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par

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USE OF PHARMACOKINETIC AND PHARMACODYNAMIC MODELLING SIMULATION TO SUPPORT DRUG DEVELOPMENT

ILLUSTRATION WITH AN EXPLORATORY ANALYSIS OF THE OBINUTUZUMAB EXPOSURE INFLUENCED BY DEMOGRAPHIC AND DISEASE PARAMETERS AND IMPACT ON CLINICAL OUTCOMES

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List of abbreviations

AUC: Area Under the Curve

BMI: Body Mass Index (kg/m²)

BSA: Body Surface Area (m²)

CLL: Chronic Lymphocytic Leukemia

CR: Complete Response

DLBCL: diffuse large B-cell lymphoma

DNA: deoxyribonucleic acid

FC Gamma-Receptor: FcγR

FDA: Food Drug Administration

FL: Follicular lymphoma

FLIPI: Follicular Lymphoma International Prognostic Index

HAs: Health Authorities

HR: Hazard ratio

iNHL: indolent Non-Hodgkin Lymphoma

IPI: International Prognostic Index

ITT: Intention to treat

IV: Intra-venous

LDH: Lactate dehydrogenase

mAb: Monoclonal antibody

MRD: Minimal Residual Disease

NONMEM: Non-Linear Mixed Effects Modelling

OS: Overall Survival

PR: Partial Response

PD: Product Development

PD: Pharmacodynamics

PET: Positron Emission Tomography

PFS: Progression Free-Survival

PK: Pharmacokinetics

PK/PD: Pharmacokinetics/Pharmacodynamics

Pop PK: Population Pharmacokinetics

PR: Partial response

ST: Stable disease

TMDD: Target-Mediated Drug Disposition

WHO: World Health Organization

Partie en Français

Cette partie en français constitue un condensé du document principal. Toutes les informations et réflexions sur le sujet travaillé ne se retrouvent pas dans cette partie. De même, les figures et graphiques ne sont pas insérés ici.

Merci de vous référer au document principal.

Introduction et problématique

Les nombreux et nouveaux challenges des laboratoires pharmaceutiques, des autorités de santé, des centres de recherche académiques sont de développer des programmes cliniques de développement de médicaments alliant efficacité et minimisation des risques pour la santé avec un but d'accès rapide aux patients.

Dans le développement de molécules, la pharmacologie clinique est une des vastes disciplines qui couvre l'étude du médicament de son développement à son utilisation après sa mise sur le marché. La pharmacologie comprend entre autres l'étude du mécanisme d'action du médicament, la définition de ses conditions d'utilisation, l'évaluation de son efficacité et de sa sécurité d'emploi.

Cette discipline comprend entre autre la pharmacodynamie, qui étudie le mécanisme d'action des médicaments et la pharmacocinétique qui étudie le devenir du médicament dans l'organisme.

Différentes approches incluant ces disciplines ont de nombreux objectifs, un design d'étude clinique approprié, une utilisation de biomarqueurs ciblée et précise, et le développement des molécules pour une médecine personnalisée. Des modèles et simulations alliant données pharmacocinétiques et pharmacodynamiques sont créés.

La modélisation pharmacocinétique et pharmacodynamique (PK/PD) fait partie du processus de développement des médicaments. Cette technique mathématique permet d'anticiper les effets et l'efficacité du dosage des médicaments sur une période donnée. De manière générale, les modèles pharmacocinétiques décrivent la façon dont l'organisme réagit à un médicament en termes d'absorption, de distribution, de métabolisme et d'excrétion. Les modèles pharmacodynamiques décrivent la façon dont un médicament agit sur l'organisme en associant la concentration de médicament à une métrique d'efficacité (ou de sécurité). Un modèle PK/PD correctement décrit constitue un outil important qui aide à la conception d'expériences et d'essais cliniques futurs.

Le processus de modélisation PK/PD comprend différentes étapes comme le traitement et la visualisation des données relatives au temps d'absorption, la création de modèles PK/PD incluant différentes variables pouvant potentiellement influencer le modèle et le paramètre PK/PD étudié, l'estimation des paramètres de modèle à l'aide d'outils mathématiques, la simulation des stratégies de dosage et scénarios hypothétiques. Le but ultime reste de justifier et soutenir un développement adéquat de la molécule.

La contribution de ces disciplines pour une efficacité et une sécurité optimale du médicament est partie intégrante dans toutes les phases de développement clinique des médicaments.

Dans ce rapport, il sera question dans un premier temps de réaliser une vue d'ensemble du concept général de modélisation et simulation PK/PD dans le développement des médicaments, puis de se focaliser sur un aspect particulier de l'utilisation de ce concept. Cet aspect sera illustré par une analyse exploratoire de paramètres influençant l'exposition à un anticancéreux dans le traitement des lymphomes, obinutuzumab, et par une étude des conséquences cliniques.

1. Généralités sur l'approche de modélisation pharmacocinétique et pharmacodynamique dans le développement clinique des médicaments

1.1. Définitions et rationnel

La modélisation pharmacocinétique et pharmacodynamique (PK/PD) joue un rôle essentiel dans le processus de développement des médicaments.

La pharmacocinétique est l'étude des actions d'une substance active contenue dans un médicament sur l'organisme après son ingestion ou son administration. La modélisation pharmacocinétique repose classiquement sur une approche dite compartimentale. Le compartiment est un espace virtuel dans lequel la molécule se distribue de façon instantanée et homogène. Le nombre de compartiments et leur enchaînement sont choisis de façon à pouvoir décrire au mieux les phénomènes observés. La molécule va s'échanger entre les compartiments suivant une cinétique définie, avec des constantes de vitesses spécifiques pour chacun d'entre eux.

La pharmacodynamie décrit les effets thérapeutiques des médicaments ainsi que leurs effets secondaires. Elle décrit également le lieu et le mécanisme d'action d'un médicament dans l'organisme. La modélisation pharmacodynamique dépend de la nature de l'effet.

Une simulation est l'utilisation d'un modèle mathématique incluant différentes co-variables pour prédire des relations entre co-variables et décrire une tendance.

Dans l'ensemble, la modélisation pharmacocinétique peut prédire le comportement pharmacocinétique des molécules. Cela permet également d'explorer les effets de paramètres physiologiques comme des paramètres démographiques (âge, genre, poids…), de déterminer et guider le choix de la dose.

Un nombre de variables (âge, sexe, poids, …) est connu pour affecter la pharmacocinétique des médicaments, en particularité les anticorps monoclonaux à cause de leur pharmacocinétique non-linéaire de disposition et d'élimination (linéaire clairance puis phénomène de saturation…). Le modèle TMDD (*en anglais, Target Mediated Drug Disposition*) consiste à décrire les paramètres pharmacocinétiques de médicaments dont la distribution et/ou l'élimination sont influencées par la fixation sur leur cible.

Les approches de population en pharmacologie concernent la pharmacocinétique et/ou la pharmacodynamie. Elle combine d'une part la modélisation, qui implique la traduction en termes mathématiques du phénomène observé et d'autre part la statistique, qui implique l'utilisation d'une population assez large. L'approche de population permet alors la mise en évidence de caractéristiques individuelles (co-variables) capables d'influencer la réponse ou l'effet et d'identifier des sous-populations à risques (sous ou surexposées) pour lesquelles un ajustement posologique serait nécessaire.

1.2. Objectifs

Le premier objectif du rapport est de définir les grandes lignes de l'utilisation de la modélisation et simulation PK/PD dans le développement clinique des médicaments.

Le deuxième objectif est d'illustrer un des aspects de la modélisation et simulation PK/PD par une analyse exploratoire des paramètres influençant l'exposition au médicament. Un essai clinique de phase III GALLIUM, est pris comme exemple, en étudiant une population de patients traités par un anticorps monoclonal, obinutuzumab, atteints de lymphomes folliculaires, sous-types de lymphomes non-Hodgkinien. Les premiers facteurs analysés sont des paramètres démographiques concernant la population étudiée, les deuxièmes facteurs sont des paramètres reliés à la pathologie traitée. Enfin, on étudiera la relation entre exposition au

médicament et efficacité clinique. Toutes les analyses ont aussi été réalisées par groupe de patients et chimiothérapie associée à l'anticorps monoclonal.

1.3. Méthodes

Dans l'approche de population, la modélisation combine un modèle de structure (modèle pharmacocinétique par exemple) à un modèle statistique. Les effets sont dits mixtes ou mélangés (effets fixes et effets aléatoires), c'est ce que traduit l'acronyme « nonmem » qui donne son nom au logiciel historique d'analyse de pharmacocinétique de population : NONMEM (NON Linear Mixed Effects Model).

Ce logiciel n'a pas été utilisé dans le cadre de ce rapport pour générer des résultats mais entre bien dans le concept général de modélisation et simulation PK/PD.

Deux types d'approches sont possibles : paramétrique et non-paramétrique. L'approche paramétrique permet d'estimer d'une part des paramètres PK/PD moyens (ou paramètres PK/PD de population) et les effets d'éventuelles covariables (effets fixes), et d'autre part les variances des effets aléatoires qui mesurent la variabilité inter-individuelle et l'erreur résiduelle (variabilité analytique et écart au modèle).

La recherche de co-variables a pour objectif d'expliquer la variabilité interindividuelle des paramètres PK/PD et d'administrer la dose la plus adéquate à chaque patient : c'est l'individualisation de dose.

Le principe de modélisation par une approche de population est de mettre en évidence les paramètres PK d'une population et sources de variabilité de cette population qui peut être issue de plusieurs études cliniques. Dans ce rapport, la population étudiée est issue d'une étude clinique de phase III, GALLIUM (voir ci-dessous). Le but ici n'est pas de rentrer dans le détail de modélisation mais d'utiliser un des aspects de modélisation et simulation PK/PD

en se basant sur une analyse exploratoire de différents paramètres influençant une donnée de PK, la concentration moyenne de médicament collectée à la fin du traitement (phase d'induction et phase de maintenance incluses).

Plus généralement et dans le cadre de ce rapport, cette concentration sera mentionnée comme l'exposition au médicament, obinutuzumab.

Inclus dans l'essai clinique GALLIUM, 408 patients disposant de données PK, traités par obinutuzumab et atteints de lymphomes folliculaires sont analysés pour cette étude exploratoire. Les analyses graphiques réalisées mettant en relation l'exposition au médicament et les différents paramètres étudiés ont été obtenues par utilisation de R studio. Ce logiciel est un logiciel de programmation et d'analyse statistique. Boxplots, scatterplots, courbes de survie Kaplan-Meier sont des exemples d'analyses graphiques réalisés dans le cadre de cette étude.

Pour l'étude exploratoire réalisée dans le cadre de ce rapport, les premières tendances et résultats ont été validés statistiquement par analyse de la p-value. Un test est significatif si la p-value est inférieure à un risque donné de 5%. D'autres tests incluant des Cox-modèles et comparant les « Hasards Ratios » et intervalles devraient compléter cette analyse pour conclure à ces premiers résultats. Ils ne seront toutefois pas présentés dans ce rapport.

De ce fait, il requiert une grande prudence avant de déduire et émettre une conclusion. Un travail est encore en cours.

Les données cliniques et démographiques sont issues d'un logiciel Spotfire, de visualisation des données et des cahiers d'observations (Case Report Form).

1.4. Exemple d'utilisation de modélisation pharmacocinétique et pharmacodynamique dans le développement clinique

Le modèle est une expression mathématique décrivant la réponse d'un système pour une entrée donnée. La modélisation pharmacocinétique et pharmacodynamique permet de relier l'exposition à l'effet (souhaité-efficacité ou indésirable-toxicité). L'objectif est donc de mettre au point une représentation réaliste du devenir et de l'effet d'un médicament dans l'organisme en fonction des modalités d'administration et des caractéristiques individuelles des patients. La modélisation est de plus en plus utilisée dans le développement des médicaments. Elle constitue une base scientifique pour l'optimisation de la dose et des schémas d'administration lors d'essais cliniques de phase II, à l'aide des caractéristiques des patients, du suivi des concentrations circulantes de médicament et de la quantification des effets observés. Ce type d'approche permet aussi d'évaluer l'efficacité/toxicité d'un traitement lors des essais cliniques de phase III. Enfin, cette approche autorise, en pratique clinique, une meilleure individualisation de dose basée sur le recueil d'observations chez le patient et le calcul de prédictions individuelles.

L'identification de co-variables (ex: poids, surface corporelle, sexe) permet d'ajuster la dose toxique par exemple. Dans le cas présent, cela servirait à décrire expérimentalement les facteurs influençant la concentration moyenne du médicament dans l'organisme.

Cette approche de modélisation et simulation PK/PD constitue une partie du dossier pour les Hautes Autorités de Santé regroupant toutes les informations requises pour un médicament et dans son développement clinique.

2. Analyse exploratoire des paramètres influençant l'exposition à obinutuzumab et étude des conséquences cliniques

2.1. Pathologie et actuels traitements

Le lymphome folliculaire est l'une des formes de lymphome non hodgkinien (LNH). La pathologie est liée à la multiplication incontrôlée de lymphocytes B anormaux. La majorité des LNH sont caractérisés par l'expression d'un antigène membranaire, le CD20. Selon la classification internationale de l'Organisation Mondiale de la Santé, LNH peuvent être divisés en agressif et indolent lymphome. Le lymphome folliculaire est le plus commun des LNH indolents (LNHi).

Les actuels traitements sont une radiothérapie, une chimiothérapie pouvant associer CHOP (cyclophosphamide, doxorubicine, vincristine, prednisone), R-CHOP (CHOP avec rituximab), CVP (cyclophosphamide, vincristine, prednisone), R-CVP (CVP avec rituximab-), R-bendamustine (bendamustine avec rituximab), ainsi que des immunothérapies, des thérapies ciblées ayant recours à des molécules plus spécifiques rituximab et obinutuzumab avec bendamustine, deux anticorps monoclonaux anti-CD20.

Obinutuzumab est un anticorps monoclonal spécialement développé pour se lier à la protéine CD20 et est exprimée sur certaines cellules B ou lymphomes B.

2.2. Développement clinique d'obinutuzumab

Obinutuzumab a été homologué en 2014 dans plusieurs pays en association avec le chlorambucil pour le traitement de première ligne de la leucémie lymphoïde chronique (étude CLL-11).

En 2016, obinutuzumab a été homologué par d'abord la Food Drug and Administration (FDA) puis la Commission européenne (CE) dans le cadre d'une association avec la bendamustine, suivie d'un traitement d'entretien par obinutuzumab pour le traitement du lymphome folliculaire récidivant. En effet, la population ciblée concerne les personnes souffrant d'un lymphome folliculaire n'ayant pas répondu à rituxumab ou à un protocole contenant rituxumab ou ayant vu leur maladie progresser pendant un tel traitement ou dans les six mois suivants.

En mai 2016, l'étude de phase III GALLIUM menée chez des patients souffrant de lymphome folliculaire non précédemment traités a atteint son critère d'évaluation primaire de manière précoce.

2.2.1. GALLIUM – étude clinique de phase III

L'étude GALLIUM a évalué l'efficacité et l'innocuité de l'association obinutuzumab plus chimiothérapie (CHOP, CVP ou bendamustine), suivie de obinutuzumab en monothérapie, en comparaison directe avec l'association rituximab plus chimiothérapie, suivie de rituximab en monothérapie. Les chimiothérapies, choisies par chaque site participant à l'étude avant l'inclusion des patients, sont des protocoles CHOP ou CVP, ou la bendamustine. Les résultats d'une analyse intermédiaire programmée ont montré que le traitement à base d'obinutuzumab permettait une survie sans progression (PFS médiane à 9 ans avec obinutuzumab) supérieure de 34% à celle observée avec le traitement à base de rituximab (PFS médiane à 6 ans). Les

événements indésirables observés sous obinutuzumab ou sous rituxumab étaient cohérents avec ceux rapportés lors d'études cliniques précédentes dans lesquelles ces traitements avaient été associés à différentes chimiothérapies.

L'essai a inclus 1401 patients atteints de LNHi non précédemment traités dont 1202 patients atteints de lymphomes folliculaires. Les autres patients étaient atteints de lymphomes à zone marginale. *Ces patients ne seront pas étudiés dans ce rapport.*

Les données de pharmacocinétiques ont été collectées à la fin du traitement (induction + maintenance).

2.3. Résultats

Tous les graphiques sont dans la partie principale de ce document. Merci de vous référer aux pages nécessaires pour avoir l'analyse complète des résultats obtenus.

Le but de ce rapport est de confirmer les résultats obtenus par le manuscrit PK d'approche populationnelle et l'analyse faite mettant en évidence les relations entre obinutuzumab et des paramètres démographiques. Seuls les principaux résultats sont mentionnés ici.

2.3.1. Paramètres démographiques et pharmacodynamiques et exposition au médicament

La première partie de cette présentation de résultats obtenus de manière exploratoire est de confirmer en partie les modèles réalisés auparavant au cours du développement clinique d'obinutuzumab. Voici ces paramètres :

- Age

L'âge ne consiste pas en un paramètre influençant l'exposition au médicament. La chimiothérapie associée à obinutuzumab n'a pas non plus d'impact sur les résultats observés.

- Genre

La tendance générale des résultats met en avant une plus importante exposition à la molécule chez les femmes. Cela est confirmé quelque soit la chimiothérapie associée.

- Poids

Les patients avec les poids les plus faibles ont une plus importante exposition au médicament. Cela est également confirmé quelque soit la chimiothérapie associée.

Ces deux paramètres démographiques (genre et poids) constituent déjà des co-variables inclus dans le modèle PK/PD dans des travaux et publications précédentes. Cette première analyse confirme bien les résultats obtenus précédemment.

D'autres paramètres pharmacodynamiques peuvent être également inclus dans le modèle.

