# Clinical study protocol

## Endobiotics for phenotyping cytochrome P450 enzymes:

## Using metabolomics to identify novel endogenous CYP2C19 activity biomarkers in healthy volunteers

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| **Study type** | Clinical Trial with Investigational Medicinal Product (IMP) |
| **Study Categorisation** | Risk Category B |
| **Study Registration** | Registration on www.clinicaltrial.gov and SNCTP |
| **Sponsor and Principal Investigator** | Pr Caroline Samer<br>Division of Clinical Pharmacology and Toxicology<br>University Hospitals of Geneva<br>Rue Gabrielle Perret-Gentil 4<br>1211 Geneva<br>Tel. 022 372 99 32<br>Email: caroline.samer@hcuge.ch |
| **Investigational Products** | Omeprazole (10 mg, Antramups®)<br>Fluvoxamine (50 mg, Floxyfral® junior)<br>Rifampicin (600 mg, Rimactan®) |
| **Protocol Version and Date** | Version 4 of 09.08.2024 |

*CONFIDENTIAL*

## Co-Investigateurs et collaborateurs, rôles dans l'étude

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|---|---|
| Mr Yahia Bennani, pharmacien, doctorant<br>Service de Pharmacologie et Toxicologie Cliniques, HUG | Conception et rédaction du protocole<br>Conception du eCRF<br>Récolte et traitement des données |
| Dr Aurélien Simona, MD<br>Service de Pharmacologie et Toxicologie Cliniques, HUG | Inclusion et suivi clinique des volontaires |
| Pr Youssef Daali, PharmD, PhD<br>Unité d'investigations pharmacologiques et toxicologiques<br>Service de Pharmacologie et Toxicologie Cliniques, HUG | Supervision de la conception et de la rédaction du protocole<br>Développement des approches métabolomiques |
| Dre Gaëlle Magliocco, pharmacienne, PharmD, PhD<br>Unité de Toxicologie et Chimie Forensiques, Centre Universitaire Romand de Médecine Légale | Supervision de la conception et de la rédaction du protocole<br>Supervision du traitement des données |
| Pr Aurélien Thomas, PhD<br>Unité de Toxicologie et Chimie Forensiques, Centre Universitaire Romand de Médecine Légale | Supervision de la conception, de la rédaction du protocole et du travail de laboratoire |
| Mme Valérie Khan-Besse, infirmière<br>Service de pharmacologie et toxicologie cliniques, HUG | Prélèvements sanguins à l'inclusion des volontaires et pendant les sessions |
| Pr Francois Curtin, MD<br>Service de Pharmacologie et Toxicologie Cliniques, HUG | Supervision du traitement des données et des analyses statistiques |

**Signature form**

I, the undersigned, have reviewed the protocol version n°4 of 09.08.2024, including Appendices. I will conduct the clinical study as described and I will adhere to GCP/ICH and all the ethical and regulatory considerations stated (Oclin and LPTh).

Pre Caroline Samer
**Sponsor and Principal Investigator** **Signature** **Date**

MD
**Title**

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# Summary

# Résumé synoptique du protocole en français

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| **Nom du promoteur** | Pre Caroline Samer, Service de Pharmacologie et Toxicologie Cliniques (HUG) |
| **Noms commerciaux des médicaments** | Antramups®, Floxyfral® junior, Rimactan® |
| **Nom des principes actifs** | Oméprazole, fluvoxamine, rifampicine |
| **Titre de l'étude en français** | Phénotypage des cytochromes P450 (CYP) à l'aide d'endobiotiques : Identification de nouveaux biomarqueurs endogènes de mesure de l'activité du CYP2C19 par métabolomique chez des volontaires sains |
| **Titre court** | Endo2C19 |
| **Version et date du protocole** | Version n°4 du 09.08.2024 |
| **Enregistrement de l'étude** | Enregistrement sur www.clinicaltrial.gov et SNCTP |
| **Catégorie d'étude** | Essai clinique de produits thérapeutiques, catégorie de risque B |
| **Investigateurs** | Investigatrice principale : Pre Caroline Samer, Service de Pharmacologie et Toxicologie Cliniques, HUG<br><br>Co-Investigateurs et collaborateurs :<br>Mr Yahia Bennani, Service de Pharmacologie et Toxicologie Cliniques, HUG<br>Dr Aurélien Simona, Service de Pharmacologie et Toxicologie Cliniques, HUG<br>Pr Youssef Daali, Service de Pharmacologie et Toxicologie Cliniques, HUG<br>Mme Valérie Khan-Besse, infirmière, service de pharmacologie et toxicologie cliniques, HUG<br>Dre Gaëlle Magliocco, Unité de Toxicologie et Chimie Forensiques, Centre Universitaire Romand de Médecine Légale<br>Pr Aurélien Thomas, Unité de Toxicologie et Chimie Forensiques, Centre Universitaire Romand de Médecine Légale |
| **Lieu de l'étude** | Partie clinique :<br>Service de Pharmacologie et Toxicologie cliniques, Hôpitaux Universitaires de Genève<br>Analyse des résultats :<br>Unité d'investigations pharmacologiques et toxicologiques, Service de pharmacologie et toxicologie cliniques, HUG<br>Unité de Toxicologie et Chimie Forensiques, Centre Universitaire Romand de Médecine Légale |
| **Calendrier de l'étude** | Début prévu pour octobre 2024 (inclusion des premiers volontaires)<br>Fin prévue pour mars 2025 (dernière session des derniers volontaires) |
| **Déclaration BPEC** | Cette étude sera conduite conformément au protocole, aux principes éthiques de la Déclaration d'Helsinki, aux directives internationales ICH-GCP ainsi qu'à la loi suisse sur la recherche humaine (LRH). |

