$ PFU) with Zaire insert, Gabon, detection with Zaire Kikwit GP
- 5: Agnandji 2016**, VSV vaccine ($3 \cdot 10^6$ PFU) with Zaire insert, Kenya, detection with Zaire Kikwit GP
- 6: Agnandji 2016**, VSV vaccine ($2 \cdot 10^7$ PFU) with Zaire insert, Kenya, detection with Zaire Kikwit GP
- 7: Agnandji 2016**, VSV vaccine ($1 \cdot 10^7$ PFU) with Zaire insert, Switzerland, detection with Zaire Kikwit GP
- 8: Agnandji 2016**, VSV vaccine ($5 \cdot 10^7$ PFU) with Zaire insert, Switzerland, detection with Zaire Kikwit GP
- 9: De Santis 2016**, ChAd3 vaccine ($2.5 \cdot 10^{10}$ VP) with Zaire Mayinga insert, Switzerland, detection with Zaire Mayinga GP
- 10: De Santis 2016**, ChAd3 vaccine ($5 \cdot 10^{10}$ VP) with Zaire Mayinga insert, Switzerland, detection with Zaire Mayinga GP
- 11: Ewer 2016**, ChAd3 vaccine ($2.5 \cdot 10^{10}$ VP) with Zaire Mayinga insert + boost MVA vaccine at D7 ($1.5 \cdot 10^8$ PFU) with multivalent insert, United Kingdom (UK), detection with Zaire Mayinga GP (Jenner method)
- 12: Ewer 2016**, ChAd3 vaccine ($2.5 \cdot 10^{10}$ VP) with Zaire Mayinga insert + boost MVA vaccine at D7 ($1.5 \cdot 10^8$ PFU) with multivalent insert, UK, detection with Zaire Mayinga GP (ADI method)
- 13: Ewer 2016**, ChAd3 vaccine ($2.5 \cdot 10^{10}$ VP) with Zaire Mayinga insert + boost MVA vaccine at D14 ($1.5 \cdot 10^8$ PFU) with multivalent insert, UK, detection with Zaire Mayinga GP (Jenner method)
- 14: Ewer 2016**, ChAd3 vaccine ($2.5 \cdot 10^{10}$ VP) with Zaire Mayinga insert + boost MVA vaccine at D14 ($1.5 \cdot 10^8$ PFU) with multivalent insert, UK, detection with Zaire Mayinga GP (ADI method)
- 15: Ewer 2016**, ChAd3 vaccine (1 to $5 \cdot 10^{10}$ VP) with Zaire Mayinga insert + boost MVA vaccine ($1.5 \cdot 10^8$ PFU) with multivalent insert, UK, detection with Zaire Makona virion
- 16: Huttner 2015**, VSV vaccine ($3 \cdot 10^5$ PFU) with Zaire insert, Switzerland, detection with Zaire Kikwit GP
- 17: Kennedy 2017**, ChAd3 vaccine ($2 \cdot 10^{11}$ PU) with Zaire insert, Liberia
- 18: Kennedy 2017**, VSV vaccine ($2 \cdot 10^7$ PFU) with Zaire insert, Liberia
- 19: Ledgerwood 2010**, Ad5 vaccine ($2 \cdot 10^9$ VP) with multivalent insert, USA, detection with Zaire GP
- 20: Ledgerwood 2010**, Ad5 vaccine ($2 \cdot 10^{10}$ VP) with multivalent insert, USA,

detection with Zaire GP

21: Ledgerwood 2014, ChAd3 vaccine ($2 \cdot 10^{10}$ PU) with multivalent insert, USA, detection with Zaire Mayinga GP

22: Ledgerwood 2014, ChAd3 vaccine ($2 \cdot 10^{10}$ PU) with multivalent insert, USA, detection with Zaire Makona GP

23: Ledgerwood 2014, ChAd3 vaccine ($2 \cdot 10^{11}$ PU) with multivalent insert, USA, detection with Zaire Mayinga GP

24: Ledgerwood 2014, ChAd3 vaccine ($2 \cdot 10^{11}$ PU) with multivalent insert, USA, detection with Zaire Makona GP

25: Martin 2006, 3 injections of DNA vaccine (2 mg) with multivalent insert, USA, detection with Zaire NP

26: Martin 2006, 3 injections of DNA vaccine (4 mg) with multivalent insert, USA, detection with Zaire GP

27: Martin 2006, 3 injections of DNA vaccine (4 mg) with multivalent insert, USA, detection with Zaire NP

28: Martin 2006, 3 injections of DNA vaccine (8 mg) with multivalent insert, USA, detection with Zaire GP

29: Martin 2006, 3 injections of DNA vaccine (8 mg) with multivalent insert, USA, detection with Zaire NP

30: Milligan 2016, MVA vaccine (10^8 TCID₅₀) with multivalent insert, UK, detection with Zaire Kikwit GP

31: Milligan 2016, MVA vaccine (10^8 TCID₅₀) with multivalent insert + boost Ad26 vaccine at D28 ($5 \cdot 10^{10}$ VP) with Zaire Mayinga insert, UK, detection with Zaire Kikwit GP

32: Milligan 2016, Ad26 vaccine ($5 \cdot 10^{10}$ VP) with Zaire Mayinga insert, UK, detection with Zaire Kikwit GP

33: Milligan 2016, Ad26 vaccine ($5 \cdot 10^{10}$ VP) with Zaire Mayinga insert + boost MVA vaccine at D28 (10^8 TCID₅₀) with multivalent insert, UK, detection with Zaire Kikwit GP

34: Milligan 2016, MVA vaccine (10^8 TCID₅₀) with multivalent insert, UK, detection with Zaire Kikwit GP

35: Milligan 2016, MVA vaccine (10^8 TCID₅₀) with multivalent insert + boost Ad26 vaccine at D56 ($5 \cdot 10^{10}$ VP) with Zaire Mayinga insert, UK, detection with Zaire Kikwit GP

36: Milligan 2016, Ad26 vaccine ($5 \cdot 10^{10}$ VP) with Zaire Mayinga insert, UK, detection with Zaire Kikwit GP

37: Milligan 2016, Ad26 vaccine ($5 \cdot 10^{10}$ VP) with Zaire Mayinga insert + boost MVA vaccine at D56 (10^8 TCID₅₀) with multivalent insert, UK, detection with Zaire Kikwit GP

38: Milligan 2016, Ad26 vaccine ($5 \cdot 10^{10}$ VP) with Zaire Mayinga insert, UK, detection with Zaire Kikwit GP

39: Milligan 2016, Ad26 vaccine ($5 \cdot 10^{10}$ VP) with Zaire Mayinga insert + boost MVA vaccine at D14 (10^8 TCID₅₀) with multivalent insert, UK, detection with Zaire Kikwit GP

40: Regules 2015, VSV vaccine ($3 \cdot 10^6$ PFU) with Zaire Kikwit insert, USA, detection with Zaire Kikwit GP

41: Regules 2015, VSV vaccine ($3 \cdot 10^6$ PFU) with Zaire Kikwit insert, USA, detection with Zaire Mayinga GP

42: Regules 2015, VSV vaccine ($2 \cdot 10^7$ PFU) with Zaire Kikwit insert, USA, detection with Zaire Kikwit GP

43: Regules 2015, VSV vaccine ($2 \cdot 10^7$ PFU) with Zaire Kikwit insert, USA, detection with Zaire Mayinga GP

44: Zhu 2015, Ad5 vaccine ($4 \cdot 10^{10}$ VP) with Zaire Makona insert, China, detection with Zaire Makona GP

45: Zhu 2015, Ad5 vaccine ($1.6 \cdot 10^{11}$ VP) with Zaire Makona insert, China, detection with Zaire Makona GP

46: Zhu 2016, Ad5 vaccine ($4 \cdot 10^{10}$ VP) with Zaire Makona insert, Sierra Leone, detection with Zaire Makona GP

47: Zhu 2016, Ad5 vaccine ($1.6 \cdot 10^{11}$ VP) with Zaire Makona insert, Sierra Leone, detection with Zaire Makona GP

Table S1: Parameters and statistics estimations of a random-effect meta-regression model (with fixed intragroup variance) for antibody titre (log 10) after Ebola vaccination according to characteristics of vaccine, population, and measurement techniques. Univariate analyses.

| Factors that may influence antibody response | Estimated β [CI 95%] | | p value |
|--|--|------------------------|-------------------|
| Vaccine platform (reference: MVA vaccine) | | | < 0.001 |
| DNA | -0.05 | [-0.90 ; 0.81] | 0.919 |
| Ad26 | 1.15 | [0.14 ; 2.15] | 0.025 |
| Ad26/MVA or MVA/Ad26 | 2.32 | [1.41 ; 3.23] | < 0.001 |
| Ad5 | 1.25 | [0.39 ; 2.12] | 0.004 |
| ChAd3 | 1.00 | [0.18 ; 1.84] | 0.017 |
| ChAd3/MVA | 0.99 | [0.09 ; 1.89] | 0.032 |
| VSV | 1.42 | [0.59 ; 2.24] | < 0.001 |
| Vaccine insert: species (reference: monovalent Zaire) | | | |
| Multivalent and other species | -0.47 | [-0.88 ; -0.05] | 0.027 |
| Vaccine insert: strain (reference: Mayinga strain) | | | 0.066 |

| | | | |
|---|-------------|----------------------|--------------|
| Kikwit | 0.52 | [-0.27 ; 1.30] | 0.198 |
| Makona | 0.78 | [0.06 ; 1.51] | 0.034 |
| Dosage (reference: low dose) | | | |
| High dose | 0.57 | [0.16 ; 0.97] | 0.006 |
| Boost (reference: no boost) | | | |
| Boost | 0.63 | [0.16 ; 1.10] | 0.009 |
| Proportion of women (reference: < 0.35) | | | 0.699 |
| 0.35-0.47 | -0.27 | [-0.87 ; 0.33] | 0.335 |
| 0.47-0.54 | -0.26 | [-0.86 ; 0.34] | 0.407 |
| ≥ 0.54 | -0.01 | [-0.62 ; 0.60] | 0.965 |
| Mean age (reference: < 32 years) | | | 0.003 |
| 32-34 | 0.58 | [0.01 ; 1.17] | 0.055 |
| 34-39 | 0.23 | [-0.30 ; 0.76] | 0.391 |
| ≥ 39 | 0.90 | [0.40 ; 1.39] | < 0.001 |
| Site of the study (reference: Africa) | | | 0.014 |
| China | 0.61 | [-0.24 ; 1.46] | 0.158 |

| | | | |
|--|-------|------------------|---------|
| Europe | 0.07 | [-0.43 ; 0.56] | 0.792 |
| USA | -0.56 | [-1.10 ; -0.021] | 0.042 |
| Year of publication (reference: < 2014) | | | |
| ≥ 2014 | 1.15 | [0.55 ; 1.76] | < 0.001 |
| Time interval between last injection and measure (reference: ≥ 28 days) | | | |
| < 28 days | 0.70 | [0.11 ; 1.29] | 0.021 |
| Measurement method (reference: maximal dilution) | | | |
| Concentration effective 90 (EC 90) | 0.32 | [-0.17 ; 0.80] | 0.200 |
| Antigen used for detection: nature (reference: glycoprotein) | | | |
| Other nature (virion, nucleoprotein) | -0.49 | [-1.35 ; 0.37] | 0.267 |
| Antigen used for detection: Ebola strain (reference: Mayinga strain), 14 missing data | | | |
| Kikwit | 0.99 | [0.57 ; 1.42] | < 0.001 |
| Makona | 0.91 | [0.32 ; 1.50] | 0.002 |

Similarity between strain used as vaccine insert and strain used for antibody detection (reference: different strains), 24 missing data

| | | | |
|-------------------|--------------|------------------------|--------------|
| Identical strains | -0.71 | [-1.25 ; -0.16] | 0.011 |
|-------------------|--------------|------------------------|--------------|

CAdVax: complex adenovirus-based vector, DNA: deoxyribonucleic acid, GP: glycoprotein, HPIV3: human parainfluenza virus 3, MVA: modified vaccinia Ankara, NDV: Newcastle disease virus, RhCMV: rhesus cytomegalovirus cytomegalovirus, VLP: virus-like particles, VRP VEEV: Venezuelan equine encephalitis virus replicon particle, VSV: vesicular stomatitis virus.

Discussion

In this chapter we focused on the antibody responses and performed the first meta-analysis of Ebola vaccine trials in humans and NPH to identify factors influencing the antibody response after vaccination. The main results were that there is a huge heterogeneity that is not explained by the factors measured in the meta-regression model and that two determinants of the immune response after a preventive vaccination have been found: the Ebola vaccine platform and the viral strain used for antibody detection.

This work gives us information to improve future Ebola vaccine clinical trials. Antigens from some viral strains may be able to better detect antibodies after vaccination, regardless of whether they are identical or not to the viral strain inserted in the vaccine. This result emphasizes the need of standardizing methods for detecting Ebola antibodies in order to be able to evaluate the efficacy of different existing vaccines under comparable conditions. A recent paper from Logue et al [6] described a new ELISA assay called Filovirus Animal Non-Clinical Group (FANG) Ebola virus immuno-assay for an optimization of standardization of the evaluation of the immunogenicity in Ebola vaccine trials. This assay has been used in recent Ebola vaccine trials (PREVAIL 1, PREVAC trial) and would allow more comparative results to be obtained between the different laboratories and clinical trials. This need for standardization should be considered also for the development of vaccines against other diseases that used ELISA for evaluation of immunogenicity.