- Taille de la tumeur avant traitement et autres paramètres pharmacodynamiques

Les patients avec une faible taille de tumeur à la base présentent une plus forte exposition au médicament. D'autres paramètres sont à prendre en compte comme le nombre de cellules B (ou lymphocytes B) et le niveau et nombre d'expression du récepteur CD20. *Ces derniers ne sont pas étudiés dans ce rapport.*

La deuxième partie de cette présentation de résultats obtenus de manière exploratoire est d'élargir l'analyse en incluant d'autres paramètres relatifs à la pathologie traitée. Voici ces paramètres :

- Index international de pronostic du lymphome folliculaire (Follicular Lymphoma International Pronostic Index ou FLIPI en anglais) à la fin du traitement

FLIPI est un index de score de pronostics concernant les lymphomes folliculaires qui permet d'évaluer et de choisir le traitement le plus approprié pour le patient. La différence de score n'influence pas l'exposition au médicament. Les résultats sont similaires quelque soit la chimiothérapie associée.

- Estimation de la présence de lésions relatives à la pathologie par Tomographie par émission de positron (ou PET en anglais) à la fin de traitement

Un PET-Scan marqué par du Fluoro-désoxy-glucose (FDG) est un test d'imagerie pour mettre en évidence la pathologie et la présence de lésions. Le traceur le plus couramment utilisé pour l'étude des tumeurs est le FDG. C'est un excellent marqueur de l'activité métabolique cellulaire permettant d'identifier les cellules tumorales à forte activité métabolique.

Une réponse positive signifie que le lymphome est toujours actif avec présence de lésions. Au contraire, une réponse négative signifie qu'aucunes lésions ne sont visibles.

Les résultats montrent une tendance différence suivant la chimiothérapie associée à obinutuzumab. Les patients traités par CHOP/CVP avec une réponse négative par PET, c'est-à-dire ne présentant pas de lésions reliées à la pathologie, auraient une plus forte exposition au médicament par rapport aux patients avec une réponse positive par PET. En revanche,

dans le groupe des patients traités par bendamustine, la différence entre les deux groupes ne semblerait pas significative.

- MRD ou maladie résiduelle minimale à la fin de traitement

Tout objectif d'un traitement est d'éradiquer le clone tumoral. L'efficacité peut être suivie par la décroissance de la masse tumorale. La maladie résiduelle est définie par la persistance dans le tissu examiné de cellules malignes en dessous du seuil de détection par les techniques conventionnelles, au terme d'une séquence thérapeutique à visée éradicatrice.

Un des objectifs de tester s'il y a présence ou non de maladie résiduelle minimale est de juger la qualité de réponse au traitement par des techniques de laboratoire qui permet de prédire la durée de rémission. La présence de maladie résiduelle minimale est une des causes majeures de rechute pour les patients atteints d'hémopathies malignes.

Les résultats montrent qu'une estimation de la maladie résiduelle négative ou positive n'influence pas l'exposition au médicament. L'interprétation est identique pour chaque groupe de patients traités par des chimiothérapies différentes.

Toutefois, cette interprétation doit être prise avec précaution étant donné le faible nombre de patients par groupe.

2.3.2. Paramètres cliniques et exposition au médicament

Différents paramètres cliniques pris en compte pour l'analyse exploratoire sont définis comme critères principaux et permettent d'estimer la réponse au traitement : réponse complète, réponse partielle, pathologie en progression, et stabilisation de la pathologie. La réponse clinique est basée sur les critères de Cheson 2007.

Un autre paramètre clinique pris en compte dans ce rapport est la survie sans progression (ou Progression-Free Survival, PFS en anglais). Cela constitue le temps depuis le début du traitement et l'apparition du premier évènement : progression de la maladie ou décès.

Prenant en compte ces deux paramètres, les résultats mettent en évidence une amélioration clinique reliée à une plus importante exposition au médicament.

Il est nécessaire d'être vigilant sur cette interprétation dans le cadre de ce rapport et de cette analyse qui reste exploratoire. D'autres facteurs doivent être inclus dans le modèle. La relation efficacité – exposition au médicament n'est pas toujours plausible et doit être interprétée avec précaution.

En associant paramètres démographiques à l'analyse, des modèles réalisés précédemment ont montré que la variabilité visible en termes d'exposition entre hommes et femmes et chez des patients avec différents poids n'impacte pas l'efficacité clinique. En revanche, en combinant les paramètres pharmacodynamiques et ceux relatifs à la maladie, le rétrécissement de la tumeur a une influence sur la survie sans progression.

Autant de paramètres étudiés révèlent la complexité de l'analyse exploratoire. Cela requiert l'ajout d'autres facteurs qui jouent un rôle en influençant l'exposition à obinutuzumab : le niveau d'expression de cibles CD20 de l'anticorps, du FC Gamma-Receptor (FcγR), récepteur impliqué dans le mécanisme d'action d'obinutuzumab. D'autres facteurs peuvent être cités en relation avec les caractéristiques de la population étudiée et des différences en termes de génétique par exemple.

3- Discussion et conclusion

Le concept de modélisation et de simulation en PK/PD constitue un outil essentiel dans le développement clinique de médicaments et fait partie intégrante du dossier contenant toutes les informations du médicament remis aux autorités de santé. Un des aspects de l'utilisation de ce concept a été illustré par une analyse exploratoire chez des patients traités par obinutuzumab, atteints de lymphomes folliculaires. Pour chaque paramètres étudiés et pouvant influencer l'exposition à obinutuzumab, les résultats restent exploratoires et requièrent d'autres discussions.

Premièrement, comme pour la majorité des anticorps monoclonaux, certains facteurs démographiques influencent l'exposition à obinutuzumab et sont déjà considérés comme des co-variables dans les modèles PK/PD d'approche de population. Les femmes présentent une plus forte exposition au médicament ainsi que les patients à plus faible poids. Des paramètres pharmacodynamiques comme la taille de la tumeur estimée avant traitement, le nombre de cellules B (ou lymphocytes B) ont un impact sur l'exposition au médicament.

Deuxièmement, l'analyse a été élargie à des paramètres relatifs au lymphome folliculaire. De façon générale, les différences en termes de FLIPI score, de réponses au PET-Scan révélant une présence de lésions ou non reliées à la pathologie, ou la présence de maladie résiduelle minimale n'ont pas d'influence sur l'exposition à obinutuzumab. Cependant, dans le groupe de patients traités par CHOP/CVP, chimiothérapie associée à obinutuzumab, les patients présentant une réponse négative au PET-Scan (pas lésions relatives à la pathologie) semblerait avoir une plus importante exposition à obinutuzumab que ceux présentant une réponse positive. Plusieurs hypothèses peuvent être émises. Les caractéristiques de population pour chaque groupe de traitement pourraient différer mais cela ne semble pas le

cas. Le mécanisme d'action des chimiothérapies pourrait également présenter des divergences. Des études sont en cours. L'environnement immunitaire pourrait également jouer un role pour chaque groupe de patients. Enfin, la supposition d'une pathologie résistante avec la présence de cellules tumorales résistantes pourrait expliquer la consommation du médicament chez les patients présentant des lésions relatives au lymphome. Ceci nécessite d'autres nombreuses analyses et réflexions sur le choix de la chimiothérapie associée à obinutuzumab.

Troisièmement, il a déjà été démontré que la variabilité observée entre hommes et femmes et de poids différents sur l'exposition à obinutuzumab n'a pas d'impact sur l'efficacité clinique du traitement. Les résultats obtenus dans ce rapport associant meilleure efficacité à meilleure exposition ne sont que exploratoires et requièrent de construire d'autres modèles plus complexes en incluant d'autres facteurs. Ces facteurs peuvent être intrinsèques et relatifs à la maladie comme relatifs à l'individu (facteurs génétiques, biologiques,…)

Toutes ces réflexions font parties de discussions et permettent la génération de réponses aux questions des Hautes Autorités de Santé à propos de l'impact du genre et du poids sur l'exposition au médicament et d'analyser l'impact sur les conséquences cliniques. Ceci constitue un exemple de l'utilisation du concept de modélisation et simulation PK/PD dans le développement clinique de médicaments.

- Core document -

This Thesis is confidential.

Introduction

For several decades, growing efforts have been made to refine pharmacokinetic/ pharmacodynamic (PK/PD) models to be applied in drug development.

The challenge for pharmaceutical industries is to be more efficient and economical in developing new drugs. Regulators aim to make fast and safe decision to approve lifesaving drugs and make it available to patients quicker. Increasing costs of drug development and reduced pipeline productivity have been growing concerns for new drug development in recent years. A number of potential reasons for this outcome have been considered.

One of the aims of clinical pharmacology is to understand the amount of drug in the body and more specifically the concentration of drug at the effect site, the site of action. The understanding of exposure clinical outcome relationships and efficacy-safety provides the basis for dose recommendations and understanding the corresponding safety margins of a molecule.

Therefore there is a call for the use of alternative tools to get answers on efficacy and safety faster with more certainty and at lower cost. Some of the alternative approaches to drug development include the use of adaptive trial design, more extensive use of biomarkers, developing personalized medicines and the use of PK/PD modelling and simulation. PK/PD modelling simulation can add value in all stages of drug development, from the preclinical discovery stage to late stage clinical development. Its use in drug development, to make crucial decisions early, may lead to significant cost reductions in both early and late drug development, during the whole lifecycle of drug development. [1]

Overall, modelling and simulation is used to make better decision about dose selection without having to do additional expensive and long dose finding studies. Finally, merits of PK/PD modelling are summarized and discussed below, in an effort to raise awareness for its

potential in drug development especially with an illustration of an anticancer drug development, obinutuzumab.

The existence of a variety of statistical techniques for handling complex PK/PD time-varying data should increase the impact of such data analysis on future drug development.
The contribution of pharmacokinetics to overall efficacy and safety assessment of new chemical entities and mature products and decision-making along their preclinical and clinical development is now well-established.

A predictive model of this kind can be used to simulate and hence design clinical trials, find initial dosage regimens satisfying an optimality criterion on the population distribution of responses, and individualized regimens satisfying such a criterion conditional on individual features, such as sex, age, etc.
There is a broad recognition, within the pharmaceutical industry that the drug development process, especially the clinical part of it, needs considerable improvement to cope with rapid changes in research and healthcare environments. Modelling and simulation are mathematically founded techniques that have been used extensively and for a long time in other areas than the pharmaceutical industry to design and develop products more efficiently. Both modelling and simulation rely on use of mathematical and statistical models which are essentially simplified descriptions of complex systems under investigation. It has been proposed to integrate pharmacokinetic and pharmacodynamic principles into drug development to make it more rational and efficient. [2]

Despite major scientific, medical and technological advances over the last few decades, a cure for cancer remains elusive.

In this report, we provide a general description of the concept of PK/PD modelling simulation in drug development and a limited overview of one aspect of the use of PK/PD modelling simulation. This one aspect is illustrated by an exploratory analysis of the drug exposure influenced by different factors regarding the population studied and the pathology treated in a phase III clinical trial. Obinutuzumab has been used as an example to treat patients with follicular lymphoma, hematologic cancer.

We limit the scope in this report further by considering models as a general concept which can include an experimental analysis using PK data and other demographic and disease factors as well as clinical outcomes.

1. Pharmacokinetic/pharmacodynamic (PK/PD) modelling simulation principle in drug development: Obinutuzumab as an example

1.1. Overview of the use of population pharmacokinetic/pharmacodynamic (PK/PD) modelling simulation in drug development

1.1.1. Definitions

Modelling simulation in drug development can be considered as a particular investigation carried out with mathematical, statistical and numerical techniques. The term PK/PD modelling refers to a data PK/PD -driven exploratory analysis based on mathematical and statistical model. A pharmacodynamic (PD) response does not generally parallel drug concentrations: pharmacokinetics (PK); therefore, models can help us understand this relationship and its change as a function of drug intake and other variables.

For a long time, the pharmacological area of **PK and PD** has been considered as separate disciplines.

On the one hand side, **PK** studies are meaningful if there is a known relationship between the described concentrations and the drug's effects and/or side effects. PK describes the drug concentration-time courses in body fluids resulting from administration of a certain drug dose pharmacodynamics the observed effect resulting from a certain drug concentration [3]. It is essential in relating the dosage of drug taken and the quantity available to exert pharmacological action at any given point in time.

On the other side, **PD** only considers concentration-effect relationships without accounting for its temporal arrangement and is thus only valid under the assumption of constant concentrations at the effect side.

They have been the focus of considerable attention because they are vital for linking PK information to measures of activity and clinical outcomes. [4]

A **simulation** is when you use a model to predict something. Instead of estimating parameters from observed data, you take the model, and a set of parameters to simulate some data. Parameters come from the molecule studied, the mechanism of action, its elimination for example, the population targeted by the drug and the characteristics associated with. By merging all the data, it permits to analyze the effect of different factors to highlight whether there is a visible trend and a link between those factors.

Basically, models are simplified descriptions of certain aspects of reality by mathematical means. In case of PK/PD modelling, the biological processes involved in the elaboration of the observed drug effect and regarded with the overall purpose to allow a quantitative description of the temporal pattern of pharmacologic effects, and even more important, a prediction beyond the existing data.

Overall as a global definition, **PK/PD modelling analysis** builds the bridge between the two disciplines, links dose-concentration relationships (PK) and concentration-effect relationships (PD). It facilitates the prediction of the time course of drug effects resulting from a certain dosing regimen. [3]

PK modelling and simulation can be used to predict the pharmacokinetic behavior of drugs in humans using clinical data. It can also explore the effects of various physiologic parameters such as age, ethnicity, or disease status on human pharmacokinetics, as well as guide dose and dose regiment selection and aid drug–drug interaction risk assessment. [5]
Indeed, modelling and simulating changes in PK in subjects may help to guide appropriate clinical dosing.

Modelling simulation analysis is initially a model built with only few patients or healthy subjects. Then, PK/PD models are continuously updated throughout different stages of drug development to incorporate relevant new data. A full simulation model will typically consist of a number of sub models which will include aspects such as dose response, time response, baseline response, other covariates response, disease progression, compliance, variability, sample size and commercial aspects. [1]

Modelling can be a complementary tool in deployment of other new approaches in drug development, such as adaptive trial design, personalized medicine and extensive use of biomarkers. Indeed, modelling is an important tool to guide adaptive study design.

In order to simulate a clinical trial, knowledge of drug action, disease progression and subject variability is required.

A **covariate distribution model** describes the distribution of the covariates in the target trial population and their relationships to other covariates. It reflects the expected frequency distribution of the various covariates such as age and bodyweight. These covariates may be tested by correlation with PK and PD parameters, which are often represented by appropriate distributions, typically arise from the random differences between individuals from one occasion to another.

The pharmacodynamics of some drugs may be subject to genetic variation with respect to the sensitivity of a drug's target to its action. Many genes which encode such target sites exhibit genetic polymorphisms which alter their sensitivity to particular pharmacological treatment. Covariate distribution models are useful in such circumstances for describing the prevalence of certain forms of genetic polymorphism and their influence on drug characteristics.

Input-output model incorporates all scientific knowledge about the disease and drug. It may include different types of models. The model used for this work is a covariate model. It serves to integrate patient-specific features (covariates such as age, weight etc) that are associated with systematic differences between individuals. Covariate models are used to predict model parameters typical of an individual with a particular combination of covariates. The distribution of demographic covariates in the trial subject sample is obtained from a model for the distribution of covariates in the target trial population. Such a model reflects the expected frequency distribution of the various covariates and more importantly, the relationships among the covariates. [5]

All these notions are used in the report.

1.1.2. Objectives and rationale

The integration of pharmacokinetic and pharmacodynamic principles into drug development has been proposed as a way of making it more efficient. The use of these principles in drug development to make scientific and strategic decisions is defined as the "pharmacokinetic-pharmacodynamic guided approach to drug development" as proposed by Holford. [3]

The objective of this report is to understand the use of PK/PD modelling simulation in drug development, to analyze the impact of the PK/PD results on clinical drug development. One of the PK/PD modelling aspects will be illustrated by the obinutuzumab example, a monoclonal antibody used in the treatment of lymphomas.

One of the main aims of PK/PD and PK clinical outcome relationships is to provide the dosing recommendation for labeling perspective. The expanded use of PK/PD modelling is

assumed to be highly beneficial for drug development as well as applied pharmacotherapy and will most likely improve the current state of applied therapeutics. [3]

A number of covariate factors, such as height, weight, age, race, and gender, are known to affect drug pharmacokinetics. It is also known that blood flow and body composition; for example, vary with age, race and gender. A sound understanding of the influence of factors such as concomitant medications, chemotherapy combined is also important to better understand the relationships with the drug exposure. They may have a potential impact on the PK/PD of inter-individual variability on pharmacokinetics. Some of these variables are used for the exploratory analysis described further. To put into context with clinical practice, understanding covariates and impact on exposure variability is important to understand the need for dose adjustments if needed, to improve the safety and efficacy of a drug agent and then provide recommendation to prescribers.

Special attention has been given to the PK/PD modelling of monoclonal antibodies (mAb) because of their highly complex pharmacokinetics with non-linear disposition and elimination. As a common property for all mAb, the elimination mechanisms which can be highlighted are a linear clearance and target mediated drug disposition (TMDD). The PK/PD models have been valuable in understanding the mechanisms behind the complex pharmacokinetics.

Hence, after defining the main uses of PK/PD modelling simulation in drugs development ***(please refer to section 1.1.3. Benefits and uses of PK/PD modelling simulation in drug development),*** we evaluated the sources of variability including the obinutuzumab exposure and different parameters related to patients included in a clinical trial, the pathology studied and the clinical outcomes.

The **first aim of this exploratory analysis with obinutuzumab** is to confirm what has been already done in the previous population PK report ***(please refer to section 1.2.1. Pop PK analysis***) including different parameters: demographic and PD parameters. It has been described that demographic parameters such as body weight and gender are covariates influencing the obinutuzumab exposure. As a basic property for mAb, the steady-stage clearance and volume parameters increase with body weight. Then, baseline tumor size, disease type as well as subtype are also considered as covariates in the model.