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| **Rationnel et Objectifs** | Le cytochrome P450 2C19 (CYP2C19) est l'enzyme responsable du métabolisme de médicaments fréquemment prescrits en clinique comme l'oméprazole, un antiacide inhibiteur de la pompe à proton (IPP), le citalopram, un antidépresseur inhibiteur sélectif de la recapture de la sérotonine (ISRS) ou le clopidogrel, un antiagrégant plaquettaire. Le CYP2C19 présente une importante variabilité interindividuelle de son activité dans la population générale due, notamment, à un fort polymorphisme génétique, avec une trentaine de variants alléliques identifiés à ce jour. Ceci résulte en cinq phénotypes distincts dans son activité : le phénotype métaboliseur lent (PM), le phénotype métaboliseur intermédiaire (IM), le phénotype métaboliseur normal (NM), le phénotype métaboliseur rapide (RM) et le phénotype métaboliseur ultra-rapide (UM).<br><br>Pour caractériser l'activité du CYP2C19, il existe différentes méthodes de phénotypage à savoir l'administration d'une substance-test métabolisée de manière spécifique par le CYP2C19 et la mesure de paramètres pharmacocinétiques, tels que l'aire sous la courbe (AUC), ou des ratios métaboliques entre les concentrations de la molécule-mère et de son métabolite. Les substances-tests les plus utilisées sont l'oméprazole (OPZ) et la méphénytoïne. Il est également possible de mesurer le phénotype de plusieurs isoformes des CYPs simultanément avec le phénotypage mixte, tel que le « Geneva cocktail » développé aux HUG. Ce dernier est composé de 6 substances-tests permettant de phénotyper simultanément 6 isoformes des CYPs, dont l'OPZ pour le CYP2C19.<br><br>Malgré une bonne tolérance de ce cocktail, certains patients peuvent être réticents à l'administration de médicaments de manière exogène et il n'est parfois pas possible de les administrer à certains patients (*e.g*. femmes enceintes, enfants, personnes âgées). Il convient par ailleurs d'attendre 2h après l'ingestion du « Geneva cocktail » avant d'effectuer la prise de sang, ce qui peut poser quelques contraintes pratiques en clinique.<br><br>L'utilisation de biomarqueurs endogènes de mesure de l'activité des CYPs permettrait ainsi de s'affranchir de ces obstacles et de rendre la pratique moins invasive et plus acceptable pour les patients. Une récente étude consacrée au CYP2D6 conduite au sein de notre service a permis d'identifier de nouveaux biomarqueurs endogènes pour cet isoforme, à savoir la solanidine, un alcaloïde aglycone très commun, et ses dérivés.<br><br>Le but de notre étude est d'identifier de nouveaux biomarqueurs endogènes pour mesurer (phénotyper) l'activité du CYP2C19 par métabolomique non ciblée.<br><br>Les volontaires sains seront stratifiés en deux groupes selon leur phénotype pour le CY2C19 : les sujets PMs et NMs-RMs-UMs. Les sujets IMs seront exclus afin d'observer des différences plus significatives au sein des deux groupes.<br><br>Objectif primaire :<br>- Partie 1 : mise en évidence de potentiels substrats et/ou métabolites endogènes du CYP2C19 par métabolomique non ciblée sur des échantillons urinaires, plasmatiques et de vésicules extracellulaires dérivées du foie prélevés avant (baseline, session 1) et après administration répétée de fluvoxamine (inhibition enzymatique du CYP2C19, session 2) ainsi qu'après administration répétée de rifampicine (induction enzymatique du CYP2C19, session 3).<br>- Partie 2 : identification des substrats et/ou métabolites endogènes du CYP2C19 parmi les composés mis en évidence dans la partie 1 par analyse semi-quantitative ciblée sur des échantillons urinaires, plasmatiques et de vésicules extracellulaires dérivées du foie des volontaires sains PMs et NMs-RMs-UMs à la baseline (i.e sans administration préalable de fluvoxamine ou de rifampicine).<br>Objectifs secondaires :<br>- Identification des substrats et/ou des métabolites endogènes du CYP2C19 à l'aide de bases de données internes ou externes, par fragmentation en LC-MS/MS ou à l'aide de standards de référence.<br>- Corrélation des concentrations plasmatiques et/ou urinaires et/ou de vésicules extracellulaires dérivées du foie d'un substrat et/ou un métabolite endogène du CYP2C19 avec le phénotype (ratio sanguin OH-oméprazole/oméprazole) et/ou le génotype du CYP2C19. |