The identification of the vaccine platform as an independent factor underlines the critical role of vaccine platform in the vaccine development. The same vaccine principle/vector can be used with inserts against other diseases, and the detailed characterization and assessment of vaccine platforms capable of being applied to swiftly develop vaccines against a variety of pathogens are of utmost importance [7]. There is need for instance to identify of the best vaccine platforms for developing novel vaccine candidates, especially against diseases identified in the Blueprint list by the WHO [8], that pose a public health risk in the absence of efficacious drugs and/or vaccines (Crimean-Congo haemorrhagic fever (CCHF), - Ebola virus disease and Marburg virus disease; - Lassa fever ; - Middle East respiratory syndrome coronavirus (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS) ; Nipah and henipaviral diseases ; Rift Valley fever (RVF) ; Zika; Disease X). In this perspective, a recent review by Fathi and al discussed insights gained from the clinical VSV-EBOV vaccine trials as well as from animal studies investigating vaccine candidates for Blueprint pathogens using the rVSV platform [7].

The huge heterogeneity can be partly explained by the fact that some factors influencing the antibody response may be missing. Main factors responsible for these variations may be human genetics factors, environmental factors, demographic factors and lately microbiome have been described as influencing vaccine response [9-13]. Geographic variations were also described for several vaccines [14]. Factors influencing the variability of the immune response after vaccination remain poorly understood. Because this is not always possible to conduct clinical trials to directly compare and this point is crucial for future trials for a better interpretation of the results. This topic is one of the subjects of research in the SISTM team.

The large non explained heterogeneity of our meta-analysis also underlines the difficulties to compare indirectly the results of different vaccine strategies conducted in different vaccine trials. Only multi arm randomized clinical trials allow a direct comparison of vaccine strategy avoiding selection and information bias. This is for example the case with two recent academic Ebola vaccine trials in which participants were randomized in different vaccine strategies and a follow up with identical procedures, in particular on the measurement of the antibody responses: PREVAIL 1 trial [15] which evaluate the immunogenicity of ChAd3-EBO-Z and the rVSV Δ G-ZEBOV-GP in Liberia and the ongoing PREVAC trial (NCT02876328) where participants are randomized between the two current most promising prophylactic vaccines rVSV Δ G-ZEBOV-GP and Ad26/MVA. In these two trials, the vaccine strategies are compared to a same placebo group. In the PREVAC trial, we have been particularly vigilant of the measure of the antibody response which is the primary endpoint of the study. The analysis of the antibody response by Filovirus Animal Non-Clinical Group (FANG) Ebola virus immunoassay [6] is currently ongoing in Liberia and a specific meeting to review the FANG assay data - in which I am involved - is organized on a regularly basis to detect a potential batch effect. A batch effect has already occurred on this type of data in an unpublished test due to a change of reagent.

References

1. Pushko P, Bray M, Ludwig GV, Parker M, Schmaljohn A, Sanchez A, et al. Recombinant RNA replicons derived from attenuated Venezuelan equine encephalitis virus protect guinea pigs and mice from Ebola hemorrhagic fever virus. *Vaccine*. 2000;19(1):142-53.
2. Wong G, Richardson JS, Pillet S, Patel A, Qiu X, Alimonti J, et al. Immune Parameters Correlate with Protection Against Ebola Virus Infection in Rodents and Nonhuman Primates. *Sci Transl Med*. 2012;4(158):158ra146.
3. Wilson JA, Hart MK. Protection from Ebola virus mediated by cytotoxic T lymphocytes specific for the viral nucleoprotein. *J Virol*. 2001;75(6):2660-4.
4. Meyer M, Malherbe DC, Bukreyev A. Can Ebola Virus Vaccines Have Universal Immune Correlates of protection? *Trends Microbiol*. 2019;27(1):8-16.
5. Engvall E, Perlmann P. Enzyme-Linked Immunosorbent Assay, Elisa: III. Quantitation of Specific Antibodies by Enzyme-Labeled Anti-Immunoglobulin in Antigen-Coated Tubes. *The Journal of Immunology*. 1972;109(1):129-35.
6. Logue J, Tuznik K, Follmann D, Grandits G, Marchand J, Reilly C, et al. Use of the Filovirus Animal Non-Clinical Group (FANG) Ebola virus immuno-assay requires fewer study participants to power a study than the Alpha Diagnostic International assay. *J Virol Methods*. 2018;255:84-90.
7. Fathi A, Dahlke C, Addo MM. Recombinant Vesicular Stomatitis Virus Vector Vaccines for WHO Blueprint Priority Pathogens. *Hum Vaccin Immunother*. 2019;1-17
8. WHO | List of Blueprint priority diseases [Internet]. WHO. [Cited Sept 5 2019]. Available from: <http://www.who.int/blueprint/priority-diseases/en/>
9. Scepanovic P, Alanio C, Hammer C, Hodel F, Bergstedt J, Patin E, et al. Human genetic variants and age are the strongest predictors of humoral immune responses to common pathogens and vaccines. *Genome Med*. 2018;10(1):59.
10. Klein SL, Jedlicka A, Pekosz A. The Xs and Y of immune responses to viral vaccines. *Lancet Infect Dis*. 2010;10(5):338–49
11. Klein SL, Marriott I, Fish EN. Sex-based differences in immune function and responses to vaccination. *Trans R Soc Trop Med Hyg*. 2015;109(1):9–15.
12. Fischinger S, Boudreau CM, Butler AL, Streeck H, Alter G. Sex differences in vaccine-induced humoral immunity. *Semin Immunopathol*. 2019;41(2):239-249.
13. Harris VC. The Significance of the Intestinal Microbiome for Vaccinology: From Correlations to Therapeutic Applications. *Drugs*. 2018;78(11):1063-1072.
14. Pasin C, Balelli I, Van Effelterre T, Bockstal V, Solfrosi L, Prague M. Dynamics of the Humoral Immune Response to a Prime-Boost Ebola Vaccine: Quantification and Sources of Variation. *J Virol*. 2019;93(18).

15. Kennedy SB, Bolay F, Kieh M, Grandits G, Badio M, Ballou R. Phase 2 Placebo-Controlled Trial of Two Vaccines to Prevent Ebola in Liberia. *N Engl J Med.* 2017;377(15):1438-1447

V. Methodology of a phase II Ebola vaccine trial

As mentioned in the preamble, I have been involved during my PhD in the Partnership for Research on Ebola VACcination (PREVAC) trial, which is an ongoing randomized phase II prophylactic vaccine clinical trial evaluating the safety and immunogenicity of three different vaccine strategies against Ebola in four West African countries (NCT02876328). As methodologist in the EUCLID/F-CRIN international clinical trial platform (a clinical trials unit hosted by several institutions, among which the University Hospital of Bordeaux, the University of Bordeaux and Inserm), I was involved in the writing of the different versions of the protocol and resolution of the methodological issues that have been encountered over time. I was observer of the Trial Steering Committee, Trial Management team meetings, and weekly coordination meetings with the site staff. I was particularly involved as member of the lab working group and the FANG assay group that have been put in place to review biweekly the analysis of the antibody response measured by FANG assay (primary endpoint of the trial). I also participate the training of the site staff on principles of the vaccine clinical trials before the first inclusions.

This chapter highlights this 3-years' work, with one published paper and another manuscript soon to be submitted: the protocol paper of the PREVAC trial; and a particular methodological reflection on the enrollment of study personnel in Ebola vaccine trial in a non-epidemic context.

1. Protocol of Partnership for Research on Ebola VACcination (PREVAC) trial

Together with a colleague from Liberia (Moses Badio, biostatistician working for the Partnership for Research on Ebola Virus in Liberia (PREVAIL) in Monrovia), I am co-leader on behalf of the entire PREVAC study group for the writing of the scientific article for the publication of the PREVAC protocol and details about the methodological rationale. The manuscript is currently being finalized for a submission planned for the end of 2019. The sociodemographic, laboratory and available antibody data at baseline will also be included in the manuscript prior to submission (not shown here).

Partnership for Research on Ebola VACCination (PREVAC): a randomized, double-blind, placebo-controlled phase 2 clinical trial evaluating three vaccine strategies against Ebola in healthy volunteers in four West African countries

Abstract

Introduction: The Ebola virus disease (EVD) outbreak in 2014-2016 in West Africa was the largest and has revived efforts to develop an effective and safe vaccine. Multiple questions regarding the safety and efficacy of EVD vaccines remain unanswered. To address these gaps in the evidence base, the Partnership for Research on Ebola Vaccines (PREVAC) trial, is being conducted. This paper describes the design, methods, and baseline results of the PREVAC trial.

Methods: The PREVAC trial is a randomized (2:1:2:1:1), double-blind, placebo-controlled phase 2 clinical trial evaluating three vaccine strategies against Ebola virus in healthy volunteers (adults and children 1-17 years) enrolled at 6 sites in four countries (Guinea, Liberia, Sierra Leone, and Mali). The three vaccine strategies being studied are the rVSVΔG-ZEBOV-GP vaccine, with and without a boost at 56 days, and the Ad26/MVA vaccine. The primary objective is to compare each of the three vaccine strategies with the pooled placebo group (3 pair-wise comparisons) for GP-EBOV antibody response at 12 months. After the prime vaccination at study entry, follow up visits occurred at day (D) 7, 14 and 28. The booster vaccine was administered on D56 with further follow-up visits at D 63, 3 months, 6 months and 12 months. Visits will continue annually until 60 months. Three versions of the protocol have been set up successively to deal with constraints related to the implementation of the trial.

Results: From April 2017 to December 2018, 5,002 participants were screened and 4,789 enrolled in all versions of the study protocol. We described here baseline characteristics, laboratory measurement of all participants included and preliminary data on antibody levels on 1035 participants.

Conclusion: PREVAC trial will evaluate the two most promising candidate vaccines in advanced stages of development and will address and evaluate unanswered questions related to safety and immunogenicity and, long-term protection in adults and children under three vaccine strategies.

Trial registration number: NCT02876328

Keywords: Ebola; Vaccine; Clinical Trials; Protocol;

Introduction

The Ebola virus disease (EVD) outbreak in 2014-2016 in West Africa was the largest since the discovery of the virus in 1976 with more than 28,000 confirmed cases of EVD and 11,000 deaths in Guinea, Liberia and Sierra Leone (ref: WHO Ebola situation report 2016). The West-African outbreak prompted the rapid evaluation of vaccine candidates that were in early development. As evidenced by the ongoing Ebola outbreak in the Democratic Republic of Congo (DRC) since August 2018, along with other public health measures, efforts to develop an effective and safe vaccine against Ebola virus disease must continue. (Levy, Lane et al. 2018)

By the end of 2015, the incidence EVD in West Africa had dramatically decreased. At that time, preliminary data from an open-label, cluster randomized ring vaccination trial conducted in Guinea, the “Ebola ca suffit” trial showed that the Merck/New Link rVSVΔG-ZEBOV-GP vaccine prevented EVD events occurring at least 10 days after randomization among close contacts immediately vaccinated compared to those who were potentially vaccinated 21 days later (Henao-Restrepo, Camacho et al. 2017). In that trial, which was conducted in adults, most EVD events occurred shortly following vaccination; no EVD events occurred more than 6 days after vaccination. The durability of vaccine protection was not assessed. Two other vaccines, the GlaxoSmithKline (GSK) ChAd3-EBO Z (replication deficient Chimpanzee adenovirus type 3-derived vector encoding the Ebola virus Zaire [EBO Z] GP) vaccine and the Johnson & Johnson (J&J) 2-dose heterologous vaccination regimen, Ad26/MVA, had completed phase 1 testing. In addition, safety and immunogenicity up to 12 months of the rVSVΔG-ZEBOV-GP and ChAd3-EBO Z vaccine had been evaluated in a phase 2, placebo-controlled trial of healthy adults in Liberia (Partnership for Research on Ebola Virus in Liberia I [PREVAIL I]) (Kennedy et al, NEJM 2017 (Kennedy, Bolay et al. 2017).

However, multiple questions regarding the safety and efficacy of EVD vaccines remain unanswered, including the durability and the immediacy of immune responses generated by different vaccine strategies with and without a booster, and the safety of vaccines, particularly in special populations.

To address these gaps in the evidence base, a phase 2 trial, the Partnership for Research on Ebola Vaccines (PREVAC) trial, is being conducted to compare three vaccine strategies with placebo in adults and children in Guinea, Liberia, Mali and Sierra Leone. The purpose of this paper is to describe the design, methods, and baseline results of the PREVAC trial.

The Partnership for Research on Ebola Vaccinations (PREVAC) was established as an international consortium at the end of the West-African outbreak in 2015 to focus on Ebola research activities to prevent or respond effectively to the next potential Ebola outbreak. The

consortium includes research and academic institutions (the French Institute for Health and Medical Research [Inserm], London School of Hygiene & Tropical Medicine [LSHTM], the US National Institutes of Health [NIH], and the Universities of Bordeaux and Minnesota), health authorities and scientists from four Ebola-affected countries (Guinea, Liberia, Sierra Leone, and Mali), nongovernmental organizations (the Alliance for International Medical Action and Leidos Biomedical Research, Inc) and pharmaceutical companies (MSD, Johnson & Johnson, and Bavarian Nordic).