Tumor size affects obinutuzumab exposure. Initial time-dependent clearance is higher in patients with high tumor size. Then, when the saturation stage is reached, with higher clearance for higher tumor burden and higher CD20 expression, exposure level decreases with elimination of target cells. This is consistent with the PK of mAb and the elimination mechanism by TMDD.

Secondly, the goal is to extend the exploratory analysis done with demographic and PD parameters by including other new parameters more related to the disease treated: follicular lymphoma. These parameters will be described further in the report.

PK data as exposure values here might be taken into account as a cause or a consequence to explain whether the factors studied influence clinical outcomes. The exposure-efficacy relationships will be described as a **third part of this exploratory analysis**.

All the results will be split into different chemo-backbones and regimens associated with the molecule.

1.1.3. Benefits and uses of PK/PD modelling simulation in drug development

PK/PD modelling simulation has many roles and impacts on clinical development.

In the pharmaceutical industry, PK/PD modelling simulation is used for purposes, such as mechanistic studies, aiding internal drug discovery or clinical development decisions, and informing regulatory communication including filing at various stages. It is mostly applied at the development stage.

Overall, modelling can add value in all stages of drug development. To use PK/PD modelling and simulation in its optimal potential for drug development, models should be developed early in program development, continuously updated and refined as more data become available. Their validation is necessary during development and they will then provide valuable support to make important decisions with an increased confidence level around the analyzed data. [1]

Modelling has a first benefit in preclinical phase. Indeed, PK/PD models are routinely applied from the early discovery stage (at the lead optimization stage of drug discovery as well as at the candidate selection stage of drug discover), where there is limited data captured for any compound of interest, to late drug development, where large amounts of data are available. For example, a drug candidate should be partially metabolized by polymorphic enzymes; then a PK/PD modelling can provide human PK prediction at clinical dose and dose schedule. Once the target effect is identified, the focus of modelling and simulation can fully move on the optimization of the study design to demonstrate robustly the effect and reduce the risk of failed study design.

Other benefits of modelling are showed in late clinical development. As we use a phase III study in this report, this phase III study provides the final confirmation of the efficacy and

safety of the tested drug in a wide patient population of interest. They provide the ultimate safety and efficacy data for approval of drug's use in clinical practice. Then, modelling and simulation can use both efficacy and safety data to build adequate models.

Modelling in Phase III can also be used to assess the impact of applicable covariates, validate the population PK/PD model, establish or confirm dose exposure-response relationship in target population, assess need for dose adjustment in special population.

Among PK data, exposure to drug is a well-known parameter determined from early stages. This is another aspect of the modelling simulation use.

Although the use of an exposure–response evaluation to replace a pivotal trial is not common, population PK modelling and exposure–response evaluations are frequently used to support registration decisions and labelling. This is because population PK modelling enables the identification of the sources of variability that ultimately have an impact on both safety and efficacy.

As a general aspect and focused on the interest for patients, modelling and simulation also play a large role in personalized medicine. Personalized medicine aims to provide more accurate predictions of individual responses to therapy based on the characteristics of the individuals.

They can be used to simulate alternative dose regimens, allowing for informed assessment of dose regimens before study conduct.

Modelling can be a complementary tool in deployment of other new approaches in drug development, such as adaptive trial design and extensive use of biomarkers. Indeed, modelling is an important tool to guide adaptive study design.

Pharmacodynamic response used in PK/PD analyses is often derived from the effects on various biomarkers. Modelling is, therefore, highly dependent on the quality of PD data and the level of confidence in biomarkers used in PK/PD analysis.

There is an obvious incentive for having a greater number of validated and qualified biomarkers to aid refinement of PK/PD models.

Overall, PK/PD modelling can be categorized into three major roles that can be used to inform regulatory communications, that have impacted clinical development decisions and that promote the mechanistic understanding of clinical observations. [4]

PK/PD modelling and simulation can be an invaluable tool for making crucial decisions for drug development. These may include decisions on compound selection, dose selection, study design, study design or patient population.

The use of PK/PD modelling and simulations for regulatory submission should be considered and discussed with the regulators as early as is feasible. [1] This is part of dossier required for Health Authorities to approve new drugs.

This summarizes the use of PK/PD modelling simulation in drug development.

Through systems biology and systems pharmacology we are likely to identify a number of different key targets in a disease, each of which may require specific pharmacological intervention in order to achieve optimal efficacious and safe treatment for patients. This is a different approach than PK/PD models this has started to become the future for modelling and simulation, the disease modelling. *This aspect will not be part of this report.*

One of the aspects of PK/PD modelling simulation is a Population PK (Pop PK) analysis which will be introduced in the following part of the report.

1.2. Introduction to a Population PK (Pop PK) analysis with the obinutuzumab example

1.2.1. Pop PK analysis

A **primary goal** of most **Population Pharmacokinetic (Pop PK)** modelling evaluations is finding Pop PK parameters and sources of variability in a population.

The Pop PK approach can be used to estimate population parameters of a response surface model in phases I, II and III of clinical drug development. The Pop PK model employs certain inferential approaches which focus on providing estimates of some or all of the components of variability along with estimates of the mean PK parameters. It is used to describe the time course of drug exposure in patients and to investigate sources of variability in patient exposure.

In general, potential covariate-parameter relationships were identified on the basis of scientific interest, exploratory analysis and exploratory graphics and were added to the full model. Only the most plausible covariates were incorporated. The link between the covariate effects and their clinical relevance was based on parameter estimates. They can be used to simulate alternative dose regimens, allowing for informed assessment of dose regimens before study conduct, one of the uses of modelling simulation already mentioned above.

The PK/PD models used, their parameter values and the use of study designs and data analysis method are included in the Pop PK analysis.

The molecule obinutuzumab will be taken into account as an illustration of those PK modelling and Pop PK concepts.

1.2.2. Obinutuzumab generalities

1.2.2.1. Obinutuzumab clinical development

Obinutuzumab is one of the treatments for follicular lymphoma patients (***please refer to section 1.2.2.1.1.2. Follicular lymphoma***). Many studies have brought obinutuzumab as an excellent molecule in hematology in the treatment of lymphomas. It has been approved in the United States to treat two common types of blood cancer. Obinutuzumab is also used in combination with chlorambucil for people with previously untreated chronic lymphocytic leukemia (CLL) based on data from the pivotal CLL11 study, which compared obinutuzumab plus chlorambucil head-to-head with rituxumab plus chlorambucil. Other studies were undertaken as GADOLIN, GOYA, and GALLIUM. The data used for this report are extracted from the GALLIUM study (***please refer to section 1.2.2.1.2. GALLIUM study***).

1.2.2.1.1. Pathology

1.2.2.1.1.1. Non-Hodgkin Lymphoma

Non-Hodgkin's lymphoma (NHL) is the most common hematologic malignancy in adults. The majority of NHLs is of B-cell origin and is characterized by the expression of a membrane antigen, CD20, which is important in cell cycle initiation and differentiation. B-lymphocytes are the cellular origin of humoral immunity, represent a substantial portion of hematopoietic malignancies and contribute significantly to autoimmunity and transplant rejection. Overall, lymphomas are characterized by an ongoing pattern of relapse and are usually incurable in their advanced stages.

According the international classification of World Health Organization (WHO), NHL can be divided into aggressive and indolent NHL (iNHL). iNHL subtypes progress slowly. They make up about 40 percent of all NHL cases in the United States. The disease type analyzed in

this report is the iNHL, especially the subtype follicular lymphoma (FL) which is the most common type of iNHL and the most common subtype of indolent B-cell lymphomas.

1.2.2.1.1.2. Follicular lymphoma

Follicular Lymphoma (FL) is the most common subtype of iNHL and is associated with follicle center B cells that typically contain the BCL-2 chromosome translocation t(14:18) which leads to overexpression of the intracellular anti-apoptotic protein BCL-2. FL is generally an indolent B cell lymphoproliferative disorder of transformed follicular center B cells. FL is characterized by diffuse lympho-adenopathy, bone marrow involvement, splenomegaly, and less commonly other extra-nodal sites of involvement. About 1 out of 5 lymphomas in the United States is a FL according American Cancer Society. The symptoms frequently described are enlargement of the lymph nodes in the cervical, axillary, abdomen, or inguinal, as well as fatigue, and weight loss. Often, patients with FL have no obvious symptoms of the disease at diagnosis.

1.2.2.1.2. GALLIUM study

GALLIUM study is an international, open label, randomized, phase III study. 1397 patients were enrolled with previously untreated FL or chemotherapy-naïve marginal zone lymphoma (MZL).

The study is divided into three phases, induction and maintenance as well as follow-up & observation. 1202 patients were analyzed with previously untreated iNHL CD20 positive (especially FL patients - *but also 200 Marginal Zone Lymphoma patients who will not be discussed in the report*) were randomized to treatment into two arms. This is a phase III head-to-head comparison of CD-20 antibody obinutuzumab versus rituximab. This study strategy was to use induction therapy with a CD20 antibody (obinutuzumab 1000 mg IV or rituximab

375 mg/m² IV) and chemotherapy (bendamustine, CHOP or CVP) for 6 months followed by maintenance therapy with the CD-20 antibody (obinutuzumab or rituximab) for 2 years.

The **primary endpoint** was 3 year-progression-free survival (PFS) and the **second endpoints** were the complete response of treatment, the overall ratio response, the efficacy-free survival and safety.

The primary analysis population for efficacy is the intend-to-treat follicular population, defined as all randomized patients with follicular histology. Patients have been analyzed according to the treatment arm to which they were randomized.

This trial was designed to establish obinutuzumab as the superior activity CD20 versus rituximab when each was combined with chemo in over 1202 patients with previously untreated advanced FL.

Results have shown that patients in the obinutuzumab arm had significantly improved Progression-Free Survival (PFS) when compared to those in the rituximab arm.

Different disease parameters were analyzed such as Positron Emission Tomography (PET) complete response rate, Minimal Residual Disease (MRD), Follicular Lymphoma International Prognostic Index (FLIPI). *PET and MRD responses are only qualitative response, yes or no. These terms will be defined further* ***(Please refer to section 3.1. Disease parameters: definitions)***.

Main results show that obinutuzumab based treatment provides a higher rate of PET-complete response at the end of induction. With obinutuzumab–chemotherapy associated, patients achieve MRD negativity at a rate of 92% at the end of induction (against 85% with rituximab).

An exploratory analysis determined that regardless of therapy choice, MRD-negative patients at the end of induction experienced greater improvement in PFS vs MRD-positive patients.

No new safety signals were observed with obinutuzumab in the FL safety population, and treatment discontinuation due to adverse events was comparable for both treatment arms.

1.2.2.2. Obinutuzumab PK

The drugs studied in this paper are two **monoclonal antibodies (mAbs)** with **specific properties**.

Typically, as for mAbs, the steady-state clearance and volume parameters increased with body weight. Linear, time dependent clearances and central volume were also higher in males. [7]

Typically, PK/PD modelling utilizes the time course of the drug concentration in plasma as a measure of internal exposure. This is important because drug concentration versus time profiles can differ widely between drugs, and for the same drug, between species and individuals.

Regarding to **the collection of PK data**, the plasma concentration-time course of a drug is determined by the pharmacokinetic process of distribution, metabolism and excretion as well as absorption in case of no systemic administration. The currently used pharmacokinetic models can basically be distinguished into compartmental, physiological and statistical models. Compartmental models are the most frequently preferred, probably due to the fact that they provide a continuous concentration-time profile in a body fluid that can be related to a continuous effect-time profile. The effect compartment concept can also easily be implemented. [3]

Modelling requires specialized software and experienced analysts.

The association between various covariates and individual parameters was evaluated by graphical exploration followed by testing within **NONMEM** (non-linear mixed effects modelling) with a stepwise covariate modelling procedure. The key element is the ability to add random variability to the model as residual error. NONMEM, a software package for population pharmacokinetic modelling, has a comprehensive library of pharmacokinetic models but requires the user to create a data file to specify doses, covariates and observation times for each subject. This development of modelling/fitting software were based on solid experimental data with more and more opportunities for measuring effects and the existence of reliable assay methodologies for assessing drug levels in biological fluids. This is a well-established mathematical sub-models.

Overall, this software is one of the main tools used in PK/PD modelling simulation. However, to undertake the exploratory analysis, other software have been used as the aim was not to build the model but confirm and extend the analysis with some co-variables already known.

The **PK of obinutuzumab** was accurately described by a two-compartment pharmacokinetic model with two clearance mechanisms, one time-dependent clearance and the other linear.

A covariate modelling approach emphasizing parameter estimation was implemented for the covariate model development. [3]

A PopPK analysis using data from six clinical trials in patients treated by obinutuzumab has already been realized including different variables and parameters. It results in the

establishment of a two-compartment model with linear and time-dependent clearance components that describes PK characteristics of the molecule in the target population.

Unlike rituximab, a fixed dose has been tested in patients treated by obinutuzumab. This is certainly more convenient for patients and clinical practice but it means that it is important to take into account inter-individual variability of PK and PD, especially focusing on body weight.

As results in PK and some are already mentioned in the section ***1.1.2. Objectives and rationale,*** obinutuzumab time course is well described by a two compartment PK model with total clearance being the sum of time-independent and time-dependent clearance pathways. Pop PK has shown that obinutuzumab catabolic clearance increased with body size while the elimination of obinutuzumab through its target, target-mediated drug disposition (TMDD) component, is not affected by body size.

The mean obinutuzumab concentration at the end of treatment (Cmean) was used to represent the obinutuzumab exposure.

Using data from GALLIUM study, the exploratory analysis was realized by looking at relationships between exposure and demographic, pharmacodynamics, disease and efficacy parameters. It was also split by chemotherapy backbones and regimens used in the treatment of follicular-patients: bendamustine and CHOP/CVP.

1.2.3. Methods

A clinical trial is characterized by different steps which include PK/PD data. From early development to late development, PK/PD data are collected and can contribute to the analysis of different studies endpoints.

PK/PD modelling simulation includes a population PK analysis and different covariates in the model. The variables and clinical data used for the exploratory analysis are described in this section.

The results are analyzed and validated by statistical methods.

1.2.3.1. Target population and pharmacokinetic data collected

The GALLIUM trial studied a large population whose age and disease characteristics are inclusive of a broad range of patient types studied in prior trials of previously untreated advanced FL.

Regarding the analysis done within this report, we only selected FL patients treated by obinutuzumab with PK data collected at the end of treatment (induction plus maintenance) in order to simulate PK model influenced by different covariates. As PK data, serum obinutuzumab mean concentrations were analyzed using a validated sandwich enzyme-linked immunosorbent assay: Cmean in µg/mL.

This analysis has been realized by splitting into different demographic and disease parameters which are or not significant covariates for the drug exposure.

Looking at **population characteristics** split into chemotherapy, patients treated by CHOP/CVP are younger and have lower weight whereas patients treated by bendamustine are older and have higher weight. Overall the difference is not significant. The population baseline characteristics studied for this exploratory analysis is similar to the whole target

population studied in GALLIUM trial. This confirms that the population is representative of the GALLIUM study.

1.2.3.2. Exposure-demographic parameters, exposure-pharmacodynamic outcomes and exposure-disease factors relationships for FL-patients (GALLIUM study)

Relationships between exposure and demographic parameters were explored in this report: age, gender, body weight, Body Surface Area (BSA), Body Mass Index (BMI).

Relationships between exposure and PD outcomes were also explored: baseline tumor size and B-cell counts.

Relationships between exposure and disease factors were analyzed: FLIPI score, PET and MRD responses at the end of treatment.

1.2.3.3. Exposure-efficacy analysis for FL-patients (GALLIUM study)

Clinical outcomes as Overall Survival (OS), Progression-free Survival (PFS) were collected over time ***(the terms will be explained further, please refer to section 3.3 Exposure-efficacy relationships and impact on clinical outcomes)***

Regarding the exposure-efficacy analysis, relationships between PFS and exposure were assessed by comparing exposure in patients with and without events (progression, relapse, or death). This determines PFS.

Relationships between clinical response and exposure were assessed by looking at patients with complete response, partial response to treatment, stable disease or progressive disease.

The clinical response is based on **Cheson 2007 response criteria.** These criteria highlight the lesions in patients for malignant lymphomas with a response assessment:

- Complete response (CR)

This means that there is a complete disappearance of all detectable clinical evidence of disease and disease-related symptoms if they were present before therapy. The spleen and/or liver should be considered normal size by imaging studies and nodules related to lymphoma should disappear.

- **Partial response (PR)**

This means that at least a 50% decrease in term of sum of the product of the diameters (SPD) for tumoral masses.

No increase should be observed in the size of other nodes, liver, or spleen.

- Progressive disease (PD)

This means that cancer is still growing, spreading or getting worse.

- **Stable disease (SD)**

A patient is considered to have SD when he fails to attain the criteria needed for a CR or PR, but does not fulfill those for PD.

Typically for lymphomas-patients the PET should be positive at prior sites of disease with no new areas of involvement on the post-treatment CT or PET. [8]

1.2.3.4. Statistical analysis

A statistical analysis method which can accommodate different follow-up times among patients with inclusion of drug concentration data as additional covariates is based on the relative risk regression approach and p-value analysis.

In a PK/PD modelling simulation and exploratory analysis, the exposure-response relationships for Progression-Free Survival (PFS) and Overall Survival (OS) are described by Cox-proportional hazards analysis models.

This statistical analysis with Cox-models has not been used to validate the results obtained for this obinutuzumab exploratory analysis.

In this report, the graphical analyses of exposure-different parameter relationships in patients who received obinutuzumab in GALLIUM study were performed on 408 patients with Cmean as drug exposure collected at the end of treatment.