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| | - Évaluation des niveaux d'expression de cfRNA du CYP2C19 issu de vésicules extracellulaires et corrélation avec le génotype, phénotype (omeprazole) et modulation de l'activité du CYP2C19 par administration d'un inhibiteur (fluvoxamine) et d'un inducteur (rifampicine).<br>- Comparer la signature métabolomique du CYP2C19 des vésicules extracellulaires dérivées du foie avec celle du plasma et de l'urine.<br>- Comparer la signature métabolomique du CYP2C19 des vésicules extracellulaires dérivées du foie avec celle des vésicules extracellulaires totales.<br>- Construction d'un modèle de régression linéaire multiple pour prédire le phénotype du CYP2C19.<br>- Validation des résultats à l'aide d'enzymes recombinantes et d'hépatocytes humains génotypés pour le CYP2C19. |
| **Méthode** | Le projet consiste en trois parties expérimentales :<br>- Partie 1 : mesure de l'impact d'un inhibiteur du CYP2C19, la fluvoxamine, sur le métabolome urinaire, plasmatique et de vésicules extracellulaires dérivées du foie<br>- Partie 2 : mesure de l'impact d'un inducteur du CYP2C19, la rifampicine, sur le métabolome urinaire, plasmatique et de vésicules extracellulaires dérivées du foie<br>- Partie 3 : mise en évidence des biomarqueurs du CYP2C19 par mesure de l'impact des polymorphismes du CYP2C19 sur les composés affectés dans les parties 1 et 2.<br><br>Il s'agit d'une étude ouverte à design croisé chez des volontaires sains répartis en deux groupes selon leur génotype du CYP2C19 (PMs et NMs-RMs-UMs). Trois sessions auront lieu pour chaque sujet :<br>**Session 1** : prélèvements plasmatique et urinaire ainsi que la détermination du ratio sanguin OH-oméprazole/oméprazole à la baseline par prise de sang capillaire.<br>**Session 2** : idem session 1 avec prise au préalable de 50 mg de fluvoxamine pendant 7 jours consécutifs (inhibiteur fort du CYP2C19).<br>**Session 3 :** idem session 1 avec prise au préalable de 600 mg de rifampicine pendant 10 jours consécutifs (inducteur fort du CYP2C19).<br><br>A chaque session, une récolte urinaire sur 24h est effectuée, suivie d'une prise de sang veineux (6 x 6 ml) le matin à jeun. Le phénotypage a ensuite lieu avec la prise d'oméprazole à 10 mg, suivie d'une prise de sang capillaire (4 x 10 µl) après 2h.<br>Après la session 1, la prise de fluvoxamine a lieu durant 7 jours consécutifs (administration au total de 7 doses de fluvoxamine à 50 mg), pouvant commencer au moment du repas de midi lors du déroulement de la session 1. La dernière prise de fluvoxamine doit avoir lieu 2h avant l'administration d'oméprazole à 10 mg lors de la troisième visite (jour 11±4j, session 2).<br>Enfin, la prise de rifampicine aura lieu au moins 6 jours après la session 2, tous les soirs durant 10 jours consécutifs (administration au total de 10 doses de rifampicine à 600 mg). La dernière prise de rifampicine doit avoir lieu le soir de la veille de l'administration d'oméprazole à 10 mg lors de la quatrième visite (jour 27±4j, session 3). |
| **Nombre de volontaires** | 40 volontaires sains : 10 PMs et 30 NMs-RMs-UMs |
| **Durée de l'étude** | Au minimum 24 jours et au maximum 32 jours par volontaire |