Methods

The PREVAC trial is a randomized, double-blind, placebo-controlled phase 2 clinical trial evaluating three vaccine strategies against Ebola virus in healthy volunteers enrolled at 6 sites in four countries (Guinea, Liberia, Sierra Leone, and Mali). Members of the PREVAC study group conducting the vaccine trial are listed in appendix.

Study objectives

The primary interest of PREVAC investigators and the two pharmaceutical companies providing the vaccine differed with respect to the timing of efficacy assessments. The primary interest of the investigators was to study the durability and immediacy of the antibody response to vaccination. The objectives of the two companies were aimed at satisfying information requirements for regulatory filings. All parties had a common interest in evaluating the safety of the vaccines, especially in children.

The durability and immediacy objective stated by the PREVAC investigators were:

Separately for adults and children:

- To compare each of the three vaccine strategies with the pooled placebo group (3 pairwise comparisons) for antibody response 12 months after randomization (durability of response). This was the primary objective.
- To compare the groups given the Ad26.ZEBOV prime vaccine and the rVSVΔG-ZEBOV-GP prime vaccine (both rVSVΔG-ZEBOV-GP groups, with and without the boost, combined) each with the placebo group for the antibody response 14 days after randomization (immediacy of response). This was a secondary objective.

The primary efficacy objective formulated by each company is shown below.

In order to facilitate Merck regulatory filings and bridging of immune responses of the rVSVΔG-ZEBOV-GP vaccine from this study to other studies and between pediatric and adult

populations, the following objective will be assessed specifically for those in the two rVSVΔG-ZEBOV-GP vaccine groups:

- To compare the rVSVΔG-ZEBOV-GP vaccine (pooled rVSVΔG-ZEBOV-GP groups) with the matched placebo group for antibody response 28 days after randomization (prime vaccination).

In order to facilitate Janssen regulatory filings of the Ad26/MVA vaccine, the following objective will be assessed specifically for those in the Ad26/MVA vaccine group:

- To compare the Ad26/MVA vaccine group with the matched placebo group for antibody response 3 months after randomization (approximately 28 days after the second dose vaccination).

Other objectives stated in the protocol are included appendix.

Study Design

Beginning in early 2017, eligible participants were to be randomized to one of the following five groups in a 2:1:2:1:1 allocation 1) Ad26.ZEBOV (prime vaccination at day 0) (0.5 mL) followed by a second dose with MVA-BN-Filo (0.5 mL) at 56 days; 2) placebo (at randomization and at 56 days) (0.5 mL); 3) rVSVΔG-ZEBOV-GP (prime at day 0) (1 mL) followed by placebo boost (1 mL) at 56 days; 4) rVSVΔG-ZEBOV-GP (prime at day 0) (1 mL) followed by rVSVΔGZEBOV-GP boost (1 mL) at 56 days; and 5) placebo (prime at day 0 and boost at 56 days) (1 mL) (Figure 1). The study design includes two placebo arms, because the Ad26.ZEBOV and rVSVΔG-ZEBOV-GP vaccines were administered at different volumes. For the primary analyses, the two placebo groups will be pooled.

A week prior to the commencement of participants enrollment for version 1.0 of the PREVAC trial protocol, an ongoing open-label study of healthcare workers (the PREPARE study; ClinicalTrials.gov Identifier: NCT02788227) evaluating the immunogenicity of the rVSVΔG-ZEBOV-GP vaccine conducted in the United States of America at NIH was suspended because of reported arthritis in 3 out of the 9 participants who had received the rVSVΔG-ZEBOV-GP vaccine from the same vaccine lot that was to be used in the PREVAC trial. This rate of arthritis was substantially higher than observed in previous studies conducted with the rVSVΔG-ZEBOV-GP vaccine in sub-Saharan Africa and especially in the “Ebola ca suffit” and Prevail I trials (Henao-Restrepo, Camacho et al. 2017). Due to sites readiness in both Guinea and Liberia, the protocol was revised to version 2.0 to exclude the rVSVΔG-ZEBOV-GP and matching placebo arm. The PREVAC trial commenced with a two-arm strategy and

randomized participants' ≥ 12 years of age to the Ad26.ZEBOV first dose (0.5 mL) followed by an MVA-BN-Filo second dose (0.5 mL) at 56 days or to matching placebo. Version 2.0 aimed to enroll up to 600 participants to allow migration quickly to the original five arm randomization scheme once the rVSV Δ G-ZEBOV-GP vaccine issue had been resolved.

After review of other safety data and information about the lot of the rVSV Δ G-ZEBOV-GP vaccine to be used, PREVAC investigators requested that a new lot of the rVSV Δ G-ZEBOV-GP vaccine be produced. That was done and provided to the sites in Guinea and Liberia in May 2017. However, on review of the certificate of analysis of this new lot of the rVSV Δ G-ZEBOV-GP vaccine it was found that the potency (9.4×10^7 pfu.mL) was higher than that used in the "Ebola ca suffit" and the PREVAIL I trial (2×10^7 pfu.mL). A limited number of children had been enrolled in previous studies of the rVSV Δ G-ZEBOV-GP vaccine, none in PREVAIL I. Therefore after discussion, it was decided to use a measured approach and give the rVSV Δ G-ZEBOV-GP vaccine at a 2-fold dilution (approximately 5×10^7 pfu.mL) which we refer to as the diluted dose. In June 2017, the protocol was revised to version 3.0 with the planned 2:1:2:1:1 randomization to 5 groups and with the rVSV Δ G-ZEBOV-GP vaccine groups being given the diluted dose. In order to protect the children, enrollment was staggered by age group, starting with children aged 12-17 and adults. After 70 children aged 12-17 were enrolled, safety data through Day 28 were reviewed by an independent Data and Safety Monitoring Board (DSMB), which identified no safety concerns and recommended opening enrollment to children aged 5-11 years. The procedure was repeated for the younger children; in each age group, a DSMB review was conducted after 70 children were enrolled and followed during 28 days, and before enrollment was opened to the next younger age group.

Variation in the potency in live virus vaccines is common. Vaccine manufacture and release for potency is based upon defined specifications and always encompasses a range with an upper and lower limit. The lower limit is determined during development and is defined by the lowest dose for which there is demonstrated efficacy. The lower limit for potency must still be valid at the end of shelf-life in order to ensure that the vaccine is still efficacious up until its defined expiry. Considering this variation in potency that would be expected when future lots of rVSV Δ G-ZEBOV-GP were produced and the logistical issues associated with dilution of the vaccine during an outbreak, it was decided that once the safety of the diluted dose of rVSV Δ G-ZEBOV-GP had been established in children, the trial would be amended to use the undiluted rVSV Δ G-ZEBOV-GP vaccine.

In April 2018, after the safety of all three vaccine strategies, including the diluted dose of rVSVΔG-ZEBOV-GP, had been determined in each of three age groups of children (1-4, 5-11, and 12-17 years) by an independent Data and Safety Monitoring Board (DSMB), the PREVAC protocol was again amended. This version of PREVAC (version 4.0) follows the design originally planned (version 1.0) and of version 3.0 except the undiluted dose of the rVSVΔG-ZEBOV-GP vaccine (9.4×10^7 pfu/mL) was to be used. The enrollment targets in version 4.0 were 1,400 children aged 1-17 years and 1,400 adults.

Study agent

The three vaccine strategies being studied are the rVSVΔG-ZEBOV-GP vaccine, with and without a boost at 56 days, and the Ad26/MVA vaccine. One (1) mL from a 3 mL syringe of the rVSVΔG-ZEBOV-GP vaccine was administered for the prime and booster vaccination. The Ad26/MVA requires a 0.5 mL administration of Ad26.ZEBOV from a 3 mL syringe for the first dose vaccination and second dose vaccination of MVA-BN-Filo (0.5 mL from a 3 mL syringe) at 56 days.

The 2-dose heterologous vaccination regimen Ad26/MVA is comprised of an Ad26.ZEBOV vaccine which consists of a single recombinant, replication incompetent human Ad26 vector, constructed to express the Ebola virus Mayinga GP. The second dose with MVA-BN-Filo at 56 days encodes the GP of Sudan virus (SUDV; formerly known as Ebola Virus Sudan), EBOV (formerly known as Ebola Virus Zaire), and Marburg Virus (MARV) Musoke, and the nucleoprotein of Tai Forest virus (TAFV; formerly known as Côte d'Ivoire ebolavirus) (0.5 mL intramuscular [IM] administration for the Ad26.ZEBOV and MVA-BM-Filo vaccines). The Ad26/MVA vaccination regimen was given at the same dose in Versions 2.0, 3.0, and 4.0 of the PREVAC protocol.

The rVSVΔG-ZEBOV-GP vaccine is comprised of a single rVSV isolate (11481 nt) modified to replace the gene encoding the VSV G envelope GP with the gene encoding the envelope GP from ZEBOV (Kikwit, 1995 strain) (1 mL IM administration). The rVSVΔG-ZEBOV-GP vaccine was not used in Version 2.0. The rVSVΔG-ZEBOV-GP dose was given as a 2-fold diluted dose (approximately 5×10^7 plaque-forming units [pfu]/mL) in Version 3.0 and was given as an undiluted dose (geometric mean of available assays 9.4×10^7 pfu/mL) in Version 4.0 of the PREVAC protocol. The doses of rVSVΔG-ZEBOV-GP used in Versions 3.0 and 4.0 are referred to as the diluted and undiluted doses, respectively.

The placebo is sterile normal saline (sodium chloride 0.9 percent for injection, United States Pharmacopeia, preservative free).

Study location

Both version 2.0 and 3.0 of the study protocol were implemented in Guinea at two sites (Landreah located in an urban area in Conakry and Maferinyah, a rural area in Forecariah region) and one site in Liberia (Redemption Hospital in Monrovia). Protocol version 4.0 was implemented in these three sites and also in two sites in Mali (Center for Vaccine Development (CVD) and the University Clinical Research Center (UCRC), in the capital Bamako) and one site in Sierra Leone (Mambolo), a rural community in Kambia.

Study Endpoints

- GP-EBOV antibody response 12 months after randomization. This endpoint will be used to compare the immunogenicity of the three vaccine strategies with placebo.
- For rVSVΔGZEBOV-GP arms only, GP-EBOV antibody response at Day 28 after vaccination will be used for regulatory purposes for comparison to other studies and for bridging children to adults.
- For the Ad26/MVA vaccine arm only, GP-EBOV antibody response at Month 3 after randomization (approximately 28 days after the second dose of vaccine) will be used for regulatory purposes.

The primary analysis will be performed separately, for adults and children, and will exclude participants with elevated antibody levels at baseline.

Antibodies to the Ebola virus GP will be measured with the Filovirus Animal Nonclinical Group (FANG) ELISA assay (Logue et al, J Virol Methods 2018). Other assays may also be used. If a correlate of protection is identified, stored sera will be used to measure the correlate and carry out comparisons of the three vaccine strategies with placebo and with one another. The precise definition of antibody responders will be defined by the Trial Steering Committee (TSC) prior to the unblinding of the study results.

An elevated baseline antibody level will be defined using the distribution of antibody levels measured from sera collected in 2004 and 2001 from 92 adults in Mali, a region where there had been no reports of ZEBOV transmission or disease during this time period. Baseline antibody levels were considered elevated in PREVAIL I if they were greater than 3 standard deviations (SD) above the mean (607 enzyme-linked immunosorbent assay units/mL based on the FANG ELISA assay). This threshold was previously used in PREVAIL 1 trial (Kennedy and al, NEJM 2018).

Study inclusion and exclusion criteria

Inclusion into the study was based on the following criteria: 1) Willingness to participate and signed informed consent/assent, 2) Age \geq 1 year, 3) Planned residency in the area of the study site for the next 12 months, and 4) Willingness to comply with the protocol requirements.

Participants were excluded from enrolment based on the following: 1) Fever $>$ 38°C, 2) History of EVD (self-report), 3) Pregnancy (a negative urine pregnancy test was required for females of child-bearing potential), 4) Positive HIV test for participants $<$ 18 years of age, 5) Reported current breast-feeding, 6) Prior vaccination against Ebola, 7) Any vaccination in the past 28 days or planned within the 28 days after randomization, 8) In the judgement of the clinician, any clinically significant acute/chronic condition that would limit the ability of the participant to meet the requirements of the study protocol.

Randomization and blinding

Participants were randomized in a 2:1:2:1:1 allocation to the 5 study arms shown in Figure 1. For each vaccination center, the randomization schedule was prepared centrally, using block randomization to ensure the desired allocation ratio for the five arms of the study for each vaccination center.

To ensure blinding, syringes were prepared at the local study pharmacies according to a centrally prepared list, and labelled with a unique Syringe IDentifier (SID) and a bar code identifier tear-off label. The staff administering the vaccine could see whether the syringe contained 0.5 mL or 1 mL, but did not know whether the syringe contained active vaccine or placebo. At the time of vaccination, the tear-off label on the syringe with the SID was attached to the baseline Case Report Form (CRF) creating the primary link between the vaccine administered and the Participant IDentification (PID). Thus, randomization occurred at the time of vaccination, by randomly assigning pre-filled syringes to participants. Study participants and clinical staff assessing the study participants for safety and laboratory outcomes remained fully blinded until all participants from the three protocol versions complete 12 months of follow-up; the syringes for the booster vaccination at day 56 were prepared at the local pharmacies and labelled with the PID, to maintain blinding of the clinical staff. The laboratories carrying out the safety and immunogenicity analyses were blinded to the vaccine assignment.