The graphical analyses of exposure-different parameters relationships have been performed on these 408 patients integrating different variables. Boxplots, scatterplots and Kaplan-Meyer curves have been realized by R studio. The most relevant plots to add in this report have been selected.

The programming has been done by using a statistical analysis with R studio: t-test, anova-test and log-rank depending on the variables number. A test is considered significant whether the p-value is lower than 5%. It is essential to say that a p-value does not provide information on clinical relevance of effect.

As a disclaimer, one of the aspects of this statistical analysis done within this report is that we cannot conclude properly to the results. A trend is only shown and it is important to be careful about the results interpretation. Other values as confidential intervals, hazard ratios

and a Cox model analysis (stratified by chemotherapy backbone and including several covariates) should have been added but the analysis' aim was not to realize a proper statistical analysis.

2. Exploratory analysis of the obinutuzumab exposure influenced by demographic and pharmacodynamic parameters and chemo backbones combined in follicular lymphoma patients in the GALLIUM study

2.1. Exploratory analysis: follicular lymphoma and treatments studied

2.1.1. Immunotherapies

Treatment modalities for FL include **chemotherapy, radiotherapy and immunotherapy** against CD20, and stem cell transplantation, but a standard treatment approach for FL has not been established.

CD20 mAbs have been classified as type I or type II depending on their functional characteristics. Type I as rituximab exhibits strong Complement-dependent Cytotoxicity (CDC) activity but weak apoptosis whereas the type II as obinutuzumab shows weak CDC and strong apoptosis.

2.1.1.1. Rituxumab

Rituximab is a chimeric murine/human monoclonal antibody (mAb) and was the first anti-CD20 mAb approved that binds specifically to the transmembrane antigen CD20. Rituximab exerts its therapeutic effect by promoting B-cell lysis, inducing a rapid and sustained depletion of peripheral CD20+ B cells by binding at this antigen.

Effects are mediated through direct induction of cell apoptosis, complement dependent cellular cytotoxicity and antibody dependent cellular cytotoxicity (CDC).

The biologic activity of rituximab has been correlated to the degree of CD20 antigen expressed on the surface of malignant B-cells and to the capacity of effector cells to bind to rituximab's Fc region. [9]

Rituximab is an anti-CD20 monoclonal antibody used for the treatment of hematologic malignancies. Rituximab plus chemotherapy CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone) has become the standard-of-care treatment FL, diffuse large B-cell lymphoma (DLBCL) and CLL.

Several studies were undertaken to establish dose and dose scheduling. The dose 375 mg/m2 was selected for further clinical evaluation. Several inter-individual variabilities were shown.

A new generation of anti-CD20 mAb designed to improve the efficacy of rituximab are currently under clinical evaluation with already approved indications.

2.1.2.1. Obinutuzumab

Obinutuzumab is a novel glyco-engineered type II humanized anti-CD20 monoclonal antibody. It has been developed to have superior efficacy to Rituximab. Obinutuzumab is glycol-engineered to enhance the blinding affinity to FC gamma Receptor (FcγR) on effector cells such as MIK cells neutrophils, and macrophages/dendritic cells. [9]

The molecule targets CD20 epitope and have an antibody-dependent cellular cytotoxicity (ADCC) activity.

It is provided as a single-dose and consists of 25 mg/mL drug substance.

Obinutuzumab has been approved in 2016 plus bendamustine chemotherapy followed by obinutuzumab alone as a new treatment for people with follicular lymphoma who did not respond to rituximab-containing regimen, or had their follicular lymphoma return after such treatment.

Overall and as a summary, both drugs have antibody-dependent cellular cytotoxicity (ADCC) activity and target CD20. However, some differences can be highlighted.

They indeed differ in two key characteristics: the nature of the IgG subclass which modifies FcγR-dependent effector functions, and the angle of CD20 epitope recognition, which modifies the ability to trigger CDC and direct cytotoxicity. Obinutuzumab has a greater direct cell death induction and ADCC/ADCP activity than rituximab.
Rituximab does not recognize the same CD20 epitope as obinutuzumab and is human IgG1 non-Fc-optimized antibody, two characteristics independent from each other and having functional and pharmacological consequences that need to be taken into consideration when trying to compare the two drugs. Rituximab translocates CD20 into lipid rafts which are the CDC activity. Obinutuzumab does not translocate CD 20 into lipid rafts, no CDC activity is involved. Obinutuzumab can better recruit MK cells and macrophages. This means that any CD20 modulation will not impact the obinutuzumab exposure.

There are also differences in terms of dosing and regimen. Rituximab success has demonstrated that the chosen dose was relevant for most patients.

By looking at these differences between these two molecules, it means that it is still needed to be careful when the two drugs are compared in order to better understand the exposure-clinical outcomes relationship.

In GALLIUM study and in FL patients obinutuzumab can be associated with chemo backbones

2.1.2. Chemo backbones and regimens combined

Rituximab- Bendamustine, Rituximab- CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone), Rituximab- CVP (cyclophosphamide, vincristine and prednisone) are currently recommended as first line treatments for follicular lymphoma. In other trials and especially the GALLIUM study, study of interest in this report, the same association has been done with obinutuzumab during the induction period.

2.1.2.1. Bendamustine

Bendamustine is a bifunctional alkylating agent. It primarily targets base excision repair pathways rather than mismatch repair pathways, and it activates DNA-damage stress responses, apoptosis, inhibition of mitotic checkpoints, and induction of mitotic catastrophe [10]. As for other alkylating agents, the toxicity of bendamustine (nausea and vomiting, alopecia or myelosuppression) is low. Bendamustine induces DNA strand breaks as well as apoptosis [10] [11].

2.1.2.2. CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone), CVP (cyclophosphamide, vincristine and prednisone)

CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone), CVP (cyclophosphamide, vincristine and prednisone) are the acronyms for a chemotherapy regimen used in the treatment of NHL.

Regarding the dose, different rhythms of administration are described according the chemotherapy combined with. It is mandatory to administer a total of six 28-day cycles of bendamustine whereas it is mandatory to administer a total of six 21-day cycles of CHOP.

Preliminary results in some studies indicate that rituximab plus bendamustine was generally more effective than rituximab plus CHOP in the first-line treatment of follicular lymphoma, other indolent lymphomas or mantle-cell lymphoma [7].

2.2. Exploratory analysis: procedure and tools

2.2.1. Rationale

Several models have been already done with the two anticancer drugs in the treatment of lymphomas such as rituximab and obinutuzumab with other clinical trials studied including patients treated by this molecule. As already mentioned, these two antibodies are similar but the mechanism of action differs. The literature has already shown relationships between rituximab PK/PD parameters and several manuscripts and publications have also demonstrated first PK/PD modelling analysis including obinutuzumab using several clinical trials.

Rituxumab is the one the drug used in the treatment of Non-Hodgkin lymphoma with chemotherapy or it may be used by itself. We already know that given the tolerability of rituximab event at higher or more frequent dosing and with extended therapy optimizing efficacy should be achievable without significant toxicity. Regarding rituximab, it is generally admitted that the dose and the schedule of its administration is largely empiric, based on the lack of dose-limiting toxicities and dose-response relationship observed in phase I. The current dosing of rituximab in follicular lymphoma (FL), a subtype of Non-Hodgkin lymphoma, is 375 mg/m2, 1 x 21 or 28 days for 6-8 cycles followed by maintenance. This is not extended to the novel humanized anti CD20 monoclonal antibody, obinutuzumab, which has been approved for use of combination with bendamustine in patients with rituximab refractory follicular lymphoma. Within the label, the recommended dose and regimen for

obinutuzumab in patients with FL consists in a fixed dose of 1000mg given on Day 1, Day 8 and Day 15 during Cycle 1 and then every 21 or 28 days on Day 1 of subsequent cycles [12] [13].

Preclinical data show that obinutuzumab has superior efficacy over rituximab at the same dose of mAb, indicating that enhanced clinical efficacy may not be simply related to the higher mAb dosing of obinutuzumab (1000 mg) compared with standard rituximab dosing (375 mg/m2 in NHL and 375 then 500 mg/m2 in CLL).

While the role of the innate immune system in "responsiveness" to rituximab-chemotherapy regimens still in debate, it is necessary to identify and evaluate other surrogate markers that can be utilized to predict clinical benefit. Our group of investigators has studied the role of neutrophils and natural killer (NK) cells in the biologic activity of rituximab. [9]

The described dose above has been determined based on efficacy and safety data and PK profile to ensure full target saturation throughout the entire dosing period.

Generating databases for population analysis is one of the most critical and time-consuming portions of the evaluation and is considered as the first step to undertake the exploratory analysis for the report. Dataset was created by selecting only patients enrolled in GALLIUM study who have PK data and especially the mean concentration at the end of treatment as Cmean (µg/mL) as the exposure to obinutuzumab. That's why the analysis has been realized in 408 follicular lymphoma-patients treated by obinutuzumab ± chemo backbones instead of those 1202 patients enrolled in GALLIUM study. Cmean (µg/mL) was calculated as the ratio of cumulative Area Under the Curve (AUC) up to the time of the last dose of the maintenance period (end of treatment) on duration of this time interval.

2.2.2. Spotfire

Spotfire is data visualization and analytics software. This is a platform which contains all the data regarding a clinical study. Tables cover data discovery, interactive data visualization, geocoding, survey analysis, social analytics, and real-time event analytics.

Spotfire TIBCO has been used for the PK/PD exploratory analysis for extracting data such as demographic parameters within patient's profile, disease parameters within baseline disease characteristics, lymphoma status, clinical outputs within tumor response, responses to treatment.

2.2.3. R studio

The influence of demographic, disease factors on drug exposure and the relationships with efficacy endpoints were analyzed using exploratory graphical analyses.

Models were evaluated graphically using R studio.

R studio is a programming language for statistical computing and graphics.

Degrees of regression were evaluated by calculating the correlation coefficient. Individual concentrations were simulated using patients' individual PK parameters, the exposure, that's to say Cmean at the end of treatment (end of induction + maintenance). Cmean was used as the mean measure of obinutuzumab exposure.

Results were computed using PK parameters from the final population PK model. Cmean was calculated as the ratio of cumulative Area Under the Curve (AUC) up to the time of the last dose of the maintenance period on duration of this time interval. Tertiles of Cmean (low, medium, high) were used to reflect variability in exposure among patients.

2.3. Demographic parameters – obinutuzumab exposure relationships

The aim was to identify whether the results obtained for this report match with the Pop PK analysis already done regarding the demographic parameters with obinutuzumab exposure example. This is important to highlight that the data have been compared from the final Pop PK model already done. The analysis has been split into chemotherapy regimen to have a look at the choice treatment effect.

For each parameter studied and analyzed, the data obtained and the graphical analyses with the Pop PK have been cross-checked. Then, the second aim was to extend the analysis including new parameters such as the disease parameters.

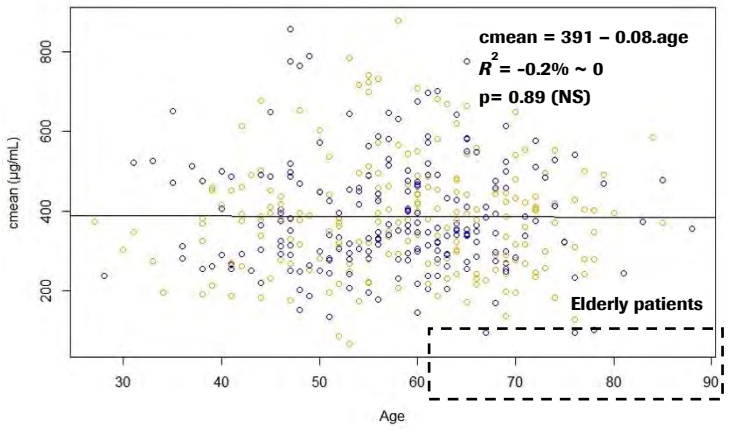
As the number of patients treated by CVP is low, we decided to take into account both patients treated by CHOP and CVP.

This part is a description of the results obtained by doing this exploratory analysis using data available on Spotfire and using R studio by creating boxplots and doing the statistical analysis.

2.3.1. Age

The first variable used within the analysis is the age. The average age is 58 years old in the target population. As GALLIUM study has enrolled more elderly patients, it was more relevant to look into this population.

A. All patients



Patients treated by obinutuzumab and Bendamustine

Patients treated by obinutuzumab and CHOP/CVP

B. Elderly patients

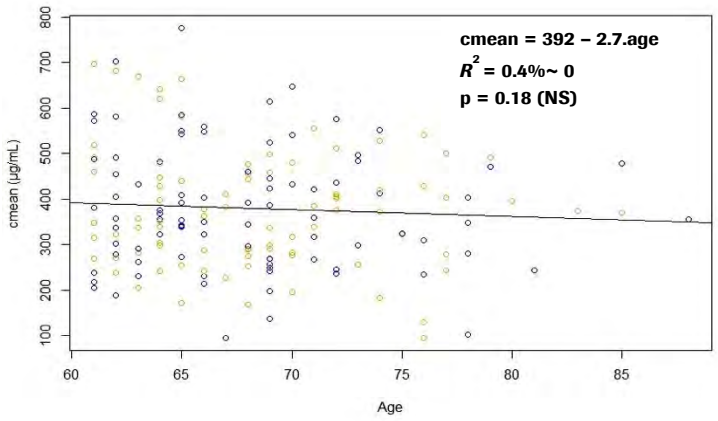


Figure 1:
A. Relationships between cmean (µg/mL) and age (years) in all patients
B. Relationships between cmean (µg/mL) and age (years) in elderly patients
R^2 represents the correlation coefficient
Cmean = a – b.age is the linear equation of the regression line and "b" is the slope coefficient
P-value is significant (***) if lower than 5% / NS means non-significant

Graphical analyses show no correlation between obinutuzumab exposure and age when all follicular lymphoma-patients are included as well as elderly patients. Indeed, the correlation coefficient is close to zero, the slope coefficient is very low (-0.08 and -2.7) as well as the p-value is non-significant (<5%). The same results could have been highlighted in patients treated by

obinutuzumab and bendamustine as well those treated by obinutuzumab and CHOP/CVP. Chemo backbone seems to do not impact the exposure.

2.3.2. Gender

The second variable analyzed is the gender.

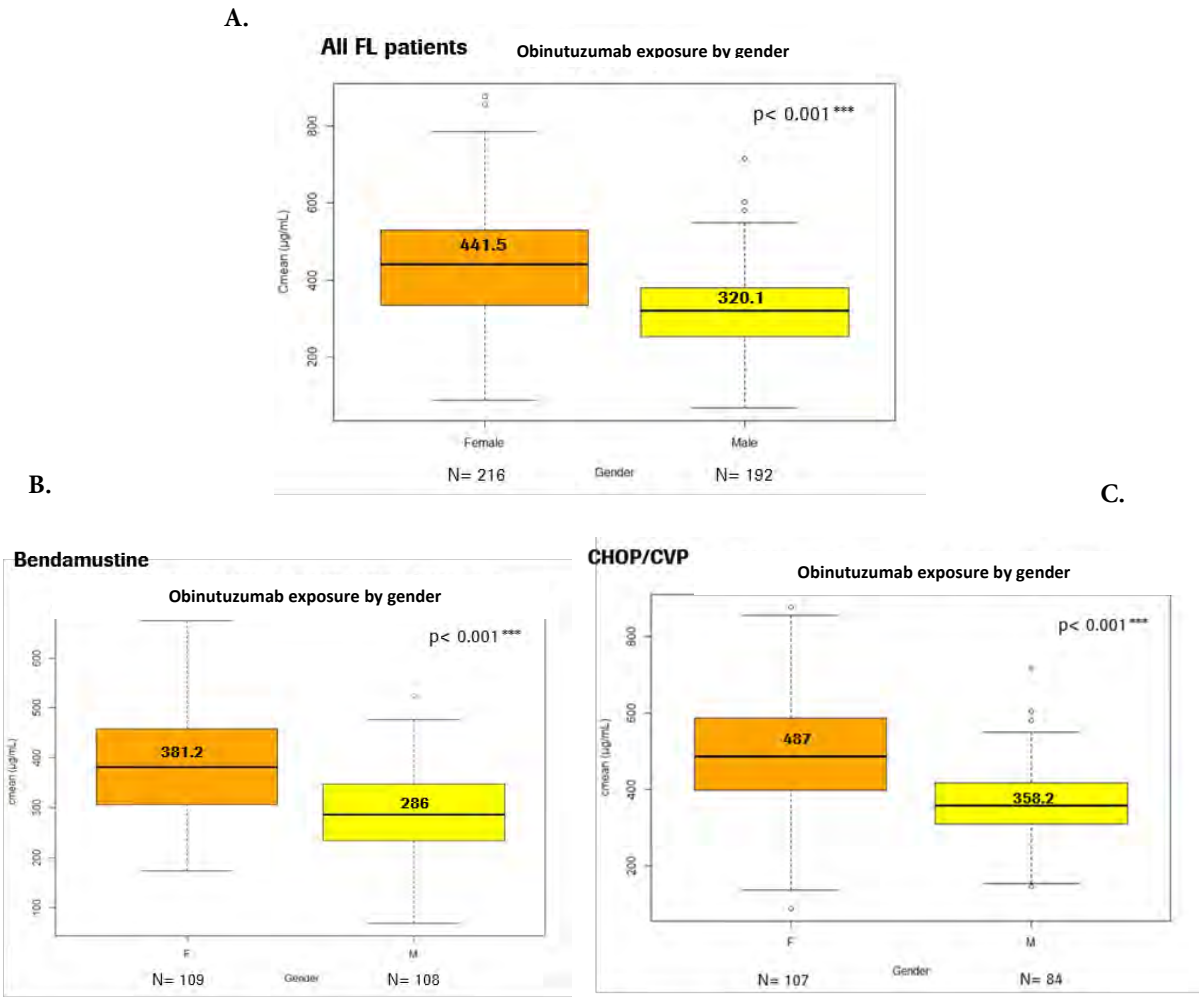


Figure 2: Relationships between cmean (µg/mL) and gender (F/female – M/males) in all patients with chemotherapy (A), patients treated by obinutuzumab and bendamustine (B) and patients treated by obinutuzumab and CHOP/CVP (C)

The Cmean median is indicated and compared (µg/mL)

P-value is significant (***) if lower than 5%

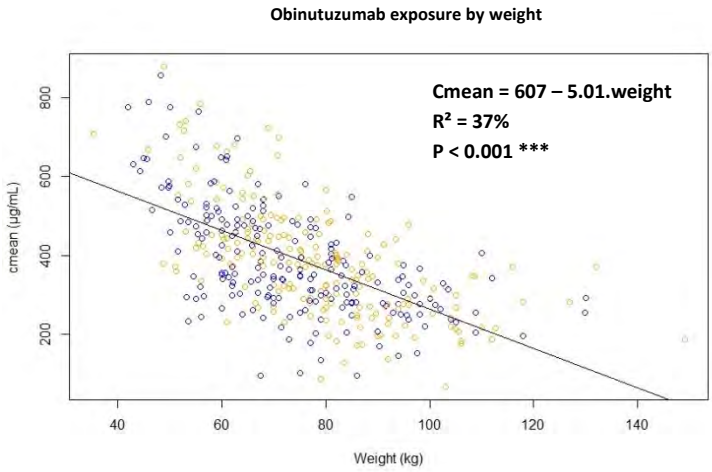
N means the number of patients

This graphical analyses show a relationship between obinutuzumab exposure and gender (p< 5%). Females seem to have higher exposure than males and the gender trend is the same with the different chemo backbones and regimens.