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| **Critères de sélection** | <u>Critères d'inclusion :</u><br>Hommes et femmes en bonne santé habituelle<br>Age : entre 18 et 65 ans<br>IMC : entre 18 et 27<br>Capables de donner leur consentement éclairé<br>Compréhension de la langue française<br>Génotype du CYP2C19 : PMs, NMs, RMs ou UMs<br>Au moins une contraception à effet barrière pendant toute la durée de l'étude et 1 mois après la fin de l'étude (en plus de la contraception hormonale habituelle le cas échéant)<br><br><u>Critères de non-inclusion :</u><br>Génotypes IMs du CYP2C19<br>Participation à un autre essai clinique interventionnel dans les 3 mois précédant l'inclusion<br>Femme enceinte ou allaitante<br>Pathologies, médicaments ou aliments qui affectent l'activité des CYP450<br>Antécédent de transplantation hépatique<br>Fumeurs réguliers de ≥ 10 cigarettes/jour<br>Altération des tests hépatiques (ASAT, ALAT, GGT, bilirubine plus de 3x la norme)<br>Insuffisance rénale (urée sérique, créatinine sérique et eGFR en dehors des normes)<br>Antécédent d'alcoolisme chronique ou d'abus de substances psychotropes<br>Allergie aux médicaments administrés<br>Pathologie psychiatrique active<br>Score de Beck ≥ 10 (question sur le suicide > 0)<br>Prise d'alcool 2 jours avant la session 1 et pendant la prise de fluvoxamine et rifampicine<br>Porteurs de lentilles de contact |
| **Produit d'étude**<br>**Dosage**<br>**Voie d'administration**<br>**Durée du traitement** | Oméprazole<br>Spécialité : Antramups®<br>Dosage : prise unique d'un comprimé de 10 mg d'Antramups® le matin à chaque session de phénotypage<br>Voie d'administration : orale<br>Durée de traitement : prise unique, à chaque session<br>Dose thérapeutique habituelle : 20-40 mg/j, selon l'indication<br><br>Fluvoxamine<br>Spécialité : Floxyfral® junior cpr pell 50 mg<br>Dosage : un comprimé de 50 mg de Floxyfral® junior avec le petit déjeuner pendant 7 jours (le dernier jour de prise étant au matin du phénotypage de la session 2, 2 h avant la prise d'oméprazole)<br>Voie d'administration : orale<br>Durée de traitement : 7 jours consécutifs (7 comprimés au total)<br>Dose thérapeutique habituelle : Dose initiale de 50 mg par jour, puis titration jusqu'à 100 mg 1x/j-150 mg 2x/j<br><br>Rifampicine<br>Spécialité : Rimactan®, caps 300 mg<br>Dosage : 600 mg/j, soit 2 capsules de 300 mg le soir pendant 10 jours (le dernier jour de prise étant le soir de la veille précédant le phénotypage de la session 3)<br>Voie d'administration : orale<br>Durée de traitement : 10 jours consécutifs (20 comprimés au total)<br>Dose thérapeutique habituelle : 600-1200 mg/j selon l'indication |
| **Critères d'évaluation** | <u>Critère d'évaluation principal :</u><br>Changement des intensités des ions déterminées par spectrométrie de masse selon le génotype (PMs versus NMs-RMs-UMs) et selon les sessions. Les ions montrant un changement de facteur ≥ 1,5 ou ≤ 0,67 seront qualifiées de |

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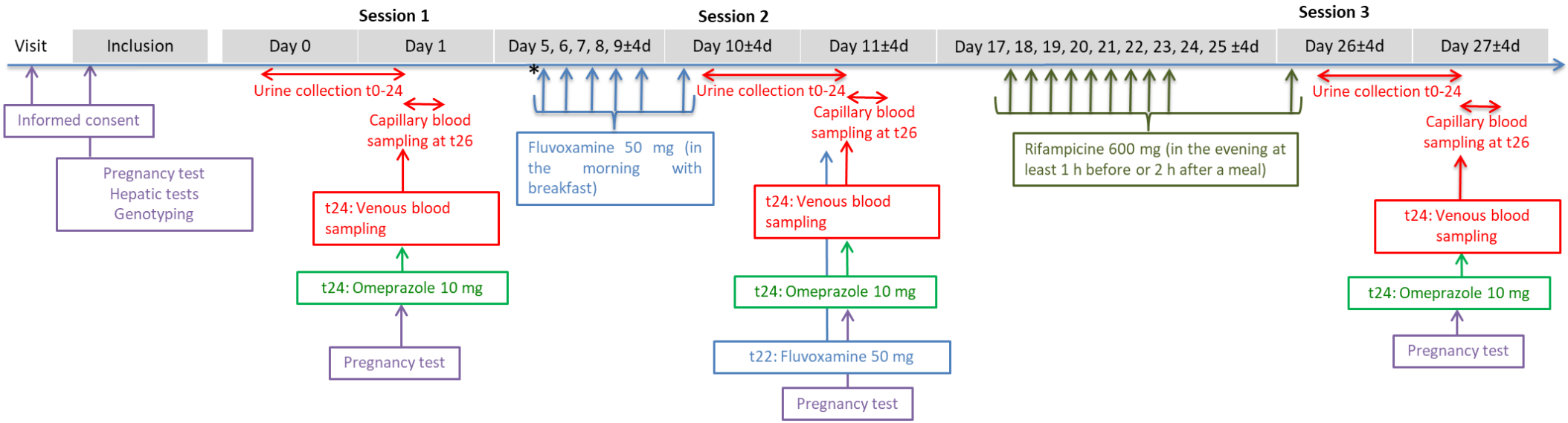
| | biomarqueurs endogènes du CYP2C19. Les signaux chromatographiques seront détectés dans l'urine, plasma et vésicules extracellulaires dérivées du foie de volontaires sains par chromatographie liquide couplée à la spectrométrie de masse à haute résolution (LC-HRMS) à chaque session pour chacun des volontaires.<br>Critères d'évaluation secondaires :<br>Corrélation du phénotype avec les métabolites statistiquement significatifs mesurés en métabolomique.<br>Modèle de régression linéaire multiple pour prédire le phénotype du CYP2C19. |
| **Evaluation de la tolérance** | Les SAEs avec issue fatale et les SUSARs seront systématiquement relevés et rapportés dans les délais appropriés à la CCER et aux autorités. |
| **Méthode statistique** | Critères primaires :<br>Analyses univariées : Volcano plot<br>Analyses multivariées : Analyse en composantes principales, régression des moindres carrés partiels<br><br>Critères secondaires :<br>Test de corrélation de Spearman |