Baseline and follow up data collection plan

The baseline visit was conducted following informed consent and eligibility assessment. Demographics and a short medical history were obtained, blood was drawn as specified by the protocol, and participants received the first dose of the vaccine (“prime vaccination”). Randomization occurred at the point of vaccination as described above. For 30 minutes after

the vaccination, participants were watched closely, injection site reactions and targeted symptoms were assessed, and possible grade 3 or 4 adverse events were recorded.

After the prime vaccination at study entry, follow up visits occurred at: 7 (\pm 3 days), 14 (\pm 3 days), and 28 (\pm 7 days) days. The booster vaccine was administered on day 56 (53 to 66 days), with further follow-up visits at 63 days (7 \pm 3 days after the booster vaccination), at 3 months (\pm 14 days), 6 months (\pm 1 month), and 12 months (\pm 1 month). Visits will continue at 24 (\pm 1 month), 36 (\pm 1 month), 48 (\pm 1 month) and 60 (\pm 1 month) months. At each follow-up visit up to Month 3, injection site reactions, targeted symptoms, and grade 3 or 4 adverse events that occurred since the previous visit are reported. At all follow-up visits, malaria and serious adverse events (SAEs) are reported, the temperature is recorded, and blood is drawn and stored for future immunogenicity assessments and other research.

For children, additional data was collected. Blood chemistries were assessed prior to the prime and booster vaccination at baseline and day 56, respectively, and 7 days after each vaccination. Also, during the first week following the prime and booster vaccination, there were scheduled daily contacts with children to assess injection site reactions, targeted symptoms and serious adverse events (SAEs), and body temperature.

Blood samples will be collected on each site and will be processed according to their final use: on-site analysis and aliquoting for further analysis. Local lab on each site will analyze blood samples to evaluate toxicity in response to vaccination at day 0 for adults and day 0, 7, and 63 for children and adolescents for liver (ALAT, ASAT), kidney (Creatinine), metabolic potassium and complete blood cell counts. Laboratory values results will be graded for severity according to the Division of AIDS (DAIDS) Adverse Event Grading tables. Serology for HIV and Syphilis will also be performed at day 0 using rapid tests. All countries will use their own normal ranges as a reference. For Guinea it has been decided to use the Ghana value from Dosoo et al (Dosoo DK, PlosOne 2012 ; Dosoo DK, et al. Am J Trop Med Hyg 2014), for Liberia those used in PREVAIL 1 (Kennedy et al, NEJM 2017), for Sierra Leone those used for Ebovac1 (NCT02509494), and for Mali the one from Khone et al (Khone B et al, J Blood Lymph 2017).

FANG assay for the measure of the antibody response will be performed at the Liberian Institute for biomedical Research (LIBR) lab in Monrovia for participants from Liberia, Guinea and Sierra Leone and at UCRC for participants from Mali. Aliquots will be shipped from sites to the lab at regular intervals (every 3 months). Quality control for intra- and inter-lab reproducibility will be performed on a regular basis. Additional antibody response testing will

be performed for specific time-points (day 0, day 28, month 3 and month 12) at Quest diagnostics Clinical Laboratories Inc., San Juan Capistrano, US (FOCUS), for validated GP-ELISA assay required for the analysis of Merk and JnJ.

All the lab test results will be transfer on a daily basis to the centralized data center (EUCLID/FCRIN Clinical Trials Platform, Inserm/Univ Bordeaux, France).

Sample size considerations and statistical analysis

Sample size was calculated to provide power to compare safety and immunogenicity separately for adults (N=1,400) and children (N=1,400). Sample size is greater than what is required to address the primary objectives because if a correlate of protection is identified, the vaccine strategies will be compared with one another for that correlate using an intention to treat analysis. Expected differences between vaccine groups may be smaller than comparisons with placebo and the correlate may have greater variability than the assay which will be used to measure antibody levels to address the primary, secondary and exploratory objectives. The larger sample size will also permit the exploration of subgroups and preserve power in the event there are more participants with elevated antibodies at baseline than anticipated.

For the planned antibody comparisons of each vaccine strategy versus placebo at 12 months, the planned sample is based on data from PREVAIL I (Kennedy et al, 2017).

With type 1 error = 0.0167 (2-sided) to adjust for the three comparisons, separately for adults and for children in all age groups combined, and power = 0.90, even if the percent with a positive antibody response at 12 months is 50% in a vaccine group, with equal allocation, approximately 30 participants per group (60 participants total) are needed assuming the percent in the placebo group with a positive antibody response is approximately 7%. With unequal allocation as for the rVSVΔG-ZEBOV-GP with the booster versus placebo comparison, a total of 63 participants (21 vaccinated with rVSVΔG-ZEBOV-GP with a boost and 42 vaccinated with placebo), a difference of 50% versus 7% can also be detected at 12 months with 87% power. These sample size estimates indicate that power for the planned subgroup analysis by age is also appropriate.

The planned sample size is also adequate for the comparisons with placebo if more than 4% of participants are antibody positive at baseline and excluded from the primary analysis, and if there is some missing data 12 months.

According to the data analysis plan, to address the primary efficacy objectives concerning immunogenicity, data from version 4.0 of PREVAC will be used. Analyses will be carried out

separately for adults and children. Antibody response for each of the three treatment groups to pooled placebo at 12 months will be assessed.

Sub studies

There are currently two (2) substudies ongoing in PREVAC. The Immunological substudy is being conducted in Guinea with the primary objective of evaluating the T cell responses induced by the 2 vaccine candidates (rVSV Δ G-ZEBOV-GP and Ad26/MVA) in adult population and on their persistence until 1 year after vaccination and more (middle/long term). The second substudy is the viral shedding of the rVSV Δ G-ZEBOV-GP vaccine that is being conducted in Liberia. The objective of the viral shedding substudy is to estimate the proportion of children who shed vaccine virus and to quantify the rVSV Δ G-ZEBOV-GP vaccine shed in children (participants aged < 18 years) after the prime and boost vaccinations.

Trial governance

The trial governance is detailed in Appendix Figure 1. The trial is being conducted under the direction of a Trial Steering Committee (TSC), which provides overall supervision for the trial on behalf of the three sponsors (Inserm, LSHTM, NIH). Members of the TSC (Appendix 3) are blinded to interim safety and immunogenicity results.

The DSMB provides independent, expert oversight for the trial. The primary rationale for establishing this DSMB is to make certain that appropriate safeguards are in place to ensure the safety of study participants and to ensure that the study is conducted with scientific rigor. The DSMB will especially closely monitor accumulating safety and immunogenicity data for adults and children in each age group (1-4, 5-11, and 12-17 years) using reports provided by an unblinded statistician from the University of Minnesota (Division of Biostatistics, Minneapolis, USA).

The day-to-day operations and management of the trial is done by the Trial Management Team (TMT). A daily close monitoring of inclusions and retention rates will be carried out in the central database and blind reports will be made available to the TMT via a secure website by a blinded statistician from EUCLID/FCRIN Clinical Trials Platform (Inserm/Univ Bordeaux, France). In coordination with the sites, the close monitoring will allow to achieve especially the same number of children in each age group and a gender balance for children and adults.

Pharmacovigilance

A centralized pharmacovigilance service is being implemented as it relates to the trial Safety management. AEs occurring in participants enrolled were report by investigators as soon as

they became aware of it. All serious adverse events (SAEs) were reported to the sponsors immediately and no later than 24 hours. The medical officer (MO) responsible of pharmacovigilance first performed a partial unblinding of a potential suspected unexpected serious adverse reaction (SUSAR) to assess whether the SAR is unexpected for either product. If the event was unexpected for the product arm assigned, the MO requested to be fully unblinded to determine whether the participant received active vaccine or placebo. This full unblinding is also performed via the web-based unblinding application monitored by the University of Minnesota. Reporting SUSAR or new safety data that could constitute a new fact to competent authorities were performed, especially the DSMB. The DSMB could recommend to the sponsors that vaccines are postponed or discontinued in an individual participant or in all participants.

Ethical and regulatory aspects

During the enrollment process, there was widespread communication including community engagement about the trial and the location of nearby vaccination centers. Potential participants in a detailed information session prior to signing of an informed consent form if they agreed to enroll. A picture booklets describing the study was used to ensure that illiterate volunteers and minors understood the study requirements and risks and benefits in addition to the use of impartial witness. In Liberia and Sierra Leone, minors aged 7-17 years signed an informed assent after their parents/guardian provided consent for their participation in the study. Minors who declined participation in the study after reviewing the assent materials were not enrolled even if their parent(s) or legal guardian consented to their participation.

The study protocol, the informed consent and assent forms, including participants' information materials were approved by ethics committees of the sponsors (INSERM IRB 00003888, LSHTM) and the implementing countries (Guinea, Liberia, Mali, and Sierra Leone) before each version of the protocol was implemented. NIH established an institutional authorization agreement with INSERM to rely upon the INSERM ethics committee. The study is registered at www.ClinicalTrials.gov (NCT02876328).

Discussion

The successful setting up and conduct of this multicenter randomized Ebola vaccines trial involving six (6) sites within four (4) West African Countries, three (3) sponsors and two (2) pharmaceutical companies is a milestone in collaborative Ebola research. PREVAC trial is to date the largest Ebola vaccine trial conducted in a non-epidemic context. It will evaluate the

two most promising candidate vaccines in advanced stages of development and will address and evaluate questions related to safety and immunogenicity and, long-term protection in adults and children under three vaccine strategies.

Prior to the design and conduct of PREVAC, most Ebola vaccine phase II trial did not involve children. (Agnandji, Fernandes et al. 2017) The involvement of children aged 1-17 in PREVAC is key in evaluating the safety profile of the different candidate vaccines in children. Considering the percentage (16%) of children who were infected with EVD during the 2014-2015 epidemic in West Africa, for which the index case was probably a 2 year-old child, and the current ongoing epidemic in DRC, this is a big step towards decision making for vaccinating children.

Whether the different vaccine approaches are able to confer longer-term protection after vaccination also remains an important question and currently only few data exist on it. PREVAC trial will evaluate the durability of immune responses with a long-term follow up of the participants up to 60 months after vaccination. These data will provide important information when considering a preventive vaccination strategy for at-risk populations, and specifically for health-care and front-line workers.

The use of placebo in a vaccine clinical trial may be difficult to implement in particularly in countries where the population have suffered from the 2014 – 2016 outbreak. However, its use remains ethical in the current non-epidemic context in the four countries of the trial. Placebo controls will be an important strength of this trial for the evaluation of safety outcomes. In the event of a new Ebola epidemic in these countries during the trial, the study design, including the use of placebos, will be re-considered. Up-to-date scientific knowledge and current recommendation by local ministries of health and WHO will be taken into consideration at the time of the redesign. In addition, if a vaccine is licensed, the labelling of vaccines with marketing authorization will be evaluated to determine whether the use of a placebo group should be maintained in the design. The inclusion of the two most promising vaccines was also a strength of the trial. The opportunity to collaborate with the respective companies producing the vaccine and the use of a same pooled placebo group were efficient given the urgency of the public health need.

There were efforts to achieve equal demographic distribution among participants enrolling. An enrollment monitoring team was established to ensure that there is diversity in the ages of children enrolling especially during enrollment into version 4.0 of the trial. This system allows

a good representation of each age cohort in the trial as done in PREVAIL I.(Kennedy, Bolay et al. 2017)

Prior to the conduct of PREVAC, only half the sites were existing and had prior experience on mobilization and community awareness programs for enrollment and retention. Community awareness of randomized Ebola vaccine trials is pivotal to recruitment and retention of eligible participants.(Enria et al, BMC Public Health 2016 ; Laverack and al, Blob Health Promot 2016) While establishing clinical trial capacity in new sites and upgrading existing sites, community awareness was an integral component that led to achieving the enrollment of adults and children sampled in a short period of time. At most sites, the number of eligible volunteers who were willing to enroll were more than the targeted number of participants to be enrolled daily. Establishing a system for that capture information on how sites implement a trial and expand community and participants' awareness are important to consider for future studies. The implementation of a clinical trial such as PREVAC will also strengthen and maintain capacity in clinical research for the personnel involved on the sites, and beyond will strengthen the capacities of involved African institutions to host and conduct training in biological, clinical and population health research and practices.

In conclusion, the results of the PREVAC trial observed will extended our knowledge of the safety and long-term immunogenicity of the two current most promising prophylactic vaccine against Ebola.