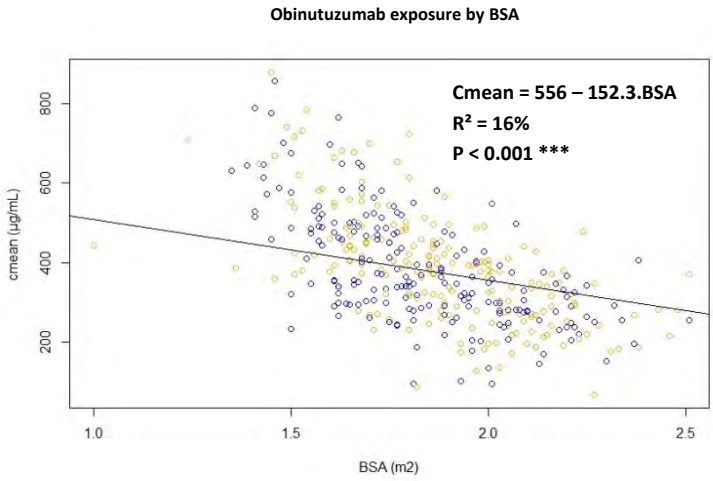
2.3.3. Body weight/ Body Surface Area (BSA)/ Body Mass Index (BMI)

The third parameter includes 3 sub-variables such as body weight (kg), Body Surface Area (BSA in m²), and Body Mass Index (BMI in kg/m²). Two figures are presented below.

A



B



○ Patients treated by obinutuzumab and Bendamustine

○ Patients treated by obinutuzumab and CHOP/CVP

Figure 3:

A. Relationships between cmean (µg/mL) and weight (kg) in all patients

B. Relationships between cmean (µg/mL) and BSA (m²) in all patients

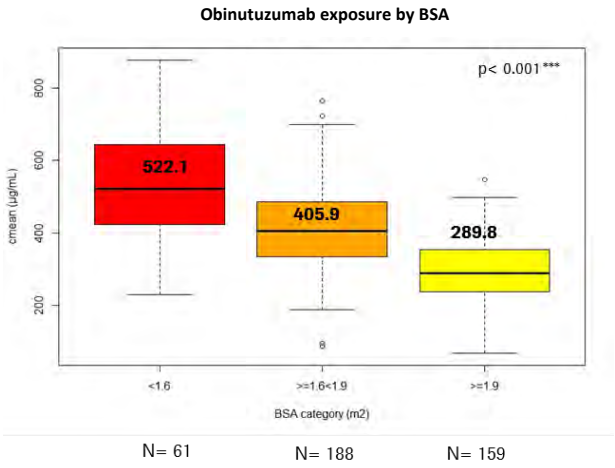
R^2 represents the correlation coefficient

Cmean = a – b.weight/BSA is the linear equation of the regression line and "b" is the slope coefficient

P-value is significant (***) if lower than 5%

The same analysis with BMI shows the same results

A.



B.

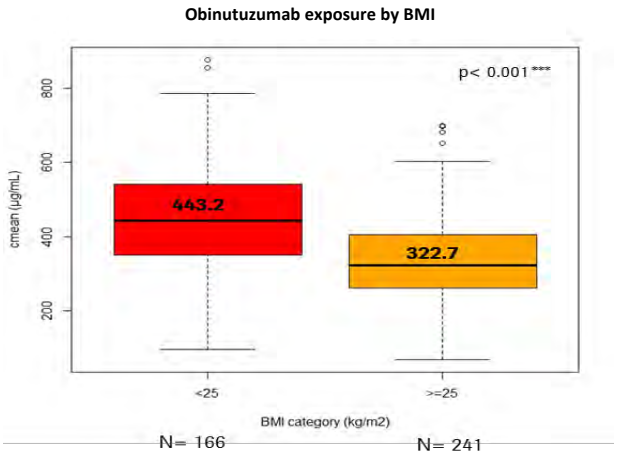


Figure 4:

A. Relationships between cmean (µg/mL) and BSA category (m^2) in all patients

B. Relationships between cmean (µg/mL) and BMI category (kg/m^2) in all patients

The Cmean median is indicated and compared (µg/mL)

P-value is significant (***) if lower than 5%

N means the number of patients

Same analysis done in patients treated by obinutuzumab and bendamustine, obinutuzumab and CHOP/CVP

Looking at body weight, BSA, BMI, obinutuzumab exposure seems to be influenced by those parameters. The p-values are significant (<5%) and R^2 shows a correlation between these variables. No matter their chemotherapy regimen added to obinutuzumab, the trend is that patients with the lowest body weight or BSA or BMI have higher exposure.

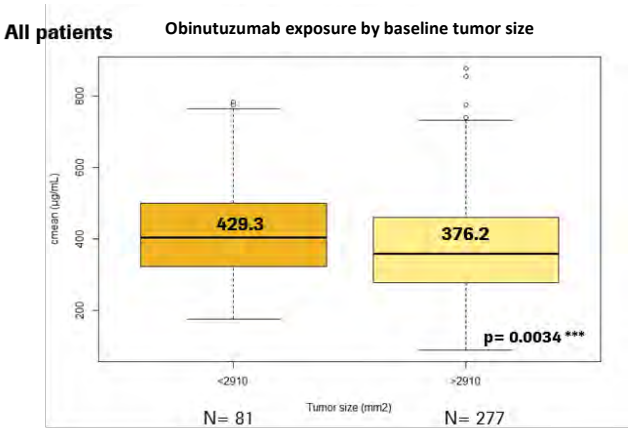
2.4. Pharmacodynamic parameters – obinutuzumab exposure relationships

2.4.1. Baseline tumor size

2.4.1.1. Baseline tumor size in all patients

The exploratory analysis supporting PK/PD modelling simulation was also relevant including pharmacodynamic parameters such as baseline tumor size.

A.



B.

C.

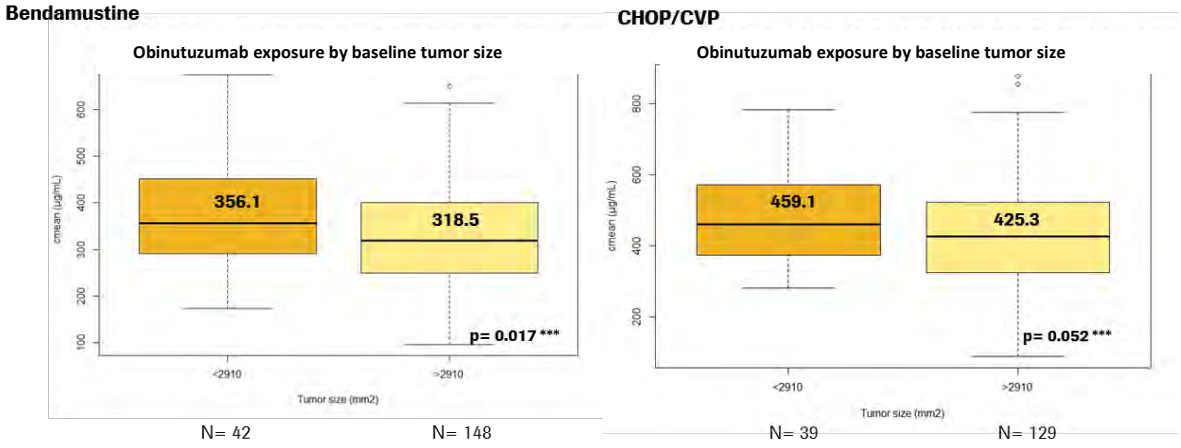


Figure 5: Relationships between cmean (µg/mL) and baseline tumor size (mm²) in all patients with chemotherapy (A), patients treated by obinutuzumab and bendamustine (B) and patients treated by obinutuzumab and CHOP/CVP (C)

The Cmean median is indicated compared (µg/mL)

P-value is significant (***) if lower than 5%

N means the number of patients

The cut-off were (<2910 mm, >2910 mm) determined by looking at the PopPK report

Based on the graphical analysis, patients with lower baseline tumor size have greater obinutuzumab exposure. The trend seems to be similar with all chemo backbones and regimens ($p < 5\%$). In previous models, it has already been demonstrated that the decline of clearance was faster for patients with lower baseline tumor size than for those with higher baseline tumor size also consistent with target-mediated drug disposition.

2.4.1.2. Baseline tumor size by gender

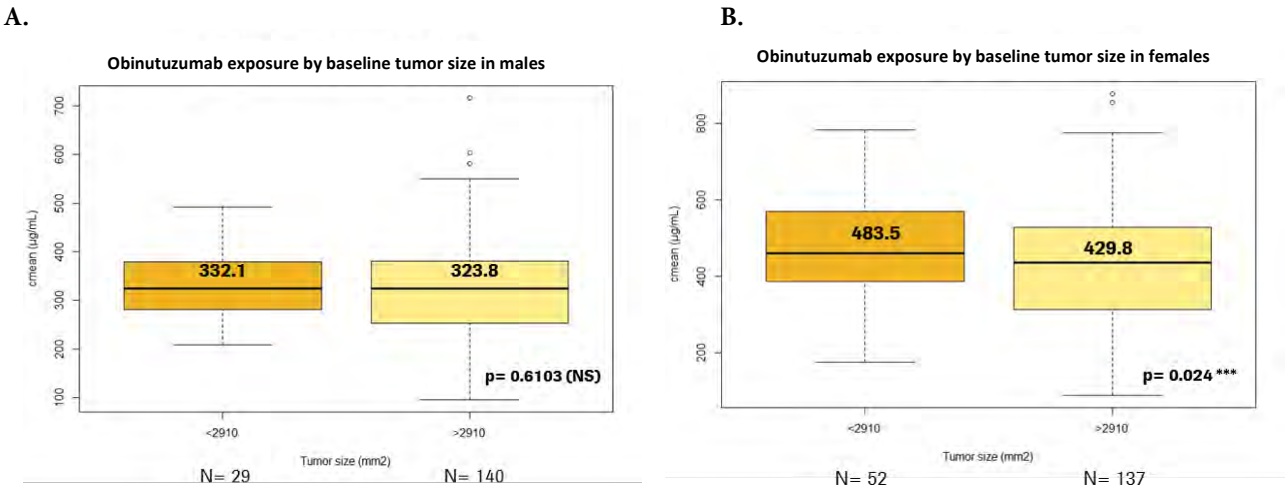


Figure 6: Relationships between cmean (µg/mL) and baseline tumor size (mm²) in all patients with chemotherapy split into gender (A: in females/ B: in males)

The Cmean median is indicated compared (µg/mL)

P-value is significant (***) if lower than 5%

N means the number of patients

The cut-off were (<2910 mm, >2910 mm) determined by looking at the PopPK report

Based on these plots and only looking at females, patients with lower baseline tumor size have higher exposure. This trend seems to not be visible in male patients. However, this needs to be careful as the total number of patients for females and males is not the same. It has already demonstrated that tumor size is a significant covariate in the previous PopPK model within the whole population.

2.4.2. Other pharmacodynamic parameters

Other parameters could have been involved within the exploratory analysis realized. In order to highlight the pharmacodynamic parameters influence, we could also look at B-cells counts as an example. It has already demonstrated that there is no effect of obinutuzumab exposure

on B-cell depletion. Another example would be to analyze the CD20 level. This is the goal of an overall and multivariate analysis.

Further investigations are needed.

3. Exploratory analysis of the obinutuzumab exposure influenced by disease parameters and chemo backbones combined in follicular lymphoma patients in the GALLIUM study – Impact on clinical outcomes

3.1. Disease parameters: definitions

3.1.1. Follicular Lymphoma International Prognostic Index (FLIPI) score

FLIPI score is a validated Follicular Lymphoma International Prognostic Index which helps in evaluating and choosing the treatments in those patients. Adverse prognostic factors are selected such as age, hemoglobin level, number of nodal areas, Ann Arbor stage and Lactate Dehydrogenase (LDH) level. This score divided into 3 levels has been used as a first disease parameter. There are two different types of score: FLIPI 1 and FLIPI 2.

- FLIPI 1

A validated prognostic index (PI) would help in evaluating and choosing these treatments. Five adverse prognostic factors were selected: age (> 60 years vs ≤ 60 years), Ann Arbor stage (III-IV vs I-II), hemoglobin level (< 120 g/L vs ≥ 120 g/L), number of nodal areas (> 4 vs ≤ 4), and serum LDH level (above normal vs normal or below). Three risk groups were defined: low risk (0-1 adverse factor, 36% of patients), intermediate risk (2 factors, 37% of patients, hazard ratio (HR) of 2.3), and poor risk (≥ 3 adverse factors, 27% of patients, HR = 4.3).

This Follicular Lymphoma International Prognostic Index (FLIPI) appeared more discriminant than the International Prognostic Index (IPI) proposed for aggressive non-Hodgkin lymphomas. Results were very similar in the confirmation group. The FLIPI may be used for improving treatment choices, comparing clinical trials, and designing studies to evaluate new treatments.

- FLIPI 2

Follicular Lymphoma International Prognostic Index 2 is a simple prognostic index based on easily available clinical data and may represent a promising new tool for the identification of patients with FL at different risk in the era of immunochemotherapy.

3.1.2. Positron Emission Tomography (PET) Scan response

The second parameter concerns the PET-Scan response. It is an imaging test in order to check for the presence of the disease in the body. The scan uses special radioactive tracers. Researches have shown that PET is accurate at predicting both progression-free survival (PFS) and overall survival (OS). In a simplified way, “patients with positive PET-Scan response” means that lymphoma is still active with visible lesions. However “patients with negative PET-Scan” response do not highlight any lesions.

3.1.3. Minimal Residual Disease (MRD) response

The third parameter concerns Minimal Residual Disease (MRD) response. One of the goals of testing MRD is to judge the quality of the response to treatment which might predict the durability of the remission. It is a term used in blood cancer meaning that small number of cancer cells or clones remains in the patients’ blood or bone marrow following the treatment. MRD is a major cause of relapse for patients with blood cancer. Patients may be left with a tiny number of cancer cells within the blood or bone barrow which may cause them to relapse. This means that MRD response is positive. On the contrary, MRD response is negative and no clones are detected in blood and/or bone marrow.

By looking at the literature, MRD negativity is also defined by the absence of BCL2 gene rearrangement in whole blood and or bone marrow in follicular lymphoma patients with evidence of BCL2 gene rearrangement. [14]

3.2. Exposure-disease parameters relationships

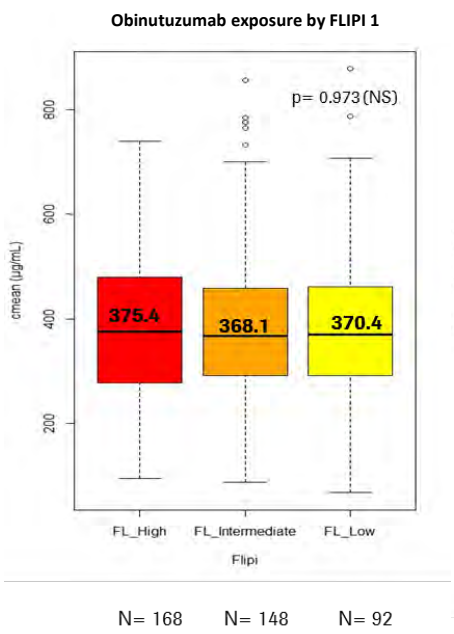
Different exploratory disease parameters regarding follicular lymphoma and those studied in GALLIUM trial are included in this analysis. FLIPI score is the first disease parameter analyzed in this report.

3.2.1. Exposure- Follicular Lymphoma International Prognostic Index (FLIPI) score

3.2.1.1. All patients analyzed

The analysis has been realized by splitting the two different FLIPI scores.

A.



B.