# STUDY FLOWCHART

| Study period | Inclusion | Session 1 | | Session 2 | | | | | | | Session 3 | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Visit (HUG) | 1 | - | 2 | - | - | - | - | - | - | 3 | - | - | - | - | - | - | - | - | - | - | 4 |
| Time (day) | -16±15d | 0 | 1 | 5±4d | 6±4d | 7±4d | 8±4d | 9±4d | 10±4d | 11±4d | 17±4d | 18±4d | 19±4d | 20±4d | 21±4d | 22±4d | 23±4d | 24±4d | 25±4d | 26±4d | 27±4d |
| Reception of signed ICF | X | | | | | | | | | | | | | | | | | | | | |
| Inclusion and exclusion criteria | X | | | | | | | | | | | | | | | | | | | | |
| Status and clinical evaluation | X | | | | | | | | | | | | | | | | | | | | |
| Venous blood sample for CYP genotyping | X | | | | | | | | | | | | | | | | | | | | |
| Hepatic and renal tests | X | | | | | | | | | | | | | | | | | | | | |
| Medical history | X | | | | | | | | | | | | | | | | | | | | |
| Pregnancy test (women only) | X | | X | | | | | | | X | | | | | | | | | | | X |
| CYP2C19 phenotyping (omeprazole administration) | | | X | | | | | | | X | | | | | | | | | | | X |
| Verifying compliance by taking back empty blisters | | | | | | | | | | X | | | | | | | | | | | X |
| Contraception (with a barrier method) | | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Administration of fluvoxamine | | | (X)* | X | X | X | X | X | X | X | | | | | | | | | | | |
| Administration of rifampicine | | | | | | | | | | | X | X | X | X | X | X | X | X | X | X | |
| Venous Blood sampling (metabolomics) | | | X | | | | | | | X | | | | | | | | | | | X |
| 24-hours urine collection (metabolomics) | | X | X | | | | | | X | X | | | | | | | | | | X | X |
| Capillary blood sampling (phenotyping) | | | X | | | | | | | X | | | | | | | | | | | X |

**The first tablet of fluvoxamine can be administrated during lunch on day 1 of session 1*

# STUDY DESIGN




**The first dose of fluvoxamine can also be administered on day 1 of session 1 during lunch*

# Abbreviations

| | |
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| ADR | adverse drug reaction |
| AE | adverse event |
| ALAT | alanine-aminotransferase |
| ASAT | aspartate-aminotransferase |
| ASR | annual safety report |
| AUC | area under the concentration curve |
| CCER | commission cantonale d'éthique et de la recherche |
| eCRF | electronic case report form |
| CYP | cytochrome P450 |
| DBS | dried blood spot |
| DDI | drug-drug interaction |
| EV | extracellular vesicle |
| FDA | US Food and Drug Administration |
| GGT | Gamma glutamyl transpeptidase |
| HUG | Geneva University Hospitals |
| IM | intermediate metabolizer |
| MR | metabolic ratio |
| NM | normal metabolizer |
| NMR | nuclear magnetic resonance |
| OClin | swiss ordinance on clinical trials on research involving humans |
| OH-OPZ | hydroxyomeprazole |
| OPZ | omeprazole |
| PM | poor metabolizer |
| RM | rapid metabolizer |
| SAE | serious adverse event |
| SUSAR | suspected unexpected serious adverse reaction |
| UM | ultra-rapid metabolizer |
| UMR | urinary metabolic ratio |

# 1. STUDY ADMINISTRATIVE STRUCTURE

## 1.1 Sponsor, Sponsor-Investigator

Professor Caroline Samer, MD
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Professor Caroline Samer supervised the conception of study's documents. She will be in charge of participants' clinical follow-up and will supervise the interpretation of data and reports relative to this study.

## 1.2 Principal Investigator(s)

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Study's investigators will include participants and be in charge of their clinical follow-up.

## 1.3 Statistician ("Biostatistician")

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## 1.4 Laboratory

Pharmacological and toxicological investigation unit, University Hospitals of Geneva
Rue Michel-Servet 1, 1206 Genève

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## 1.5 Monitoring institution

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### 1.6 Any other relevant Committee, Person, Organisation, Institution

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Professor Youssef Daali supervised the conception of study's documents. He will supervise laboratory work as well as data acquisition and analyses.

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Professor Aurélien Thomas supervised the conception of study's documents. He will supervise laboratory work as well as data acquisition and analyses.

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Mr Yahia Bennani wrote study's documents. He will perform information sessions, collect participants' data and handle data processing and interpretation.

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Dre Gaelle Magliocco supervised the conception of study's documents and will supervise data processing.

Mrs. Valérie Khan-Besse, nurse
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Mrs Valérie Khan-Besse will be in charge of blood sampling during participants' inclusion and sessions.