Declarations

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Acknowledgements

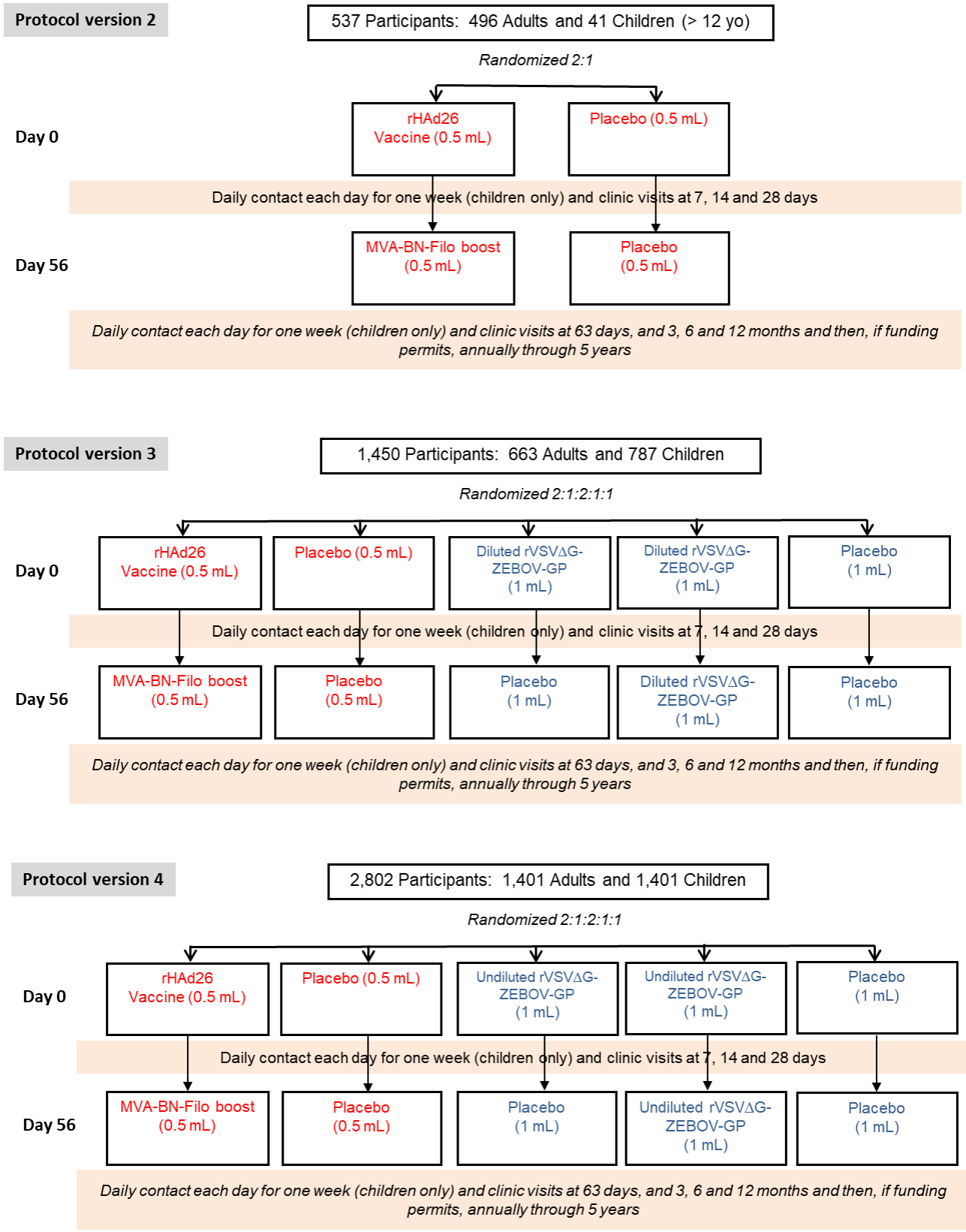
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References

- Agnandji ST, Fernandes JF, Bache EB, Obiang Mba RM, Brosnahan JS, Kabwende L, et al. Safety and immunogenicity of rVSV Δ G-ZEBOV-GP Ebola vaccine in adults and children in Lambaréné, Gabon: A phase I randomised trial. *PLoS Med.* 2017;14(10):e1002402.
- Henao-Restrepo AM, Camacho A, Longini IM, Watson CH, Edmunds WJ, Egger M. Efficacy and effectiveness of an rVSV-vectored vaccine in preventing Ebola virus disease: final results from the Guinea ring vaccination, open-label, cluster-randomised trial (Ebola Ça Suffit!). *Lancet.* 2017;389(10068):505-518.
- Kennedy SB, Bolay F, Kieh M, Grandits G, Badio M, Ballou R, et al. Phase 2 Placebo-Controlled Trial of Two Vaccines to Prevent Ebola in Liberia. *N Engl J Med.* 2017 377(15): 1438-1447.
- Lévy Y, Lane C, Piot P, Beavogui AH, Kieh M, Leigh, et al. Prevention of Ebola virus disease through vaccination: where we are in 2018. *Lancet.* 2018;392(10149):787-790.
- Logue J, Tuznik K, Follmann D, Grandits G, Marchand J, Reilly C, et al. Use of the Filovirus Animal Non-Clinical Group (FANG) Ebola virus immuno-assay requires fewer study participants to power a study than the Alpha Diagnostic International assay. *J Virol Methods.* 2018;255:84-90.
- Dosoo DK, Kayan K, Adu-Gyasi D, Kwara E, Ocran J, Osei-Kwakye K, et al. Haematological and biochemical reference values for healthy adults in the middle belt of Ghana. *PLoS One.* 2012;7(4):e36308.
- Dosoo DK, Asante KP, Kayan K, Adu-Gyasi D, Osei-Kwakye K, Mahama E, et al. Biochemical and hematologic parameters for children in the middle belt of Ghana. *Am J Trop Med Hyg.* 2014;90(4):767-73.
- Kone B, Maiga M, Baya B, Sarro Y, Coulibaly N, Kone A, et al. Establishing Reference Ranges of Hematological Parameters from Malian Healthy Adults. *J Blood Lymph.* 2017;7(1).
- Enria L, Lees S, Smout E, Mooney T, Tengbeh AF, Leigh B, et al. Power, fairness and trust: understanding and engaging with vaccine trial participants and communities in the setting up the EBOVAC-Salone vaccine trial in Sierra Leone. *BMC Public Health.* 2016;16(1):1140. 10.
- Laverack G, Manoncourt E. Key experiences of community engagement and social mobilization in the Ebola response. *Glob Health Promot.* 2016;23(1):79–82

Figure 1. PREVAC study design of each version of the protocol



APPENDIX

PREVAC study team

1- Trial Steering Committee (TSC)

Voting Members: Abdoul Habib Beavogui, Geneviève Chêne, Seydou Doumbia, Hélène Espérou, Brian Greenwood, Stephen Kennedy, Cliff Lane, Bailah Leigh, Yves Lévy, James Neaton, Jerome Pierson, Peter Piot, Samba Sow, Deborah Watson-Jones, Yazdan Yazdanpanah (Chair).

Non-Voting Members: Augustin Augier, Beth-Ann Coller, Sandrine Couffin-Cardiergues, Alpha Diallo, Christine Lacabaratz, Maarten Leyssen.

Permanent Observers: Boni Ale, Moses Badio, Eric Barte de Saint Fare, Cécilia Champion, Siew Pin Chai, Laurie Connor, Mahamadou Diakite, Eric D'Ortenzio, Moussa Moïse Doumbia, Suzanne Fleck, Birgit Grund, Oumar Guindo, David Ishola, Mark Kieh, Daniela Manno, Kim Offergeld, Cynthia Osborne, Sushma Patel, Stephany Pong, Laura Richert, Cynthia Robinson, Céline Roy, Christine Schwimmer, Jakub Simon, Mili Tapia, Renaud Vatrinet, Deborah Wentworth, Jimmy Whitworth, Aurelie Wiedemann.

2- Independent Data and Safety Monitoring Board (DSMB)

Lisa Cooper (chair), Salim Abdulla, David DeMets, Albert Faye, Scott Hammer, Amadou Traoré, Ann Sarah Walker

3- PREVAC Study Team (TSC, TMT and WG members and participants)

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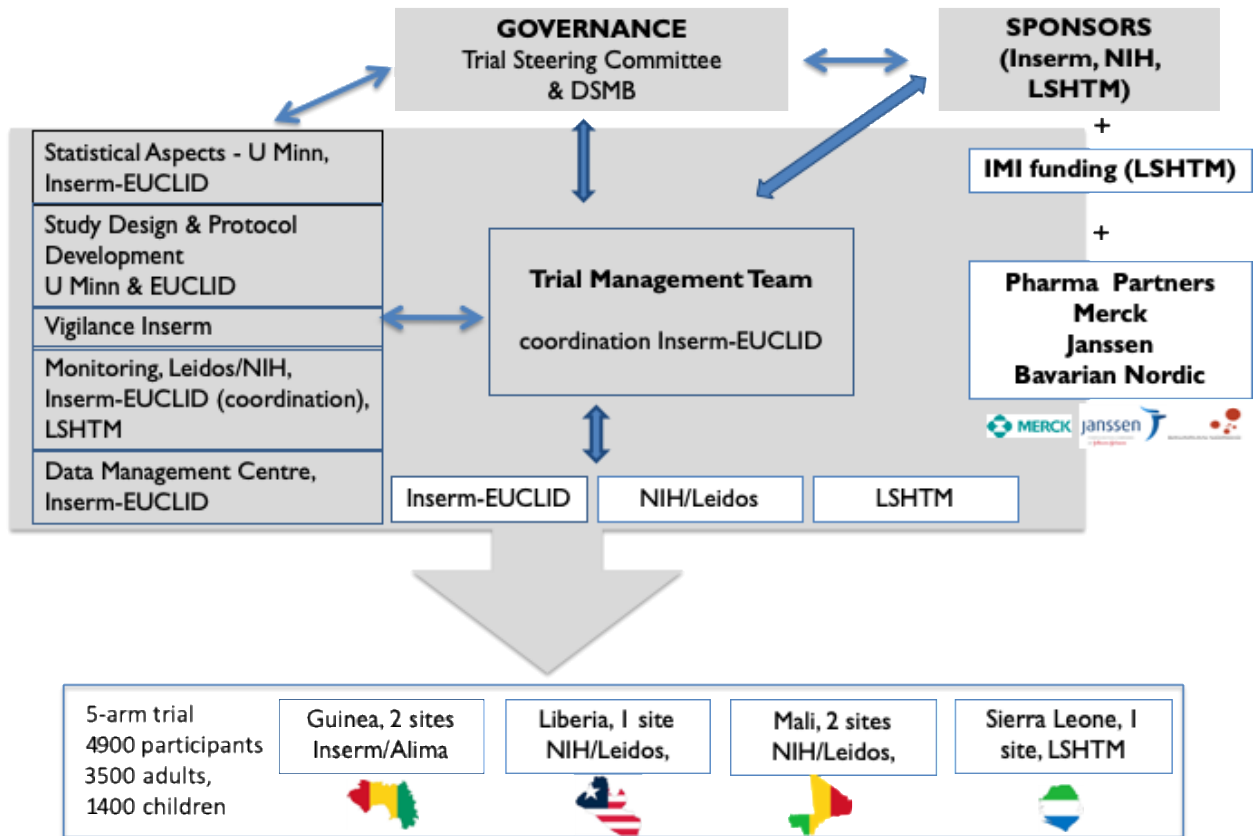
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Secondary objectives

The following secondary objectives will be assessed. Unless otherwise stated, the objectives will be addressed separately for adults and children:

- To compare the groups given the Ad26.ZEBOV prime vaccine and the rVSVΔG-ZEBOV-GP prime vaccine (both rVSVΔG-ZEBOV-GP groups combined) each with the pooled placebo group for the antibody response 14 days after randomization (prime vaccination) (immediacy of response).
- To compare each of the vaccine groups versus the pooled placebo group for the antibody response profile using measurements at 7, 14, 28, 56, 63 days and at 3, 6 and 12 months after randomization.
- To compare each of the vaccine groups with the pooled placebo group for SAEs at 12 months
- To compare the groups given the Ad26.ZEBOV prime vaccine and the rVSVΔG-ZEBOV-GP prime vaccine (both rVSVΔG-ZEBOV-GP groups combined) each with the pooled placebo group for the percent with injection site reactions and AEs graded for severity, including targeted symptoms, during the first week following randomization (including the daily contacts for children only).
- To compare the groups given the Ad26.ZEBOV prime vaccine and the rVSVΔG-ZEBOV-GP prime vaccine (both rVSVΔG-ZEBOV-GP groups combined) each with the pooled placebo group for percent reporting injection site reactions and AEs graded for severity, including targeted symptoms, following prime vaccination at the vaccination visit, and through 7, 14, and 28 days after the prime vaccination.
- To compare each of the vaccine groups with the pooled placebo group for changes from baseline in biochemical markers and complete blood count (CBC) measurements at 7 days after randomization (children only).
- To compare the rHAd26/MVA and rVSVΔG-ZEBOV-GP boost strategies with the pooled placebo group for changes from baseline in biochemical markers and CBC measurements at 63 days after randomization (children only).
- To compare the rHAd26/MVA and rVSVΔG-ZEBOV-GP boost strategies with the pooled placebo for percent with injection site reactions and AEs graded for severity, including targeted symptoms, immediately following the booster vaccination and through month 3 (approximately 35 days after the booster vaccination).
- To compare the three vaccine strategies versus the pooled placebo group for long-term antibody response at 24, 36, 48 and 60 months following randomization.
- To compare the long-term safety at month 24, 36, 48 and 60 following the three vaccine strategies with the pooled placebo group.
- To compare antibody responses and safety outcomes of each of the vaccination strategies versus the pooled placebo group in subgroups defined by age, gender, country, whether the volunteer is a close contact of an Ebola case, the presence of laboratory abnormalities at baseline, and has specific co-morbidities (in particular HIV and nutritional status as measured by body mass index).
- For adults and children combined, to compare antibody responses and safety outcomes for each of the vaccination strategies versus placebo.