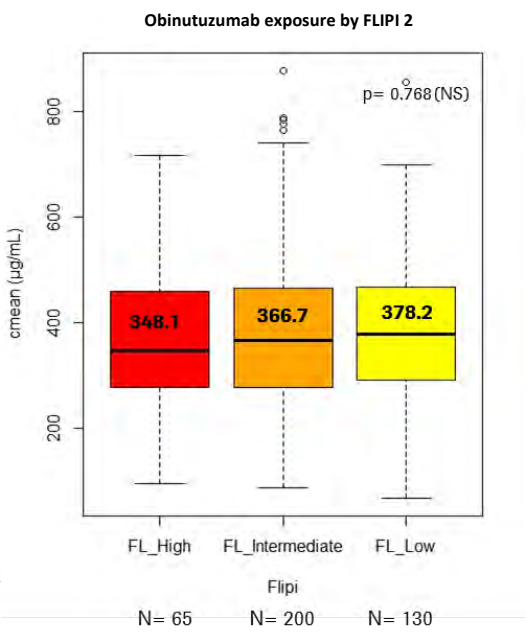


Figure 7: Relationships between cmean (µg/mL) and FLIPI 1 (A) and 2 (B) scores (high/intermediate/low) in all patients

The Cmean median is indicated compared (µg/mL)

P-value is significant (***) if lower than 5% / NS means non-significant

N means the number of patients

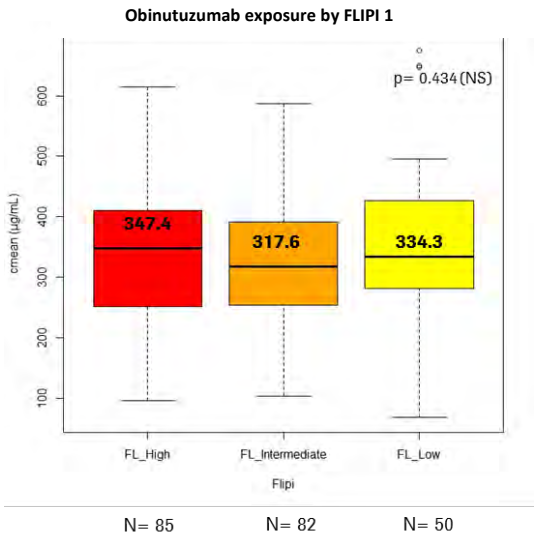
Same analysis done in patients treated by obinutuzumab and bendamustine, obinutuzumab and CHOP/CVP (see below)

Looking at the plots, FLIPI 1 and 2 scores does not impact obinutuzumab exposure.

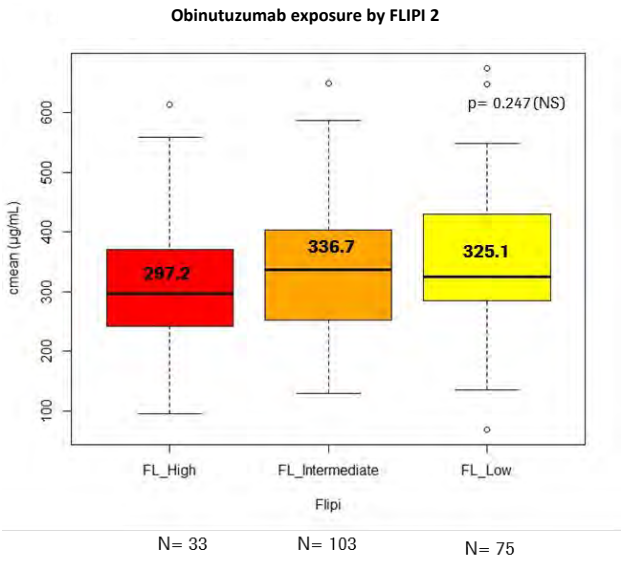
3.2.1.2. By chemo backbone

- Bendamustine

A.

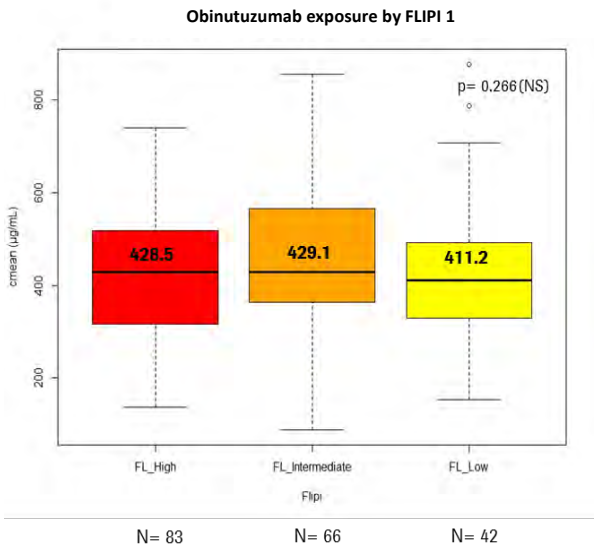


B.



- CHOP/CVP

C.



D.

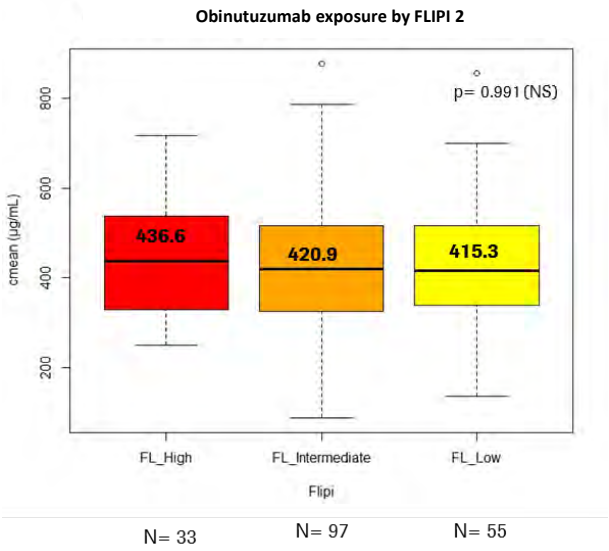


Figure 8: Relationships between cmean (µg/mL) and FLIPI 1 and 2 scores (high/intermediate/low) in patients treated by bendamustine (A and B) and by CHOP/CVP (C and D)

The Cmean median is indicated compared (µg/mL)

P-value is significant (***) if lower than 5% / NS means non-significant

N means the number of patients

Graphical analyses show no correlation between obinutuzumab exposure and FLIPI score no matter the index's level. The p-value is non-significant (<5%). The same analysis has been done in patients treated by obinutuzumab and bendamustine as well as patients treated by obinutuzumab and CHOP/CVP. Chemotherapy seems to not influence exposure.

3.2.2. Exposure- Positron Emission Tomography (PET) Scan response

3.2.2.1. All patients

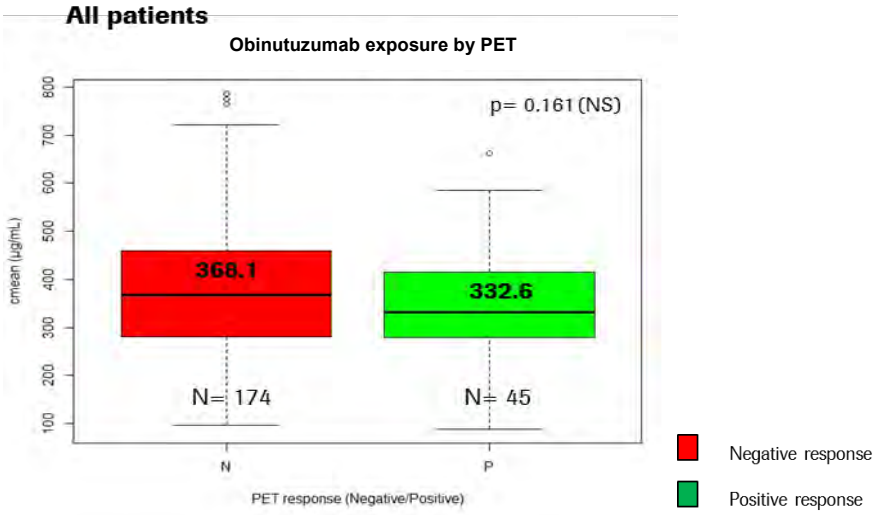


Figure 9: Relationships between cmean (µg/mL) and PET response (N: negative/ P: positive) in all patients

The Cmean median is indicated compared (µg/mL)

P-value is significant (***) if lower than 5% / NS means non-significant

N means the number of patients

Same analysis done in patients treated by obinutuzumab and bendamustine, obinutuzumab and CHOP/CVP (see below)

When all patients analyzed, it would seem that PET response either positive or negative does not influence obinutuzumab exposure.

3.2.2.2. By chemo backbone

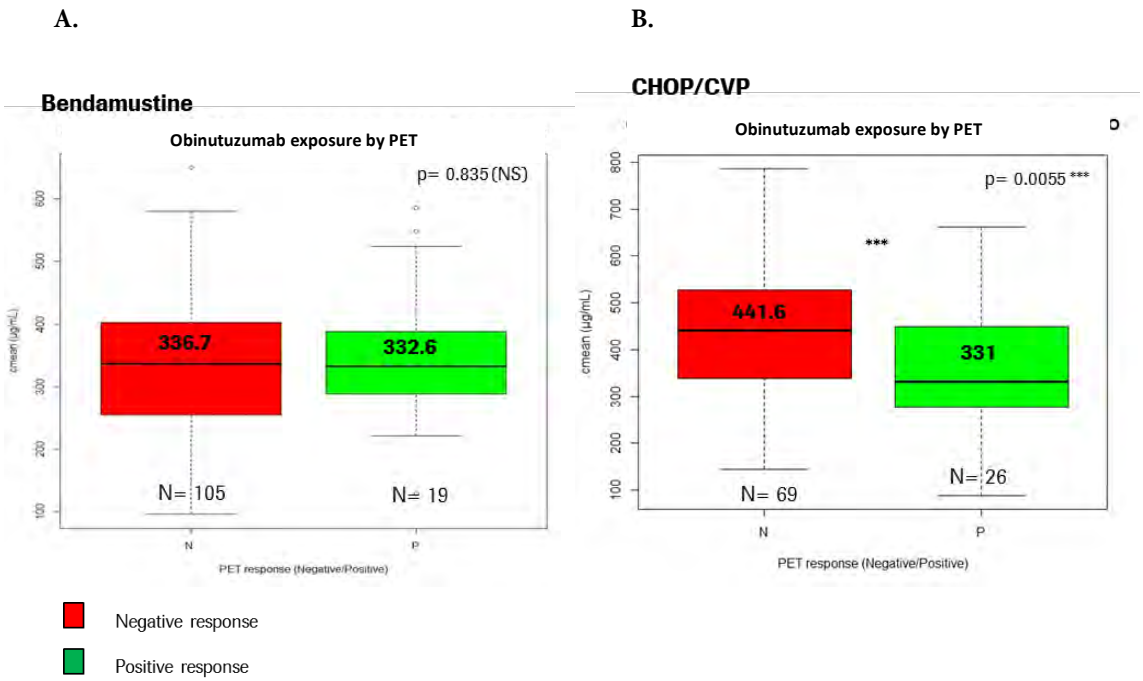


Figure 10: Relationships between cmean (µg/mL) and PET response (N: negative/ P: positive) in patients treated by bendamustine (A) and CHOP/CVP (B)

The Cmean median is indicated compared (µg/mL)

P-value is significant (***) if lower than 5% / NS means non-significant

N means the number of patients

Analyzing only patients treated by CHOP/CVP, negative PET response at the end of treatment appears to be related to higher exposure whereas the relationship between PET response and exposure seems to not be influenced whether all follicular lymphoma-patients and patients treated by bendamustine are analyzed. This trend has to be confirmed.

3.2.3. Exposure- Minimal Residual Disease (MRD) response

3.2.3.1. All patients analyzed

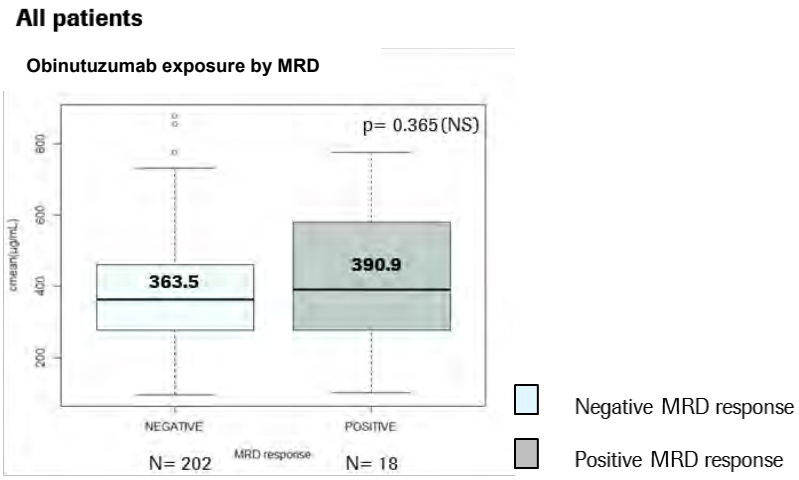


Figure 11: Relationships between cmean (µg/mL) and MRD response (Negative/ Positive) in all patients

The Cmean median is indicated compared (µg/mL)

P-value is significant (***) if lower than 5% / NS means non-significant

N means the number of patients

Same analysis done in patients treated by obinutuzumab and bendamustine, obinutuzumab and CHOP/CVP (see below)

Looking into the figure 11, obinutuzumab exposure reveals itself comparable in all follicular lymphoma patients with positive or negative MRD response. The p-value is not significant but taking into account the number of patients, this is more relevant to be careful with the interpretation.

3.2.3.2. By chemo backbone

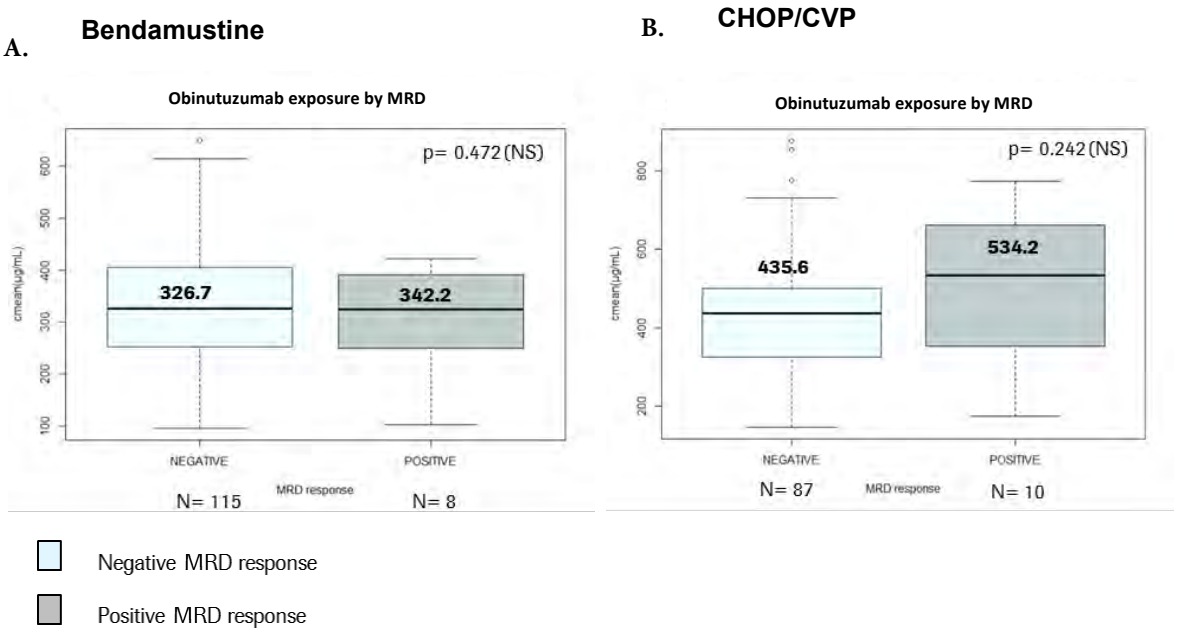


Figure 12: Relationships between cmean (µg/mL) and PET response (N: negative/ P: positive) in patients treated by bendamustine (A) and CHOP/CVP (B)

The Cmean median is indicated compared (µg/mL)

P-value is significant (***) if lower than 5% / NS means non-significant

N means the number of patients

The same analysis has been done in patients treated by obinutuzumab and bendamustine as well as patients treated by obinutuzumab and CHOP/CVP. Chemotherapy seems to not influence exposure. The same precaution has to be taken here with the low number of patients.

Based on **demographic parameters** already studied, age does not impact the exposure whereas gender and body weight/BSA/BMI do. It confirms what it has been done in the previous popPK model. Gender and body weight are considered a covariates in the model and they influence the obinutuzumab exposure. It is established that there are differences in terms of drug's volume of distribution between males and females most likely to body weight and BSA differences. Those differences exist for most of the drugs.

Regarding the **pharmacodynamic parameters**, baseline tumor size seems to have an impact on exposure whether the analysis is realized with the whole target population and with different chemos backbones and regimens. The difference seen when genders are split has to be interpreted with precaution. As it has been previously described, baseline tumor load is another covariate influencing the model with obinutuzumab exposure.

Regarding the **disease parameters**, the pathology's prognostic with FLIPI score and MRD response seem to do not influence Cmean as well as PET Scan response in all patients treated and in those treated specifically by obinutuzumab – bendamustine. We still have a difference between PET Scan responses in terms of exposure in patients treated by CHOP/CVP. Those patients with negative PET response have higher exposure. This raises the question of chemotherapy's choice regarding the toxicity and efficacy for example. However, an exposure difference does not mean that the clinical outcomes are impacted. This will be discussed further. There are some differences regarding baseline characteristics if we look at patients treated by bendamustine and those treated by CHOP/CVP but they are not meaningful and significant. Hence, it does not explain the difference trend observed.