# 2. INTRODUCTION

## 2.1 Drug variability

The response of one individual to its treatment is the result of its efficacy at the therapeutic target (pharmacodynamics, PD) and its disposition in the body (pharmacokinetics, PK).[1]

Pharmacokinetics is the result of different stages: absorption of the drug into systemic circulation, tissue distribution, metabolism and elimination. Metabolism and elimination are the main PK causes of drug variability. While elimination occurs primarily in the faeces or by renal route, metabolism is mainly mediated by hepatocytes in the liver. Hepatocytes contain various enzymes whose main function is the transformation of a lipophilic drug into a hydrophilic metabolite with high excretion capability.[2]

## 2.2 Cytochromes P450

Cytochromes P450 (CYP450) constitute the main enzyme family responsible for oxidative metabolism (phase I) of major drugs and xenobiotics.[3] In human, 57 functional genes have been identified. These are grouped in 18 families and 44 sub-families.[4] Most of them have endogenous functions such as biosynthesis of steroid hormones, prostaglandins and bile acids.[5] Drugs are metabolized by only 12 CYP isoenzymes, which belong to families 1-3. The most important isoenzymes in drug metabolism are CYP3A4/5 (which metabolizes 30.2% of drugs), CYP2D6 (20%), CYP2C9 (12.8%), CYP1A2 (8.9%), CYP2B6 (7.2%), and CYP2C19 (6.8%).[6]

## 2.3 Presentation and variability of CYP2C19

Despite its relatively low abundance in the liver (<4%), CYP2C19 metabolizes a large number of commonly used drugs such as proton pump inhibitors (e.g. omeprazole), antidepressants (e.g. citalopram), benzodiazepines (e.g. diazepam), the antiplatelet prodrug clopidogrel and mephenytoin.[7] A huge variability in the activity of CYP2C19 has been observed in the general population. Indeed, the AUC of omeprazole, a known specific substrate of CYP2C19, is four to twelve times higher in poor metabolizers (PM) than in normal metabolizers (NM).[8–11]

Five main phenotypes of CYP2C19 activity have been described to date. Normal metabolizers (NM) represent most of the population. Some people have a faster metabolism capacity and are thus called rapid metabolizers (RM). Others have an even faster metabolism and are called ultrarapid metabolizers (UM). In contrast, a significant proportion of the population metabolizes CYP2C19 substrates slowly. Those people with an impaired CYP2C19 function are known as poor metabolizers (PM). Finally, there is an intermediate phenotype, which reflects a distinctly

reduced function of CYP2C19. People with this phenotype are called intermediate metabolizers (IM). The proportion of these five phenotypes varies between ethnic groups, indicating a genetic component in the variability of CYP2C19. Currently, more than thirty alleles have been identified and described by the Pharmacogene Variation (PharmVar) Consortium.[12–14] Table 1 below shows the phenotypes predicted on the basis of the different diplotypes.

**Table 1**: Genotype-based phenotype prediction for CY2C19 and phenotype frequency in Europe according to the Clinical Pharmacogenetics Implementation Consortium (CPIC) classification.[12,14]

| Predicted phenotype | Phenotype frequency in Europe | Genotype | Examples of diplotypes |
|---|---|---|---|
| UM | 4.64% | Two increased function alleles | *17/*17 |
| RM | 27.12% | One normal function allele with one increased function allele | *1/*17, *11/*17, *13/*17, *17/*38 |
| NM | 39.61% | Two normal function alleles | *1/*1, *1/*18, *11/*38, *15/*28 |
| IM | 26.11% | One non-function allele with one increased function allele or one normal function allele | *1/*2, *1/*3, *2/*11, *4/*38 |
| PM | 2.39% | Two non-functional alleles | *2/*2, *3/*3, *7/*37, *22/*37 |

CYP2C19 is also subject to variability due to environmental, physiological and pathological factors. Inhibition by many drugs has been reported (such as with fluvoxamine)[15,16], as well as induction (such as with rifampicin)[17]. Finally, CYP2C19 activity might also be influenced by pregnancy, oral contraceptives and gender.[18–20]

### 2.4 Assessment of CYP2C19 activity

Two approaches can be used to assess CYP2C19 activity: genotyping and phenotyping methods. Genotyping is based on DNA analysis and polymorphisms detection.[21] Its major drawback is the incapacity to measure the influence of the environmental factors on CYP2C19 activity. Moreover, some rare variants may not be screened and an allele may be erroneously categorized as functional.[22]

To characterize real-time activity of these enzymes, phenotyping is the preferred approach as it takes into account simultaneously genetic, endogenous and environmental factors.[23] Phenotyping consists of the administration of a probe drug specifically metabolized by an

isoenzyme. Omeprazole (OPZ)[24–26] and mephenytoin[27,28] are the most common probes used to assess CYP2C19 activity. The assessment of different PK parameters of the probe drug and its metabolite, or the determination of the metabolic ratio (MR) of the probe drug and its metabolite enable to define an individual metabolic profile. Thus, it provides relevant information about *in vivo* enzymatic activities that may be applied in clinical practice. Phenotyping tests are either selective or mixed, also known as a 'cocktail' phenotyping method. Selective phenotyping involves the administration of one probe drug whereas mixed phenotyping allows the determination of the activity of multiple enzymes concurrently.[29]