- To carry out operational research which will include ethnographic, participatory and/or qualitative (i.e., focus groups and individual interviews) studies to: 1) identify issues relevant to understanding and acceptability of the trial, the social issues surrounding informed consent, with the primary goal of informing efforts to ensure autonomous fully informed individual consent and assent for minors; 2) describe participants' and caregivers' experience in the trial, and identify barriers and develop solutions to support trial adherence in a culturally sensitive and ethically appropriate way; and 3) understand prevailing representations and affects surrounding the epidemic (including rumors), Ebola and other vaccines, the trial and other relevant phenomena in order to ensure effective communication around the trial.
- In a subsample of adults in Guinea, T cell and memory B cell responses for the three vaccine strategies versus placebo will be compared (see Appendix D).
- In a subsample of children, to compare the rVSV Δ G-ZEBOV-GP vaccine strategies with the pooled placebo group for shedding of rVSV-ZEBOV-GP RNA (see Appendix E).



Appendix. Figure 1. Organization of the operations of the PREVAC trial

2. Enrolling study personnel in Ebola vaccine trials: from guidelines to practice in a non-epidemic context


Setting up a large international vaccine trial as PREVAC can raised specific methodological challenges, especially in the West-African context a few months after the end of the largest Ebola outbreak. This was the case, for example, with the question of the enrollment of study personnel in the vaccine clinical trial, which could have been done in clinical trials conducted during the epidemic period. To respond to this question and to define an appropriate course of action for the PREVAC trial, a methodological and ethical reflection has been conducted. This work has been published as a commentary in *Trials*.

COMMENTARY

Open Access

Enrolling study personnel in Ebola vaccine trials: from guidelines to practice in a non-epidemic context



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Abstract

Background: Enrolling participants in clinical trials can be challenging, especially with respect to prophylactic vaccine trials. The vaccination of study personnel in Ebola vaccine trials during the 2014–2016 epidemic played a crucial role in inspiring trust and facilitating volunteer enrollment. We evaluated the ethical and methodological considerations as they applied to an ongoing phase 2 randomized prophylactic Ebola vaccine trial that enrolled healthy volunteers in Guinea, Liberia, Sierra Leone, and Mali in a non-epidemic context.

Methods: On the assumption that the personnel on site involved in executing the protocol, as well as community mobilizers (not involved in the on-site procedures), might also volunteer to enter the trial, we considered both ethical and methodological considerations to set clear rules that can be shared a priori with these persons. We reviewed the scientific and gray literature to identify relevant references and then conducted an analysis of the ethical and methodological considerations.

Results: There are currently no regulations preventing a clinical investigator or site staff from participating in a trial. However, the enrollment of personnel raises the risk of undue influence and challenges the basic ethical principle of voluntary participation. The confidentiality of personal medical information, such as HIV test results, may also be difficult to ensure among personnel. There is a risk of disruption of trial operations due to the potential absence of the personnel for their commitment as trial participants, and there is also a potential for introducing differential behavior of on-site staff as they obtain access to accumulating information during the trial (e.g., the incidence of adverse events). Blinding could be jeopardized, given knowledge of product-specific adverse event profiles and the proximity to unblinded site staff. These aspects were considered more relevant for on-site staff than for community mobilizers, who have limited contact with site staff.

Conclusion: In a non-epidemic context, ethical and methodological considerations limit the collective benefit of enrolling site staff in a vaccine trial. These considerations do not apply to community mobilizers, whose potential enrollment should be considered as long as they meet the inclusion criteria and they are not exposed to any form of coercion.

Keywords: Clinical trials, Low- and middle-income countries, Ebola vaccine, Ethics, Trial participants, Research participants, Health workers, Site staff, Employees

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Introduction

The 2014–2016 Ebola virus disease outbreak in Guinea, Liberia, and Sierra Leone included more than 28,000 cases and more than 11,000 deaths [1]. The scale of the outbreak was unprecedented and prompted the development of prophylactic vaccine candidates [2–4]. A number of clinical vaccine trials were conducted in the affected countries during the epidemic [4–7]. Although these studies made many advances during the epidemic, further research is required to prevent and limit the impact of further outbreaks, such as the current outbreak in the Democratic Republic of the Congo [8]. Therefore, Ebola vaccine clinical trials continue to be conducted in a non-epidemic context.

Recruiting and enrolling participants in any clinical trial can be challenging, but this is especially true in the case of prophylactic vaccine trials; participants are volunteers from the community and do not have the specific disease, but rather are at hypothetical risk thereof. The Ebola epidemic context bred a well-documented climate of fear and mistrust [9, 10]. Therefore, setting up vaccine trials during the epidemic required the development of unique, highly sensitive communication (community engagement) strategies, specific to the socio-cultural context, to engage local populations in interventional research and thus facilitate study enrollment. One of the strategies used to inspire trust and combat rumors was the enrollment of “study staff,” i.e., local healthcare professionals working at the study site, as exemplified by the PREVAIL 1 trial in Liberia [5]. Nevertheless, in a non-epidemic context where there is no indication for emergency vaccination with vaccine candidates still being under development, the enrollment of study staff raises ethical and methodological concerns.

These considerations have been examined while setting up the Partnership for Research on Ebola Vaccinations (PREVAC) trial, a randomized phase 2 double-blind vaccine trial evaluating the immunogenicity and the safety of three prophylactic vaccine strategies against Ebola (NCT02876328). In this trial, healthy adult and children (aged ≥ 1 year) volunteers have been enrolled in Guinea and Liberia since March 2017, and in Sierra Leone and Mali, since respectively May and July 2018; the trial is ongoing. For this prophylactic vaccine clinical trial conducted in West Africa, study personnel of the PREVAC trial comprise the clinical study site staff and the community mobilizers, both employed and paid by the trial sponsors or their delegates. The clinical study site staff comprises the medical team, nurses, pharmacists, lab technicians, administrative personnel, logistics personnel, and communications staff. The community mobilizers are involved in recruitment and follow-up of participants within the communities. In the case of PREVAC, they were recruited based on a profile of community workers and community champions. Figures of exemplarity within

their communities (e.g., football/soccer coach, nurse) were encouraged to apply for these positions because of their strong community involvement.

In this article, we examined the ethical and methodological considerations of enrolling study personnel in Ebola vaccine trials in a non-epidemic context. We then defined the most appropriate course of action for the implementation of the PREVAC trial in Guinea.

Methods

We performed a review of the scientific literature using the PubMed electronic database, and of the gray literature [11] using Google searches for documents published on the Internet (in English and French), to identify relevant references regarding the enrollment of study personnel in clinical trials. Key words used for the literature search were “enrollment”, “study personnel”, “staff”, and “clinical trials”. For the review of the scientific literature, the selection was performed on the title and abstract, and then in a second step by reading the full article. For the gray literature, as it is impossible to screen all retrieved results from Google searches, we screened the most relevant results based on the Google relevancy ranking on the first 15 pages. The search was performed on March 2017 with a publication date limit of the same date. We then examined the methodological and ethical considerations pertaining enrolling study personnel in a prophylactic Ebola vaccine trial outside of an epidemic context.

As community mobilizers are rarely present at the vaccination sites (mainly being present only to provide information for the purposes of the participant consent process) and are not involved in the vaccination process, we assumed that some of the ethical and methodological considerations would not apply to them. Therefore, we distinguished between the two types of personnel—clinical study site staff and community mobilizers—for the evaluation of ethical and methodological considerations.

We then reached a consensus on how to proceed in the context of PREVAC in Guinea in March 2017 after studying all the ethical and methodological considerations. This decision was made in a multidisciplinary fashion involving the sponsor, the investigator coordinator of the study, the clinical trial unit responsible for the trial methodology, anthropologists, and study staff in the field.

Results

Assessment of the ethical and methodological considerations

We found no regulations preventing a clinical investigator or site staff from participating in a trial. No relevant biomedical literature was identified in PubMed among 325

articles retrieved. Our main sources were thus reference sources pertaining to the protection of human subjects such as the Declaration of Helsinki and the Belmont Report [12, 13], International Ethical Guidelines for Biomedical Research Involving Human Subjects [14], and the Institutional Review Board Guidebook of the Office for Human Research Protections (USA), especially Chapter 6 which concerns special classes of subjects [15].

The ethical and methodological features and challenges associated with enrolling study personnel in a prophylactic Ebola vaccine trial are summarized in Table 1.

Respect for persons is a fundamental principle of medical research involving human subjects. As defined in the Belmont Report [13], respect for persons incorporates that individuals should be treated as autonomous agents and requires that subjects, to the degree that they are capable, be given the opportunity to choose what

Table 1 Ethical and methodological considerations according to the type of study personnel to be potentially included in a prophylactic Ebola vaccine clinical trial conducted in West Africa outside of the context of an active epidemic

| Principles | Considerations | Explanation | Affected study personnel | |
|---|---|--|---------------------------|----------------------|
| | | | Clinical study site staff | Community mobilizers |
| Ethical principles | | | | |
| Respect for persons: no research without informed consent of those involved, respect of autonomy, the requirement to protect those with diminished autonomy | Direct or indirect undue influence regarding participation in the trial | As with other participants in a clinical trial, the participation of study staff must always be voluntary | Yes | Yes |
| Beneficence: do not harm and maximize possible benefits and minimize possible harms | No particular consideration: the risks and benefits of the research are the same for all the participants | | | |
| Justice: respect for the principle of equality of human beings, fair treatment during investigations | Breach of confidentiality regarding medical information | Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information | Yes | No |
| Methodological principles | | | | |
| Subject selection | Eligibility criteria | As with other participants, any study staff enrolled must meet the eligibility criteria and participate in the information session, and give informed consent | Yes | Yes |
| Selection of an adequate control group | No particular consideration | | | |
| Number of subjects: statistical assessments of sample size | No particular consideration | | | |
| Response variables: primary and secondary endpoints | No particular consideration | | | |
| Methods to minimize or assess bias: randomization, blinding, compliance | Imperfect blinding | Maintaining blinding could be difficult due to the proximity to unblinded site personnel and potential knowledge of product-specific adverse events | Yes | No |
| | Dropout rate differences between arms | The study staff enrolled as participants may have access to accumulating information during the trial to which other participants do not typically have access (e.g., overall adverse event rates) | Yes | No |
| Analysis: the study protocol should have a specified analysis plan that is appropriate for the objectives and design of the study | No particular consideration | | | |
| Other | Disruption of trial operations | During their participation in trial activities as participants, the staff are not available to perform their professional duties | Yes | Yes |

shall or shall not happen to them. This opportunity is provided when adequate standards for informed consent are satisfied. Participation must always be voluntary, and the volunteer has the right to refuse to participate in a study or to withdraw consent to participate at any time. Regarding this point, our consideration concerns the risk of direct or indirect undue influence because staff may feel pressured to join the clinical trial due to the hierarchical relationship with the employer. This consideration concerns clinical study site staff as well as community mobilizers, who are both in a dependent relationship with the employer.

The second ethical consideration is related to the principle of justice (i.e., respect of the principle of equality of human beings, fair treatment during investigations), where there is a risk of a breach of confidentiality of medical information. For example, confidentiality could be difficult to ensure in the case that a colleague has to inform a participant about a positive human immunodeficiency virus (HIV) test result. This concern, however, applies only to clinical study site staff, as community mobilizers do not work directly with other study staff.

Regarding the ethical principle of beneficence (i.e., do not harm and maximize possible benefits and minimize possible harms), no particular consideration was raised for the staff, as the risks and benefits of the research are the same for all the participants.

Regarding the methodological aspects, we identified three different points to consider. The first one is a logistical risk of disruption of trial operations due to the potential absence of the personnel for their commitment to trial activities as participants. As participants, all included personnel must adhere to the same trial procedures and follow-up as the other participants. This affects both clinical study site staff and community mobilizers, as it requires their availability as participants during working hours and conflicts with their professional duties as trial staff.

One of the methods used to minimize bias in clinical trials is blinding, which allows maintaining the same follow-up procedures and measurement of endpoints in the different randomized groups. The second methodological risk we identified concerns imperfect blinding in the case of single- or double-blind clinical trials, due to the potential knowledge of product-specific adverse events and to the proximity to unblinded site personnel. Vaccines are prepared on site in the pharmacy by unblinded personnel, and complete blinding is often impossible due to differences in color or viscosity between the active vaccine and placebo, even when using opaque syringes. Despite all precautions taken to achieve blinding, site staff are often familiar with subtle differences between the tested interventions that are not apparent to the participants.

The third methodological point concerns the risk of non-differential dropout rates, as the study staff enrolled as participants may have access to accumulating information during the trial to which other participants do not typically have access. For example, staff may be aware of the overall incidence of adverse events in the trial and are more likely to discontinue their participation in the trial on that basis. If blinding is maintained, this can lead to non-differential dropout rates between arms and therefore a loss of information and power. In the case of imperfect blinding, these dropout rates can be differential. These last two concerns do not apply to community mobilizers, who have a more distant relationship with the study site, are not involved in on-site procedures, and lack access to accumulating confidential information during the trial.