At this point, the statistical analysis could logically help in an experimental way but we are still confronted with very low numbers of patients. Moreover if the exposure is not impacted by several factors, this does not mean that survival and overall the clinical outcomes are not either. Then the logical further step has been to analyze other parameters leading to **clinical exploratory analysis.**

Let's assume that the project has already engaged lots of discussions about and it will engage further many topics to think about.

The next main aspect of this analysis has been to identify whether exposure has an impact on efficacy parameters.

3.3. Exposure-efficacy relationships and impact on clinical outcomes

In confirming and knowing these previous results, the third aim has been to explore whether the obinutuzumab exposure has an impact on clinical outcomes.

Please note that to have a scientific and a rigorous conclusion, we would need to include further factors as a multivariate analysis in order to conclude to any relationships between the variables and exposure. The only one analysis with exposure as Cmean at the end of treatment (induction and maintenance) is not relevant enough but provides an important trend to further analyze and confirm.

3.3.1. Clinical response at the end of treatment

To adjust for multiple statistical testing of the primary and key secondary efficacy endpoints, different endpoints such as response to treatment, are tested in the report: Complete Response (CR), Partial Response (PR), Progressive Disease (PD), Stable Disease (SD) rates at the end

of treatment in the FL population based on tumor assessment and PFS in the overall population as well as split into demographic, PD and disease parameters.

Taking into account these parameters, please see below the relationships between clinical response and obinutuzumab exposure.

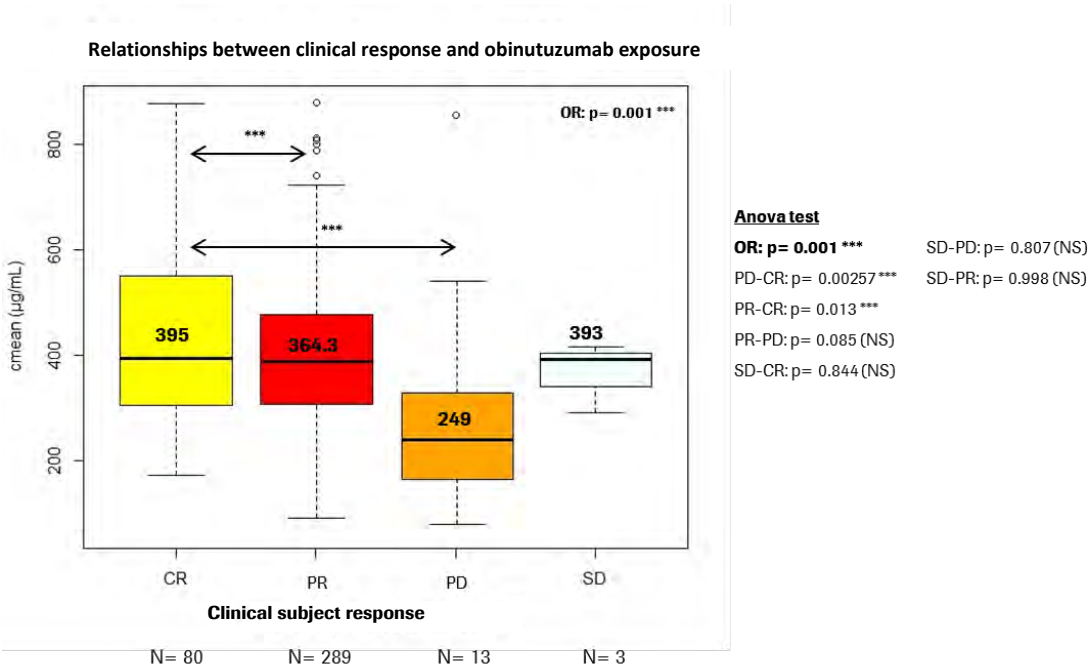


Figure 14: Relationships between cmean (µg/mL) and clinical response at the end of treatment in all patients
(CR: complete response/ PR: partial response/ PD: progressive disease/ SD: stable disease/ OR: overall response)
The Cmean median is indicated compared (µg/mL)
P-value is significant (***) if lower than 5% / NS means non-significant
Anova test is used to analyze the different combinations
N means the number of patients

Comparing patients with CR and PR as well as CR and PD, there is a significant improvement and a better clinical outcome which is related to a higher Cmean.

Hence, based on the clinical responses at the end of treatment and this exploratory analysis, higher plasma obinutuzumab levels suggest a greater efficacy and a favorable outcome in all

FL patients. Other factors should be included in the model because it has already demonstrated that a higher exposure to a drug does not always mean a better efficacy.

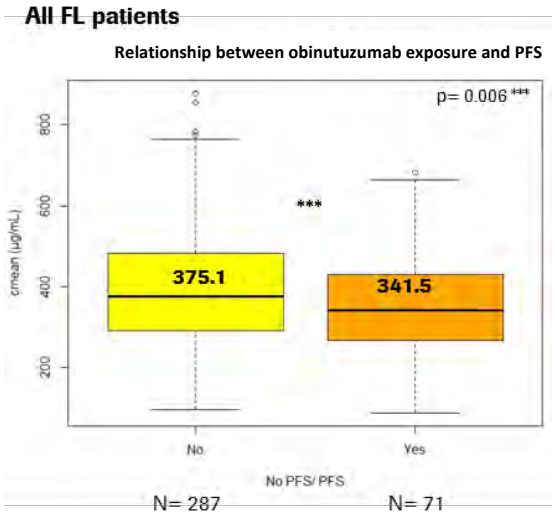
3.3.2. Progression-free survival (PFS) at the end of treatment

3.3.2.1. Progression-free survival (PFS) event

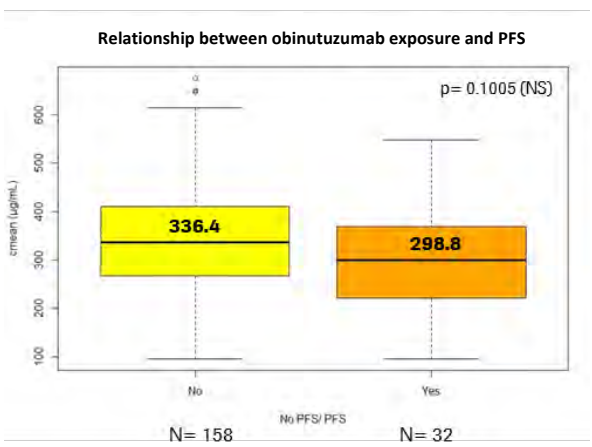
Progression-free Survival (PFS) is the length of time during and after the treatment of a disease, such as cancer, that a patient lives with the disease but it does not get worse. When looking at the data, PFS event "YES" includes patients with Progression Disease and patients who died.

(Please refer to next page)

A.



B.



C.

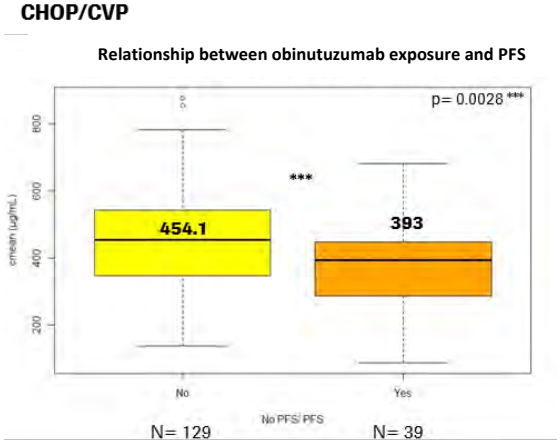


Figure 15: Relationships between cmean (µg/mL) and PFS event (No/ Yes) in all patients with chemotherapy (A), patients treated by obinutuzumab and bendamustine (B) and patients treated by obinutuzumab and CHOP/CVP (C)

The Cmean median is indicated compared (µg/mL)
P-value is significant (***) if lower than 5% / NS means non-significant
Anova test is used to analyze the different combinations
N means the number of patients

The Cmean median is indicated compared (µg/mL)
P-value is significant (***) if lower than 5% / NS means non-significant
N means the number of patients
Same analysis done in patients treated by obinutuzumab and bendamustine, obinutuzumab and CHOP/CVP

Looking at the figure 15, it would seem that there is a relationship between exposure and PFS depending on the chemo backbone combined. The impact of exposure on PFS would be visible with patients treated by CHOP/CVP but not bendamustine. This only one analysis is

not relevant enough to highlight any relationships between those factors. This needs further investigations.

3.3.2.2. Progression-free survival (PFS) by demographic and pharmacodynamic parameters

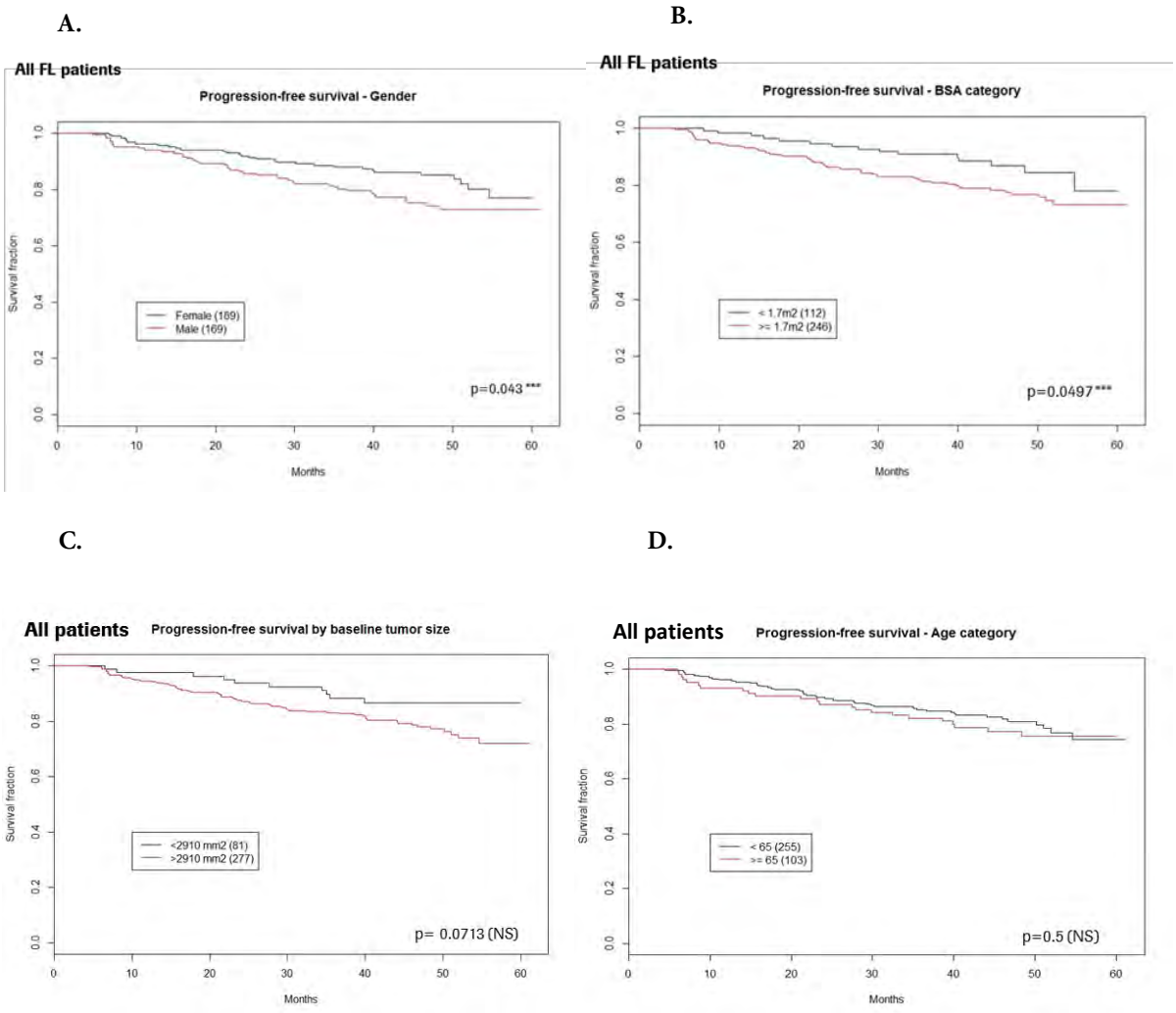


Figure 16: Impact of gender (A), BSA (B), baseline tumor size (C), age (D) on PFS in all patients treated by obinutuzumab with chemotherapy

The Cmean median is indicated compared (µg/mL)

P-value is significant (***) if lower than 5% / NS means non-significant

Anova test is used to analyze the different combinations

N means the number of patients

Looking into the figures 16 A and B, females and patients with lower BSA seem to have a significant survival improvement when all patients are analyzed. However looking into PFS according to baseline tumor size, the survival improvement seems to not be significant when the baseline tumor size is split into two categories. *This is the description of the results obtained for this report.*

However, it has to be taken into account that in previous models a different interpretation is described. It has been demonstrated than the variability we see in exposure on gender and body weight does not have an impact on efficacy. It has been showed that tumor burden has an influence on PFS.

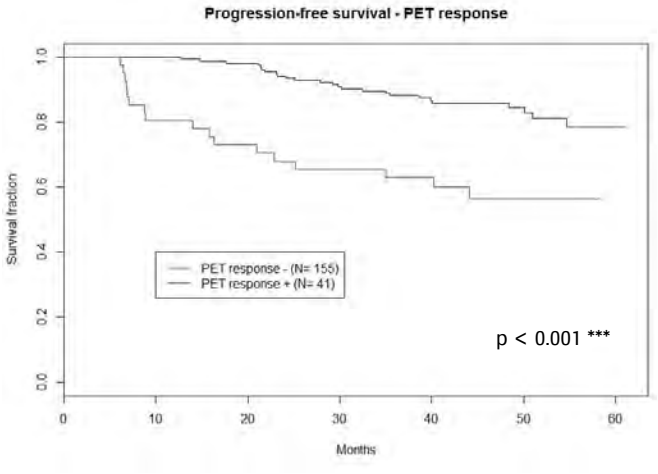
3.3.2.3. Progression-free survival (PFS) by disease parameters and by chemo backbones and regimens

It has been demonstrated and this is clear that patients with PET and MRD negative responses have improved clinical outcomes. The p-values are significant and the curves do not overlap. The PFS trend is the same with the different chemo backbones and regimens.

We already showed in the report that obinutuzumab exposure is influenced by PET response in patients treated by CHOP/CVP.

(Please refer to the next page)

A.



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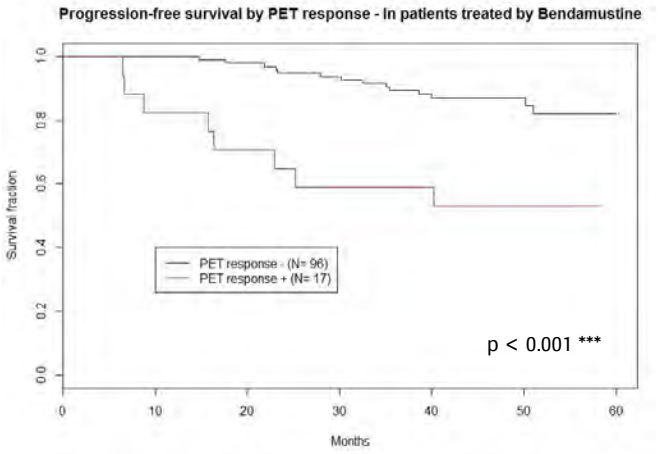

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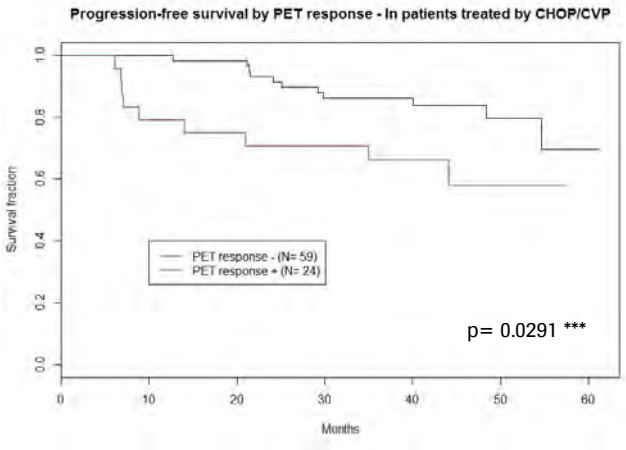

Figure 18: Progression free-Survival (PFS) by PET response in all patients with chemotherapy (A), patients treated by obinutuzumab and bendamustine (B) and patients treated by obinutuzumab and CHOP/CVP (C)

P-value is significant (***) if lower than 5% / NS means non-significant

Anova test is used to analyze the different combinations

N means the number of patients

The Cmean median is indicated and compared (µg/mL)

P-value is significant (***) if lower than 5% / NS means non-significant

N means the number of patients

To confirm that chemotherapy seems to do not impact PFS, other analyses were done using the same disease parameters.