The "Geneva Cocktail" has been developed by the Clinical Pharmacology and Toxicology Department at Geneva University Hospitals, to simultaneously assess the activity of six CYP isoenzymes (i.e. caffeine (CYP1A2), bupropion (CYP2B6), flurbiprofen (CYP2C9), omeprazole (CYP2C19), dextromethorphan (CYP2D6) and midazolam (CYP3A)). This cocktail combines the use of low-dose probes and the dried blood spots (DBS) sampling technique.[30]

However, the main disadvantage of phenotyping with probe drugs is the potentially invasive nature of the procedure using exogenous substances. Indeed, their intake may not be well received by patients and study subjects. In addition, there may still be some security issues when administrating medications (e.g. dosage errors) and it is also necessary to wait few hours before DBS sampling after the cocktail intake which may be clinically impractical. Furthermore, it can hardly be administered to vulnerable populations (i.e. pregnant women, children and the elderly).[22]

Therefore, replacing the probe drugs used in routine phenotyping with endogenous biomarkers may be more acceptable to patients and study volunteers, avoid any delay before sampling and completely eliminate any risk associated with exogenous substances.[22]

### 2.5 CYP450 endogenous biomarkers

The search for CYP450 biomarkers is on the rise and endogenous ones are already promising for certain isoforms. Regarding CYP3A, cortisol and its metabolite 6-beta-hydroxycortisol as well as cholesterol and 4-beta-hydroxycholesterol are commonly used as endogenous probes despite some drawbacks.[31,32] Furthermore, it has been shown that CYP3A specifically catalyzes 1-beta-hydroxylation of deoxycholic acid[33], which has recently been investigated in our lab by Magliocco et al[34]. 5-omega and omega-1-hydroxylated medium-chain acylcarnitines have also been identified as novel CYP3A biomarkers by Kim B. et al. using an untargeted metabolomics approach.[35] Concerning CYP1A2, the 6-hydroxymelatonin/ melatonin MR in urine is considered as a potential endogenous metric.[22] Indeed, CYP1A2 is the major enzyme involved in the 6-hydroxylation of melatonin and was recently investigated in our department in a clinical study by Magliocco et al. However, the suitability of such potential endogenous biomarker has yet to be clarified.[36] For CYP2D6, many studies have been conducted and

several endogenous compounds could be used (e.g. codeine/morphine, 5-methoxytryptamine/serotonin). We most recently discovered and identified through untargeted metabolomics and NMR, solanidine and its metabolite 3,4-seco-solanidine-3,4-dioic acid.[22,37,38]

### 2.6 CYP2C19 endogenous biomarkers

In contrast, CYP2C19 still lacks endogenous probes that could be used clinically. Arachidonic acid (AA) and its metabolites have been identified as potential endogenous biomarkers for CYP2C19.[22,39,40]

Despite this interesting finding, clinical validation remains necessary and other CYP450s, in particular CYP2C8 and 2C9, are also responsible for AA metabolism, which would compromise the specificity of the endogenous probe and the discovery of novel endogenous biomarkers of CYP2C19 is needed.[22]

### 2.7 Metabolomics

Metabolomics refers to the study of small molecules (<1500 Da) present in a biological sample (biofluid, cell, tissue, organ or organism). Liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) is the universal method to perform metabolomics analysis because of its sensitivity, selectivity, resolution, high throughput, mass accuracy and capacity to generate MS/MS spectra. Nuclear magnetic resonance (NMR) as well as gas-chromatography coupled to mass spectrometry (GC-MS) are also frequently used.[41] All these methods are complementary to each other and enable to expand the diversity and number of metabolites detected.

Metabolomics is commonly used in biomarkers discovery to understand, for example, response to environmental influences, therapy or diseases.[42] It combines the measure of the environment and genetics. Thus, the metabolome could be also seen as a high-resolution phenotype.[43]

Untargeted metabolomics is thus seen as a "*qualitative or semiquantitative analysis of the largest possible number of metabolites from a diversity of chemical and biological classes contained in a biological specimen*".[44] In this project, untargeted metabolomics analysis using LC-HRMS will be used on plasma, urinary and liver-derived extracellular vesicles samples in order to measure the impact of genetics and environmental modulation of CYP2C19 on the metabolome.

### 2.8 Primary objective

The primary objective of this study is to identify and validate an endogenous substrate and/or metabolite of CYP2C19 activity using untargeted metabolomics and semi-quantitative targeted analysis in healthy volunteers.

### 2.9 Secondary objective

- Determination of the chemical structure of the discovered metabolites with internal or external databases, LC-MS/MS fragmentation, reference standards or NMR.
- Correlation of the relative abundance (or absolute concentration if the molecule has been identified and is commercially available) in urine and/or plasma and/or liver-derived extracellular vesicles of the discovered metabolite with the phenotype (blood $MR_{OH\text{-}OPZ/OPZ}$).
- Evaluation of the expression levels of CYP2C19 cell-free RNA (cfRNA) from extracellular vesicles and correlation with genotype, phenotype (omeprazole) and modulation of CYP2C19 activity by administration of an inhibitor (fluvoxamine) and an inducer (rifampicin).
- To compare CYP2C19 metabolomic signature of liver-derived extracellular vesicles with that of plasma and urine.
- To compare CYP2C19 metabolomic signature of liver-derived extracellular vesicles with that of total extracellular vesicles.
- Establishment of a multivariate linear regression model to predict CYP2C19 phenotype.
- Validation of the results with recombinant enzymes and human hepatocytes genotyped for CYP2C19.