Regarding selection of subjects (eligibility criteria), which is an important aspect of a clinical trial, study staff enrolled must meet eligibility criteria, go through an information session, and give informed consent, just as the other participants do.

Definition of the appropriate course of action for implementing the PREVAC trial in Guinea

Given the ethical and methodological concerns we have described, the study team in Guinea decided not to enroll investigators or site staff as participants in the trial. As community mobilizers are prominent figures who are trusted within the community, and given that most of the methodological and ethical factors did not apply to them, we allowed their enrollment in the trial under the following conditions: First, participation had to be voluntary and not related to the terms of employment. This fundamental principle had to always be well explained to the potential participants before consent was obtained; this was mandatory. Second, like all other participants, they had to meet the eligibility criteria and attend an information session, provide consent, and enroll in the participant tracking process. Finally, all site staff were reminded about their professional commitment to secrecy, especially pertaining to the confidentiality of medical information.

In October 2018, when the inclusion of participant in the PREVAC trial in Guinea ended, a total of 10 community mobilizers had been included in the trial, representing 13% of all community mobilizers working at Guinean sites.

Discussion

The enrollment of study staff threatens two of the fundamental ethical principles of medical research involving human subjects: voluntary participation and privacy/confidentiality. It also raises logistical and methodological

concerns that could jeopardize trial operations and blinding and result in increased dropout rates.

Our literature review has highlighted a lack of specific references regarding the participation of study staff to a trial, in both the scientific literature and the regulatory documents. Nevertheless, our review of scientific literature was limited to the PubMed database. There is currently no regulatory prohibition banning a clinical investigator or site staff from participating in a trial. Indeed, it would be difficult to draw up a general rule with regards to this. The public health but also socio-cultural context must be considered when determining the course of action that should be taken during the implementation of a clinical trial, especially in low-income settings and in emergency situations [8]. Regulatory authorities should consider making general or specific recommendations according to the study context, particularly in the case of a community trial for which the staff may meet the eligibility criteria. In the absence of specific rules, this matter should be addressed by the sponsor, and the opinion of an ethics committee on this question could also be sought before beginning the trial. Several strategies can be deployed to strengthen the protection of employees and minimize bias in this context [16]: Measures that will be implemented to protect the rights of employee participants should be described before the implementation and be reviewed by an ethics committee; supervisors should not directly recruit subordinates for research participation, or an independent party could monitor the informed consent process.

Building trust in the communities in West Africa as well as in other regions of the world is an essential step for the successful implementation of any community trial, including vaccine trials. The community engagement strategies developed and successfully implemented in previous vaccine trials in the epidemic Ebola context supported the tremendous effort to rapidly conduct much-needed health research on prophylactic vaccine candidates. In the epidemic context, the enrollment of study staff probably contributed to mobilize the communities, but this alone may not justify the implementation of such an initiative. During an epidemic, vaccination can potentially prevent the spread of a disease with high incidence and lethality, and a major benefit of vaccinating study staff could exist, provided that candidate vaccines have a risk-benefit ratio that has been sufficiently evaluated and that the study is well designed. These considerations could also apply to other emergent infectious diseases with high incidence and lethality and without effective prevention measures or available therapeutic treatments.

Conclusion

The enrollment of study personnel can play a key role in facilitating the enrollment of participants in a community

trial and may provide potential personal benefits, especially in an epidemic context where the risk-benefit ratio is likely to be different from the non-epidemic one. In a non-epidemic context, methodological and ethical considerations suggest the need for increased caution. We conclude that study personnel not involved in on-site procedures and who meet the eligibility criteria without risk of coercion can be considered for enrollment as participants. Our structured analysis provides a framework for the systematic examination of the specific pros and cons during the preparation and implementation of prophylactic vaccine trials.

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Authors' contributions

EL realized the review of the literature. EL, GC, and LR analyzed the methodological and ethical considerations. All authors participated in the reflection on the subject of this article and read and approved the final manuscript.

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Competing interests

The clinical trial was conducted with the support of Janssen, Bavarian Nordic, and Merck Sharp & Dohme Corp. (MSD), who provided the vaccines. YY declares that he has been a board member receiving consultancy fees from AbbVie, Bristol-Myers Squibb, Gilead, MSD, Johnson & Johnson, Pfizer, and Viiv Healthcare in the past 4 years outside the subjects of the meetings (on HIV and HCV mostly); however, all these activities were stopped in November 2016. The other authors declare that they have no competing interests.

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References

1. Ebola Situation Report - 30 March 2016 | Ebola [Internet]. <http://apps.who.int/ebola/current-situation/ebola-situation-report-30-march-2016>. Accessed 24 Nov 2017.
2. Osterholm M, Moore K, Ostrowsky J, Kimball-Baker K, Farrar J, Wellcome Trust-CIDRAP Ebola Vaccine Team B. The Ebola Vaccine Team B: a model for promoting the rapid development of medical countermeasures for emerging infectious disease threats. *Lancet Infect Dis*. 2016;16(1):e1–9.
3. Lévy Y, Lane C, Piot P, Beavogui AH, Kieh M, Leigh B, et al. Prevention of Ebola virus disease through vaccination: where we are in 2018. *Lancet*. 2018;392(10149):787–90.
4. Kennedy SB, Bolay F, Kieh M, Grandits G, Badio M, Ballou R. Phase 2 placebo-controlled trial of two vaccines to prevent Ebola in Liberia. *N Engl J Med*. 2017;377(15):1438–47.
5. Henao-Restrepo AM, Camacho A, Longini IM, Watson CH, Edmunds WJ, Egger M, et al. Efficacy and effectiveness of an rVSV-vectored vaccine in preventing Ebola virus disease: final results from the Guinea ring vaccination, open-label, cluster-randomised trial (Ebola Ça Suffit!). *Lancet*. 2017;389(10068):505–18.
6. Doe-Anderson J, Baseler B, Driscoll P, Johnson M, Lysander J, McNay L, et al. Beating the odds: successful establishment of a phase II/III clinical research trial in resource-poor Liberia during the largest-ever Ebola outbreak. *Contemp Clin Trials Commun*. 2016;4:68–73.
7. Mooney T, Smout E, Leigh B, Greenwood B, Enria L, Ishola D. EBOVAC-Salone: lessons learned from implementing an Ebola vaccine trial in an Ebola-affected country. *Clin Trials*. 2018;15(5):436–43.
8. External Situation Report 34. Ebola virus disease, Democratic Republic of the Congo. 2019. [Internet]. https://apps.who.int/iris/bitstream/handle/10665/311507/SITREP_EVD_DRC_20190326-eng.pdf?ua=1. Accessed 30 Mar 2019.
9. Enria L, Lees S, Smout E, Mooney T, Tengbeh AF, Leigh B, et al. Power, fairness and trust: understanding and engaging with vaccine trial participants and communities in the setting up the EBOVAC-Salone vaccine trial in Sierra Leone. *BMC Public Health*. 2016;16(1):1140.
10. Laverack G, Manocourt E. Key experiences of community engagement and social mobilization in the Ebola response. *Glob Health Promot*. 2016;23(1):79–82.
11. Conn VS, Valentine JC, Cooper HM, Rantz MJ. Grey literature in meta-analyses. *Nurs Res*. 2003;52(4):256–61.
12. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA*. 2013;310(20):2191–4.
13. National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research. The Belmont report: Ethical principles and guidelines for the protection of human subjects of research. Bethesda: The Commission; 1978.
14. Council for International Organizations of Medical Sciences (CIOMS). International Ethical Guidelines for Health-related Research Involving Humans, fourth edition. Geneva: CIOMS; 2016.
15. Office for Human Research Protections (OHRP). IRB Guidebook. [Internet]. <https://www.hhs.gov/ohrp/education-and-outreach/archived-materials/index.html>. Accessed 24 Nov 2017.
16. Resnik D. Employees as research participants: ethical and policy issues. *IRB: Ethics Hum Res*. 2016;38(4):11–6.

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VI. Outlook and conclusions

This thesis focused on the methodological aspects of clinical vaccine research, exemplified by HIV and Ebola vaccine trials.

In chapter 3, we investigated the dynamics of the immune responses post-vaccination. We showed that early sampling time points should be considered in future clinical trials to better understand the role of the early CD4 helper T cells and to evaluate their predictive role in the immune response to vaccines. We also developed a new bivariate modelling approach for the analysis of the ICS response with a good statistical performance and should be the new statistical standard method for ICS analyses. Hopefully, this work should have a direct impact on the assessment on the ICS response in vaccine clinical trials.

In chapter 4, we showed that the antibody response after preventive vaccination against Ebola virus disease is very much variable and not fully explained by covariates. This emphasizes the interest of harmonizing measurement methods and study designs. Furthermore, it indicates the need of randomized multi arm Ebola vaccine trials for accurate comparison of immunogenicity between different vaccine strategies such as PREVAC.

In chapter 5, we detailed the protocol of the PREVAC trial, a phase 2 randomized controlled vaccine trial evaluating three different vaccine strategies against Ebola in West Africa. We then have addressed the question of the enrollment of study personnel in the vaccine clinical trial in a non-epidemic context and defined an appropriate course of action for the PREVAC trial. We showed that in a non-epidemic context, ethical and methodological considerations limit the collective benefit of enrolling site staff in a vaccine trial. These considerations do not apply to community mobilizers, whose potential enrollment should be considered as long as they meet the inclusion criteria and they are not exposed to any form of coercion.

Further work will be required to confirm or continue the work of this thesis. As each chapter of this thesis contains its specific discussion section, we will only briefly recapitulate the main features here, and then focus the rest of this outlook on a broader discussion of methodological challenges in modern vaccine research.

First, we have shown that the evaluation of the immune response to HIV vaccine is challenged by at least two aspects: the timing of sampling and the analysis of the signal taking

into account the background. Hence, the relationship found between CD4+ T cell help and CD8+T cell response should be confirmed in other studies, although the repeated blood sampling available in HVTN 068 is exceptional. The demonstration of this relationship is the first step for the construction of a mechanistic model of the response to vaccine. The model would be the description of biological process with the interaction between the early CD4+ T cell response and the later CD8+ T cell response, summarized with differential equations.

The development of an extension of the bivariate model to take into account longitudinal ICS data in vaccine clinical trials is an obvious next step. The model would be also adapted for the analysis of other functional cellular assays with a background response, such as EliSpot responses for instance. Indeed, we are currently using this bivariate model for analyzing cellular proliferation responses in samples from an Ebola vaccine trial.

More broadly, many methodological challenges remain to be solved in modern vaccine research. For decades, an empirical vaccine design approach has been used to develop efficacious vaccine against pathogens. However, the current obstacles to HIV vaccine development plead for rational strategies that more systematically consider pathogen biology and dynamics within the host [1,2]. For HIV, as well as for Ebola and other disease of the blueprint WHO list [3], there is a critical need to define signatures and mechanistic correlates of protection to rationally guide the design of protective vaccine strategies [2]. System vaccinology approaches can help in better understanding and predicting the response to vaccines as demonstrated in the context of yellow fever, flu and many other vaccines [4-7]. The idea of system vaccinology is to integrate the massive data generated by high-throughput technologies (eg. transcriptomic, proteomic, flow cytometry, multiplex data) and population characteristics (socio-demographics and coinfections) to isolate the main markers/signatures associated to the vaccine response [8-10]. This enable a holistic view of the immune system and its many components, combining high throughput immunological methods, complex statistical analysis and good knowledge of immunology. Unlike conventional techniques, which generate a limited number of measurements, high throughput technologies produce tens or hundreds of thousands of measurements per sample, leading to in an ever-increasing number of data where the number of dimensions is much larger than the sample size [9]. This requires appropriate statistical and bioinformatics methods for high dimensional data with high number of correlated parameters. A critical step is a down-selection with the identification of the most important predicting markers and to reduce the computational complexity, using dimension reduction technics such as sparse group partial least square approaches [11,12]. Then, a mechanistic model for instance of the response can be built and hopefully predict the individual

long-term response, based on mathematical description of biological process with differential equations. In the SISTM team, colleagues have produced several publications highlighting how within-host mechanistic models could play an important role in predicting vaccine efficacy and in improving treatment regimens, notably in HIV and Ebola [13-15]. Identification and validation of an early correlate of later immune responses via system vaccinology also allow early prediction of whether an individual, or group of individuals is likely to be a poor responder and then to preconize subsequent interventions to test in this subset (such as change in vaccination strategy or additional boosts) [16].