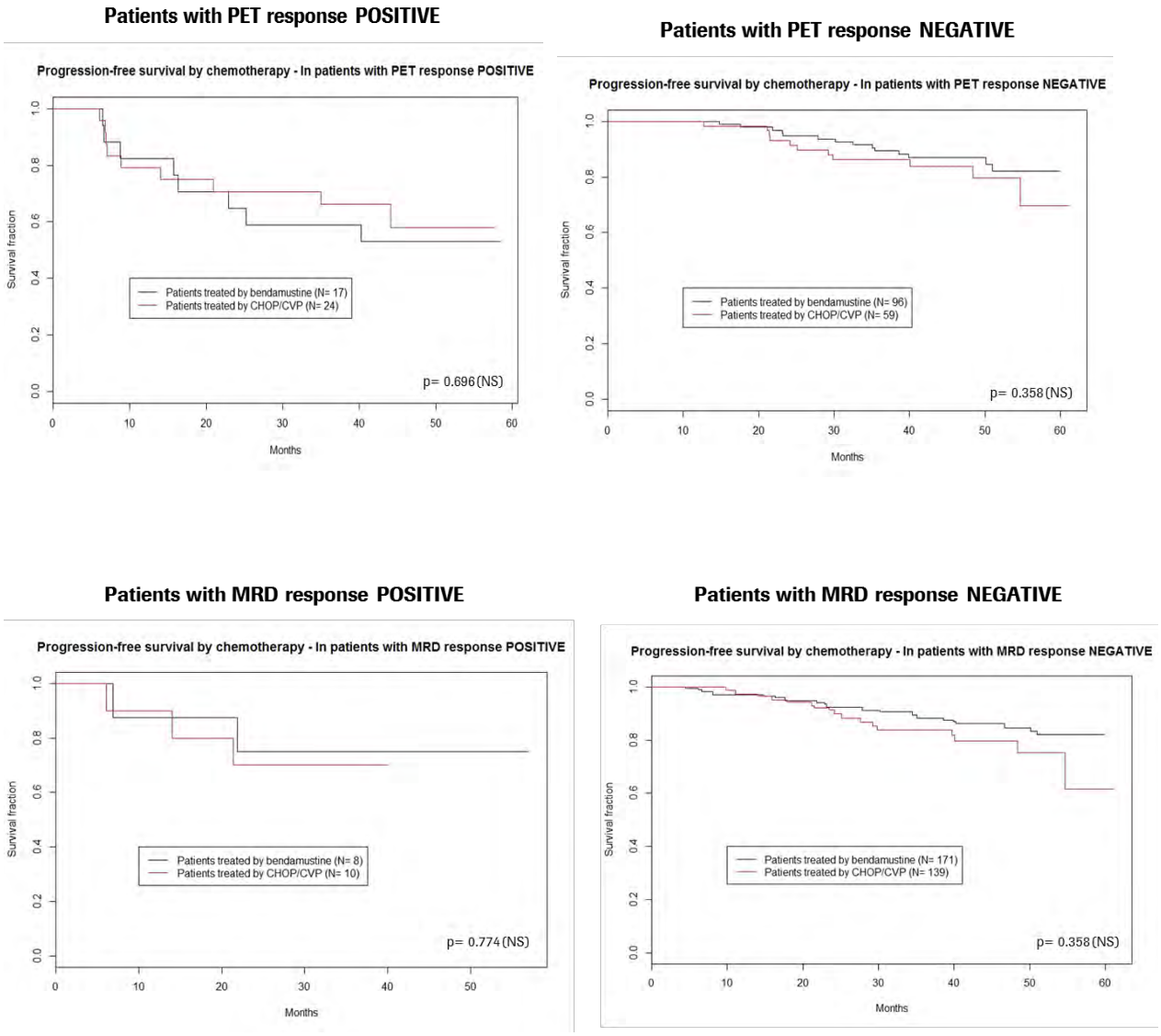

Figure 19: Impact of chemotherapy combined with obinutuzumab on PET and MRD responses (Negative/Positive) and PFS in all patients

The Cmean median is indicated compared (µg/mL)

P-value is significant (***) if lower than 5% / NS means non-significant

Anova test is used to analyze the different combinations

N means the number of patients

Analyzing this figure, it seems that the choice of treatment does not have an impact on PFS if we look at PET and MRD positive and negative responses separately. In patients with MRD and PET positive responses, the p-values are not significant and the curves overlap. In patients with negative responses, the trend looks similar.

This analysis has demonstrated that a PK model integrating the mean concentration describes the value at the end of treatment and shows correlation with demographic, disease, efficacy parameters. This experimental PK model is consistent with previous models and studies already realized.

With regards to those factors analyzed, the discussion stays open and other key factors could be added to the analysis.

3.4. Several factors could be involved- Perspectives

3.4.1. Pathology characteristics

3.4.1.1. CD20 level

Obinutuzumab is an anti-CD20 mAb where PK is considered as a surrogate marker of CD20 occupancy. The recommended dose of obinutuzumab in patients with B-cell lymphoma have been determined based on safety, efficacy and PK profil to ensure the full saturation of the target. [12]

Several factors are important in identifying an appropriate target antigen. Ideally, the antigen should be found only on tumor cells. For hematologic diseases, the use of antigens restricted to B- or T-cell lineages has been the most successful approach. For optimal activity, the target antigen should be present on all the cells from the malignant clone. A high density of tumor antigen on the cell surface will allow better targeting, and the antigen should be stable on the cell surface, not shed or secreted, because the presence of soluble antigen will prevent

the antibody reaching the target cell and result in faster clearance. Generally, modulation (internalization of the antigen following binding) is detrimental for antibodies that act by interacting with the host immune system (eg, anti-CD20 antibodies).

CD20 is not tumor-specific, but is B-cell specific. It is present in high density on most B-cell malignancies, does not internalize or modulate on antibody binding. Thus the antibody will remain bound to the cell surface without any degradation, continuously exposing the tumor cell to immune-mediated destruction. Importantly, the CD20 antigen does not appear to undergo mutation and has rarely been observed to lost following mAb therapy suggesting that antigen loss or mutation is not frequent mechanism of tumor cell resistance. Unfortunately, CD20 is not critical to the cell and can be deleted with no apparent effect on the tumor cell.

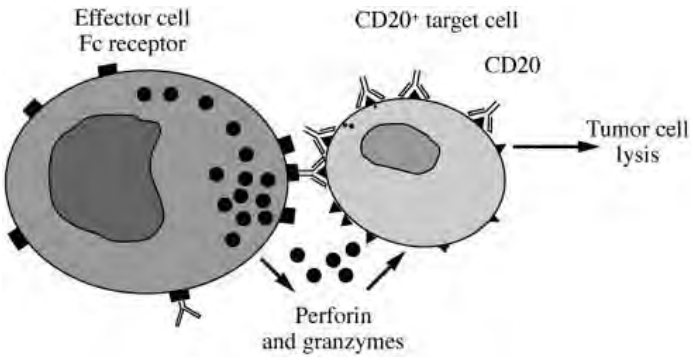

Figure 20: Illustration of the ADCC mechanism as a main activity of obinutuzumab

Antibody-coated tumor cells engage the Fc receptors on NK cells or macrophages to elicit an ADCC response, which elicits lysis of the antibody-coated tumor cells. See figure above to explain the mechanism. [15]

A full understanding of the difference between obinutuzumab and rituximab would require knowing the exact structure of the membrane CD20 and the spatial arrangement of the tetramers at the membrane surface. This could be a parameter to be added in the model.

3.4.1.2. FC Gamma-Receptor (FcγR) expression level

It is difficult to examine the efficacy of human immunotherapies in vivo and to identify the molecular mechanisms that mediate CD20 due to the complexities of carrying out mechanistic studies in human.

CD20 mAbs engage the innate mononuclear phagocytic network and deplete blood and tissue B cells through Immunoglobuline FcγR-dependent and complement-independent mechanisms.

Several studies have provided molecular understanding of the different roles carried out by each FcγR during innate immune responses and the actions of pathogenic antibodies of different isotypes.

The capacity of monoclonal antibodies to interact with FcγRs is an important factor influencing the efficacy of CD20 mAb therapies in human as explained previously. Polymorphisms influence and are correlated with the efficiency of B cells and tumor depletion during CD20 monoclonal antibodies therapy in lymphoma patients.

The current studies also indicate that it may be important to consider disease- and tissue-specific targeting effects when manipulating FcγR expression or function for therapeutic benefit. [16]

The difference in terms of drug exposure including gender could be explained by looking at the FcγR. It could possibly be related to intrinsic differences between females and males in the molecular biology of B-cells malignancies or FcγR polymorphisms.
It is quite difficult to determine whether adjusting the dose of obinutuzumab would improve efficacy outcomes.

Understanding to complex network of immune mechanisms of destruction of the lymphoma cells is necessary in development of new monoclonal antibodies. This is topic that would need further investigations.

3.4.1.3. Baseline B-cell counts

In previous models, relationships have been explored between obinutuzumab Cmean values and changes from baseline in observed B cell counts and tumor size.
Other studies have shown no effect of obinutuzumab exposure on B-cell depletion. In all obinutuzumab exposure, B-cells counts decreased rapidly from baseline after the start of obinutuzumab treatment and remained depressed for the whole observation period.

Models relating changes in clearance to the observed depletion of circulating B cells have also been tested but there were not able to explain the observed dependency of clearance on time. B-cells in circulation are eliminated very rapidly while the observed time-dependence of clearance has a longer characteristic time scale. [12]

For all exposure groups, peripheral neutrophil and B-cell counts decreased from baseline after the start of obinutuzumab administration and remained low for the duration of the study.

3.4.1.4. Expression of the antiapoptic Bcl-2

Some studies have shown that Bcl-2 expression could be related to clinical outcomes.

In patients with other type of lymphomas (Diffuse Large B-cell Lymphoma, DLBCL), a study showed that PFS and OS of patients with high Bcl-2 expression were significantly inferior to those of patients with low expression of Bcl-2. High expression of Bcl-2 was associated with poor PFS and OS only in patients with low international prognostic index (IPI). IPI is another prognostic index regarding these other types of lymphomas. In multivariate analysis, high expression of Bcl-2 was a significant independent prognostic factor of poor PFS and OS along with high IPI. In a way, the expression of Bcl-2 may be a useful prognostic factor, especially in those patients with IPI low.

The high expression of Bcl-2 was associated with advanced Ann Arbor stage. Ann Arbor staging is the staging system for lymphomas, both in Hodgkin's lymphoma and non-Hodgkin’s lymphoma. The stage depends on both the place where the malignant tissue is located and on systemic symptoms due to the lymphoma.

In the present study, no significant correlation was found between the expression status of the drug resistance-related proteins and the clinico-pathological characteristics except for the association of high Bcl-2 expression with advanced Ann Arbor stage, consistent with the results of previous reports.

[17]

Regarding the disease studied in this report, FL occupies the immune system, surviving and proliferating mainly in the germinal centers (GCs) of lymph nodes. One protective factor evident in the majority of cases is the up-regulated expression of the anti-apoptotic Bcl-2 protein via different translocations.

Genome sequencing is revealing mutational events in a proportion of cases of FL, with several located in histone-modifying genes.

It would be of interest to determine whether these are present in early disease and might therefore contribute to lymphomagenesis.
A hypothetical bridge could be constructed between this expression, drug exposure and potential clinical outcomes.

Current problems with MRD in FL patients may concern the availability of the marker. The translocation regarding Bcl-2 is not an universal marker and is not available in all FL patients. It also may concern the location of the tumor, MRD response depends on the tumor's compartment, bone marrow, peripheral blood and lymph nodes. [18] Hence, MRD response assessment by PCR is reduced within the lymph nodes and tissues. MRD response might be non-relevant especially in FL patients whether the tumor is spread to nodes or tissues. In fact, MRD is considered as a good marker in CLL patients because of the B-cells location within the bloodstream. These points are still important to rise before building a model and looking into Bcl-2 expression as a covariate in the model.

3.4.2. Patient characteristics and genetic

In addition to factors involved in pathology characteristics, several other factors more related to patient characteristics and their genetics should be taken into account such as the transcriptional differences related to sex and age and different key genes involved and related to tumor progression. [19]
Several studies have been done looking at DLBCL-patients but this should encourage analyzing FL-patients.

Older age is an adverse prognostic factor that correlates with inferior survival in DLBCL. This is likely in part attributable to poorer performance status and inability to tolerate therapy. Potential molecular perturbations may contribute to inferior outcomes. It's not well defined. A study has been realized to identify key genes and the related signaling networks that were most strongly associated with age and sex that predicted tumor progression.

The impact of both the upstream regulators and key genes was associated with tumor progression by determining through the literature how each factor impacts tumor progression. Tumor progression describes the outcome of tumor growth predicted from biological factors.

There were several distinct genes associated with older age. Older age is also associated with decreased metabolism and telomere functions and also increased immune-related pathways as shown using a network presentation of the gene sets associated with these functions. Some genes were modulated by sex regardless of age.

There was an overall downregulation of genes for older females versus older males with an overall upregulation of genes for younger females. This provides indication of an overall global difference in the biology signature occurring with age. [20]

This analysis is really complex but this could add a valuable explanation regarding differences in terms of exposure by gender in the elderly population.

All these factors regarding the patient and the disease would require more discussions to be part of the model. Undertaking a functional/pharmacological comparison between obinutuzumab and rituximab calls for extreme caution before drawing any conclusion.

Conclusion and perspectives

Pharmacokinetic/pharmacodynamic (PK/PD) modelling and simulation can be used as a tool to provide answers on efficacy risk balance of medicinal products.

One of the aspects of the use of PK/PD modelling simulation in drug development has been illustrated by an exploratory analysis in FL patients treated by obinutuzumab in the GALLIUM trial.

For each parameters studied and for each group of patients, even if some numbers of patients by group were small, here are some exploratory results that would need further discussion.

The exploratory analysis is consistent with some aspects covered by the obinutuzumab PopPK analysis already done. Similarly to most mAbs, some factors influence the obinutuzumab exposure and are considered as covariates in the model. Regarding the demographic parameters for the targeted population in GALLIUM trial, gender and body weight influence the exposure. Females have higher exposure and patients with lowest body weight also have higher exposure. Some PD parameters such as baseline tumor size and B-cell counts have an impact on the exposure.

With regards to disease parameters studied, no difference has been showed in terms of exposure with different FLIPI score, PET and MRD responses. We might see an effect of chemotherapy with an exposure difference in patients treated by CHOP/CVP combined with obinutuzumab and in terms of PET response. Patients with PET negative response treated by CHOP/CVP have higher exposure. By looking at the population characteristics for each treatment associated, there is no significant difference. However, looking into mechanisms of actions for the 2 different chemotherapies, we could see some differences. Other aspects

related the autoimmune environment including potential new component related to the immune system could also impact the exposure and be associated with the choice of chemotherapy.

Resistant disease would be another factor to be taken into account which could suppose that seeding cells are present to explain a relapse disease. It could be the case for patients receiving CHOP/CVP. Resistant tumor could consume the drug and reduced exposure contributes to inferior outcome. This would need further investigation and no conclusion can be made through this exploratory analysis.

Regarding the relationships between efficacy endpoints and exposure, the results presented in the report show some discrepancies with the previous models. There were no apparent relationships between obinutuzumab exposure and efficacy parameters for patients with FL receiving bendamustine whereas the analysis of obinutuzumab exposure-efficacy relationships for patients with FL receiving CHOP/CVP suggested that an increase in exposure might lead to an improvement in efficacy parameters. This should be taken with precaution because it has been shown that efficacy is not related to exposure in previous models.

Many other factors make an impact on obinutuzumab exposure but intrinsic disease and individual factors such as genetic factors, biology aspects, concomitant medications and parameters expressing the degree of disease resistance are also impactful on outcome and/or on exposure.

All these reflections are part of many discussions and could generate potential answers to questions from the Health Authorities regarding the impact of gender and body weight on

exposure for example and the relationships between drug exposure and clinical outcomes. This is one important aspect of the use of PK/PD modelling simulation to support drug development.

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USE OF PHARMACOKINETIC AND PHARMACODYNAMIC MODELLING SIMULATION TO SUPPORT DRUG DEVELOPMENT - ILLUSTRATION WITH AN EXPLORATORY ANALYSIS OF THE OBINUTUZUMAB EXPOSURE INFLUENCED BY DEMOGRAPHIC AND DISEASE PARAMETERS AND IMPACT ON CLINICAL OUTCOMES

ABSTRACT:
Pharmacokinetic and pharmacodynamic (PK/PD) modelling simulation plays an increasingly important role in drug development to characterize the efficacy and the safety of the drugs. One aspect of the use of PK/PD modelling simulation has been illustrated with obinutuzumab, a monoclonal antibody used in lymphomas and associated with chemotherapy. The exploratory analysis shows that obinutuzumab exposure is influenced by gender, body weight and baseline tumor size. However, other parameters related to the disease treated do not impact the exposure. Other intrinsic patient characteristics and differences (e.g., genetic factors, drug target level) are likely related to efficacy. Overall exposure cannot be correlated to clinical outcomes but results show some differences in patients depending on the chemotherapy regimen associated with obinutuzumab. The consequences remain to be clarified.

APPLICATION EN MODÉLISATION PHARMACOCINÉTIQUE ET PHARMACODYNAMIQUE AU DÉVELOPPEMENT CLINIQUE DES MEDICAMENTS : ILLUSTRATION PAR UNE ANALYSE EXPLORATOIRE DES PARAMÈTRES INFLUENÇANT L'EXPOSITION À OBINUTUZUMAB ET ÉTUDE DES CONSÉQUENCES CLINIQUES

RESUME:
Le concept de modélisation et simulation en pharmacocinétique et pharmacodynamique (PK/PD) joue un rôle important dans le développement clinique des médicaments pour caractériser l'efficacité et la sécurité du médicament. Un aspect de l'utilisation de ce concept a été illustré par l'exemple d'un anticorps monoclonal, obinutuzumab, dans le traitement des lymphomes folliculaires. L'analyse exploratoire montre que l'exposition à obinutuzumab est influencée par le genre, le poids du patient et la taille de la tumeur avant traitement. D'autres facteurs relatifs à la pathologie traitée n'impactent pas cette exposition. Quant aux caractéristiques intrinsèques du patient, de la pathologie et de son environnement, les conséquences cliniques peuvent être influencées. Dans l'ensemble, l'exposition au médicament n'est pas corrélée à l'efficacité clinique mais dans le cas d'obinutuzumab et du choix de la chimiothérapie associée, des résultats restent à discuter.

DISCIPLINES administratives: Clinical Science Oncology and Clinical Pharmacology

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MOTS-CLES: Pharmacokinetic/pharmacodynamic modelling simulation, drug exposure, variability, clinical outcomes, oncology product, lymphoma, patients, pharmaceutical company

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