While many of the current licensed human vaccine are believed to confer protection through the induction of antigen-specific antibodies (e.g. hepatitis, human papillomavirus, polio, influenza, yellow fever, pneumococcus) [17-22], humoral profiling efforts have been considerably more limited - most often focusing on the antibody titer and neutralization activity post-vaccination - in regard to the considerable advances in cellular profiling approaches made thanks to the development of high high-throughput immunological assays (e.g. robust transcriptional and multiparameter cytometric profiling). The experience of HIV vaccine development and specifically the research of correlate in the RV144 trial [23] have underlined the critical importance of looking at the more specific qualitative features of the humoral response that were suggested to be key indicators of protective efficacy [2]. Surprisingly the vaccine did not induce broadly neutralizing antibody responses and instead non-neutralizing antibodies that recognize a specific region of the HIV envelope (ENV) variable region 1 and 2 (V1V2) were associated with reduced risk of infection [24,25]. This pointed out that today, measuring the level of antigen-specific antibody titer alone or with a very limited number of humoral characteristics is not sufficient. There is a critical need to measure more data to better understand additional functional role of antibodies as antibody dependent cellular toxicity (ADCC), antibody dependent cellular phagocytosis (ADCP), and antibody dependent complement deposition [26]. This is the aim of the new field called "system serology", which is the counterpart of system vaccinology focused on the humoral response. System serology would allow to examine many of the antibody features, using broad assessments of the enormous diversity that exists within the humoral response. The aim is to better understand the mechanism behind the different antibody responses and functions, and to identify humoral signatures and mechanistic correlates of protection induced by vaccination, and key targets for futures vaccine development [26]. A systems serology approach systematically assesses biophysical antibody profiles (antigen target and subclassing, Fc-receptor and antigen affinity, Glycans) and functional profiles (eg. ADCC, ADCP) [26]. Some of the experimental assays used in system serology to evaluate these antibody features are common to those used to

evaluate the cellular response. For instance ICS is used for the evaluation of ADCC [27]. Some others are specific to the evaluation of the antibody responses (eg. complement assay, bead assay, virion assay, Surface plasmon resonance, High-performance liquid chromatography) [28-34]. The statistical analysis of these new complex data certainly raises new methodological challenges, and – as we did for the ICS assay in this thesis - it is first necessary to fully understand the nature of these data and the specificities of each technique. Then, these data will have to be analyzed by integrating the other data generated in trials through multi-omics approaches. This should help in estimating the importance of each characteristic on the establishment of the immune response.

Our implication in Ebola vaccine trials (Ebovac 2, PREVAC trial) will be a great opportunity to open up our research to system serology. I will be involved in the integrative analysis of the data from the Ebovac 2 (NCT02416453) projects and especially the integration of the B cell responses in mechanistic modelling in the near future. There are great challenges, but also opportunities to better understand the antibody-omics platform and to develop methodological solutions for the analysis pipeline of these data in system serology approaches. This constitutes key targets for accelerating future vaccine development.

References

1. Burton DR, Ahmed R, Barouch DH, Butera ST, Crotty S, Godzik A, et al. A Blueprint for HIV Vaccine Discovery. *Cell Host Microbe*. 2012;12(4):396–407.
2. Ackerman ME, Barouch DH, Alter G. Systems serology for evaluation of HIV vaccine trials. *Immunol Rev*. 2017;275(1):262–270.
3. WHO | List of Blueprint priority diseases [Internet]. WHO. [Cited Sept 5 2019]. Available from: <http://www.who.int/blueprint/priority-diseases/en/>
4. Querec TD, Akondy RS, Lee EK, Cao W, Nakaya HI, Teuwen D, et al. Systems biology approach predicts immunogenicity of the yellow fever vaccine in humans. *Nat Immunol*. 2009;10(1):116–25.
5. Nakaya HI, Hagan T, Duraisingham SS, Lee EK, Kwissa M, Roupheal N, et al. Systems Analysis of Immunity to Influenza Vaccination across Multiple Years and in Diverse Populations Reveals Shared Molecular Signatures. *Immunity*. 2015;43(6):1186–98.
6. Dunachie S, Berthoud T, Hill AVS, Fletcher HA. Transcriptional changes induced by candidate malaria vaccines and correlation with protection against malaria in a human challenge model. *Vaccine*. 2015;33(40):5321–31.
7. Li S, Roupheal N, Duraisingham S, Romero-Steiner S, Presnell S, Davis C, et al. Molecular signatures of antibody responses derived from a systems biological study of 5 human vaccines. *Nat Immunol*. 2014;15(2):195–204.
8. Pulendran B, Li S, Nakaya HI. Systems Vaccinology. *Immunity*. 2010;33(4):516–29.
9. Hagan T, Nakaya HI, Subramaniam S, Pulendran B. Systems Vaccinology: Enabling rational vaccine design with systems biological approaches. *Vaccine*. 2015;33(40):5294–301.
10. Raeven RHM, van Riet E, Meiring HD, Metz B, Kersten GFA. Systems vaccinology and big data in the vaccine development chain. *Immunology*. 2019;156(1):33–46.
11. Liqueur B, de Micheaux PL, Hejblum BP, Thiébaud R. Group and sparse group partial least square approaches applied in genomics context. *Bioinformatics*. 2016;32(1):35–42.
12. Sutton M, Thiébaud R, Liqueur B. Sparse partial least squares with group and subgroup structure. *Stat Med*. 2018 15;37(23):3338–56.
13. Prague M, Commenges D, Drylewicz J, Thiébaud R. Treatment Monitoring of HIV-Infected Patients based on Mechanistic Models. *Biometrics*. 2012;68(3):902–11.
14. Thiébaud R, Drylewicz J, Prague M, Lacabaratz C, Beq S, Jarne A, et al. Quantifying and Predicting the Effect of Exogenous Interleukin-7 on CD4+T Cells in HIV-1 Infection. *PLoS Comput Biol*. 2014 May;10(5):e1003630.
15. Pasin C, Balelli I, Van Effelterre T, Bockstal V, Solforosi L, Prague M, et al. Dynamics of the Humoral Immune Response to a Prime-Boost Ebola Vaccine: Quantification and Sources of Variation. *J Virol*. 2019;93(18).
16. Poland G, Ovsyannikova I, Kennedy R. Personalized Vaccinology: A Review. *Vaccine*. 2018;36(36):5350–7.
17. Jack AD, Hall AJ, Maine N, Mendy M, Whittle HC. What level of hepatitis B antibody is protective? *J Infect Dis*. 1999;179:489–92.
18. Frazer I. Correlating immunity with protection for HPV infection. *Int J Infect Dis*. 2007;11(Suppl 2):S10–6.
19. Faden H, Modlin JF, Thoms ML, McBean AM, Ferdon MB, Ogra PL. Comparative evaluation of immunization with live attenuated and enhanced-potency inactivated trivalent poliovirus vaccines in childhood: systemic and local immune responses. *J Infect Dis*. 1990;162:1291–7.
20. Wheatley AK, Kent SJ. Prospects for antibody-based universal influenza vaccines in the context of widespread pre-existing immunity. *Expert Rev Vaccines*. 2015;14:1227–39.
21. Mason RA, Tauraso NM, Spertzel RO, Ginn RK. Yellow fever vaccine: direct challenge of monkeys given graded doses of 17D vaccine. *Appl Microbiol*. 1973;25:539–44.
22. Lee LH, Frasch CE, Falk LA, Klein DL, Deal CD. Correlates of immunity for pneumococcal conjugate vaccines. *Vaccine*. 2003;21:2190–6.

23. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, Prem Sri N, Namwat C, de Souza M, Adams E, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. *N Engl J Med*. 2009;361:2209–20.
24. Haynes BF, Gilbert PB, McElrath MJ, Zolla-Pazner S, Tomaras GD, Alam SM, Evans DT, Montefori DC, Karnasuta C, Sutthent R, et al. Immunecorrelates analysis of an HIV-1 vaccine efficacy trial. *New Engl J Med*. 2012;366:1275–86.
25. Zolla-Pazner S, deCamp A, Gilbert PB, Williams C, Yates NL, Williams WT, Howington R, Fong Y, Morris DE, Soderberg KA, et al. Vaccine-induced IgG antibodies to V1V2 regions of multiple HIV-1 subtypes correlate with decreased risk of HIV-1 infection. *PLoS ONE*. 2014;9:e87572.
26. Chung AW, Alter G. Systems serology: profiling vaccine induced humoral immunity against HIV. *Retrovirology*. 2017 Dec 21;14(1):57.
27. Chung AW, Ghebremichael M, Robinson H, Brown E, Choi I, Lane S, et al. Polyfunctional Fc-effector profiles mediated by IgG subclass selection distinguish RV144 and VAX003 vaccines. *Sci Transl Med*. 2014;6(228):228ra38.
28. Chung AW, Crispin M, Pritchard L, Robinson H, Gorny MK, Yu X, BaileyKellogg C, Ackerman ME, Scanlan C, Zolla-Pazner S, Alter G. Identification of antibody glycosylation structures that predict monoclonal antibody Fc-effector function. *AIDS*. 2014;28:2523–30.
29. Ackerman ME, Moldt B, Wyatt RT, Dugast AS, McAndrew E, Tsoukas S, Jost S, Berger CT, Sciaranghella G, Liu Q, et al. A robust, high-throughput assay to determine the phagocytic activity of clinical antibody samples. *J Immunol Methods*. 2011;366:8–19.
30. Guan Y, Pazgier M, Sajadi MM, Kamin-Lewis R, Al-Darmarki S, Flinko R, Lovo E, Wu X, Robinson JE, Seaman MS, et al. Diverse specificity and effector function among human antibodies to HIV-1 envelope glycoprotein epitopes exposed by CD4 binding. *Proc Natl Acad Sci U S A*. 2013;110:E69–78. 117.
31. Richard J, Veillette M, Brassard N, Iyer SS, Roger M, Martin L, Pazgier M, Schon A, Freire E, Routy JP, et al. CD4 mimetics sensitize HIV-1-infected cells to ADCC. *Proc Natl Acad Sci U S A*. 2015;112:E2687–94.
32. Lan J, Yang K, Byrd D, Hu N, Amet T, Shepherd N, Desai M, Gao J, Gupta S, Sun Y, Yu Q. Provirus activation plus CD59 blockage triggers antibody-dependent complement-mediated lysis of latently HIV-1-infected cells. *J Immunol*. 2014;193:3577–89.
33. Klasse PJ. How to assess the binding strength of antibodies elicited by vaccination against HIV and other viruses. *Expert Rev Vaccines*. 2016;15:295–311.
34. Luo Y, Lu Z, Raso SW, Entrican C, Tangarone B. Dimers and multimers of monoclonal IgG1 exhibit higher in vitro binding affinities to Fcγ receptors. *MAbs*. 2009;1:491–504.

Appendice

Appendix 1: List of publications and communications not related to the thesis

Articles

Boyer A, Cadier G, Accoceberry I, Lhomme E, Ricard C, Gruson D, Blanchard E. Guidelines for antifungal therapies in ICU patients: not so bad ! Clin Microbiol Infect. 2019 [Epub ahead of print]

Zordan C, Monteil L, Haquet E, Cordier C, Toussaint E, Roche P, Dorian V, Maillard A, Lhomme E, Richert L, Pasquier L, Akloul L, Taris N, Lacombe D. Evaluation of the template letter regarding the disclosure of genetic information within the family in France. J Community Genet. 2019 [Epub ahead of print]

Peuchant O, Lhomme E, Krêt M, Ghezzoul B, Roussillon C, Bébéar C, Perry F, de Barbeyrac B; and the Chlazidoxy study group. Randomized, open-label, multicenter study of azithromycin compared with doxycycline for treating anorectal Chlamydia trachomatis infection concomitant to a vaginal infection (CHLAZIDOXY study). Medicine (Baltimore) 2019;98(7):e14572.

Fitzgerald FC*, Lhomme E*, Harris K, Kenny J, Doyle R, Kityo C, et al. Microbial translocation does not drive immune activation in Ugandan children with HIV. J Infect Dis. 2019;219(1):89-100.

Boyer A, Goret J, Clouzeau B, Romen A, Prevel R, Lhomme E, Vargas F, Hilbert G, Bébéar C, Gruson D, M'Zali F. Tailoring the empirical antimicrobial therapy in VAP subjects by mean 1 of a 10 hour E-test based approach. Resp Care 2018. [Epub ahead of print]

Montagni I, Koman J, Lhomme E, Tzourio C. Efficacy of an online video to promote health insurance literacy among students. Journal for Communication Studies. 2018: 29-48.

Richert L, Lhomme E, Fagard C, Lévy Y, Chêne G, Thiébaud R. Recent developments in clinical trial designs for HIV vaccine research. Hum Vaccin Immunother. 2015;11(4):1022-9.

Yvert M, Diallo A, Bessou P, Rehel JL, Lhomme E, Chateil JF. Radiography of scoliosis: Comparative dose levels and image quality between a dynamic flat-panel detector and a slot-scanning device (EOS system). Diagn Interv Imaging. 2015 ; 96(11):1177-88.

Bullier B, MacGrogan G, Bonnefoi H, Hurtevent-Labrot G, Lhomme E, Brouste V, et al. Imaging features of sporadic breast cancer in women under 40 years old: 97 cases. Eur Radiol. 2013; 23(12): 3237-45

Communications in conferences with a peer-review committee

Posters

European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) 2018
Coignard-Biehler H, Bahuaud M, Lhomme E, Georget A, Bakouboula P, Kara L, Rossi B, Lefebvre M, J Beytout, F Lainé, X Duval, F Galtier, Michel M, Chene G, Batteux F, Lortholary O*, Launay O. Immunogenicity and safety of a sequential pneumococcal vaccination in splenectomized adults: a multicenter, prospective study.

Conference on Retroviruses and Opportunistic Infections (CROI)| Seattle, USA
Fitzgerald F*, Lhomme E*, Harris K, Kenny J, Doyle R, Kityo C, Walker AS, Thiebaut R, Klein N and the CHAPAS3 Trial Team. Microbial translocation does not drive immune activation in Ugandan children with HIV.