## 3. STUDY DESIGN

### 3.1 Overall description

The study will be an open-label study with a total of 40 healthy volunteers fulfilling inclusion/non-inclusion criteria, divided into 2 study groups: 10 healthy volunteers PMs and 30 NMs-RMs-UMs for CYP2C19 and participating in the three sessions:

- Session 1: urine and venous blood sampling. Determination of blood $MR_{OH\text{-}OPZ/OPZ}$ at baseline (no inhibitor administrated).
- Session 2: urine and venous blood sampling. Determination of blood $MR_{OH\text{-}OPZ/OPZ}$ after pre-treatment by fluvoxamine (strong inhibitor of CYP2C19).

- Session 3: urine and venous blood sampling. Determination of blood $MR_{OH\text{-}OPZ/OPZ}$ after pre-treatment by rifampicin (strong inducer of CYP2C19).

**Information:**

At this step, the volunteer will have an interview with one of the co-investigators at the Clinical Pharmacology and Toxicology department and the study will be explained in detail. A sufficient time will be allowed before the inclusion session to guarantee sufficient time for reflection.

**Inclusion:**

Volunteers willing to participate and having signed the informed consent form (ICF) will undergo a clinical evaluation with a physician including liver and kidney tests, body mass index, genotyping (except if CYP2C19 genotype is already known) and a pregnancy test (women only). Women will be asked to use at least one physical contraceptive barrier (i.e. male or female condom, diaphragm, spermicide) throughout their study participation and 1 month after the end of the study, in addition of their hormonal contraception if applicable. In case of heavy menstrual bleeding, women will have to be recruited before or after their menstrual period to avoid the presence of blood in urine. In addition, all volunteers will be asked to avoid alcohol consumption on days of taking study drugs and 48 hours before day 0 of session 1. A volunteer code for the study will be assigned to each volunteer included in the study.

Only volunteers fulfilling the inclusion/non-inclusion criteria will be allocated to a study group.

**Sessions course:**

Each session includes urine collection over a full 24-hour period that will start in the morning of day 0 for session 1, day 10±4d for session 2 and day 26±4d for session 3. It will end in the morning of day 1 for session 1, day 11±4d for session 2 and day 27±4d for session 3. Since most of this part will be performed at home, subjects will be given precise instructions on how to perform it. The last urine stream for each session will be collected in the morning after an overnight fast. Urine will be collected into a 24-hour collection container. This will be stored in a cooler with ice packs and/or a refrigerator.

Immediately after the 24-hour urine collection, venous blood (6 x 6 ml) will be collected and one 10 mg tablet of omeprazole (Antramups®) administrated. Women will only receive omeprazole after verifying that they are not pregnant (urine test). 2 hours after omeprazole intake, capillary blood sampling (DBS sampling) will be performed. Volunteers will then be free to leave the HUG. Breakfast will be served after capillary blood sampling.

No wash-out period is necessary between the first and second session as fluvoxamine treatment lasts one week, which is sufficient to eliminate omeprazole administered in the first session, as well as its metabolites. Indeed, omeprazole's half-life elimination is generally less than 1 hour, and does not exceed 3 hours in PMs healthy subjects[45,46]. However, a minimum of six days is necessary between session 2 and session 3. This time gap is required because of the half-life elimination of fluvoxamine being 19,5 hours on average after multiple intakes[47–49].

Concerning the second session, participants will be instructed (orally and through reception of an instruction card) to take one 50 mg tablet of fluvoxamine (Floxyfral® junior) every morning for one week (7 doses in total) with the breakfast. If the third visit (session 2) happens exactly 7 days after the second visit (session 1), the drug must be taken during the lunch of session 1 (day 1) in order to have a total of 7 doses of fluvoxamine administrated. Otherwise, the treatment must start one week before the third visit (session 2) and the last dose must be taken 2 hours before omeprazole intake during the day 1 of session 2.

Concerning the third session, participants will also be instructed to take two 300 mg tablets of rifampicin (Rimactan®) every evening for 10 days (10 doses in total) before the fourth visit. This regimen will begin at least 6 days after the third visit (session 2) and the last rifampicin intake will be done at the evening preceding the fourth visit (session 3).

Volunteers will be asked to back empty blisters of the drugs that they took home in order to verify compliance.

The total study duration will not exceed 32 days. Therefore, the first dose of fluvoxamine and rifampicin must be taken, at the latest, 9 days and 21 days after day 1 of session 1, respectively (see study design page 13).

The order of sessions is purposely not randomized due to unnecessity. Indeed, as it is solely a PK study performed on paired samples, there are no possible biases requiring randomization.

### 3.2 Primary and secondary endpoints

**Primary endpoint:**

Fold-change in the intensities of features determined by mass spectrometry according to genotype (PMs versus NMs-RMs-UMs) and according to sessions. Metabolic features showing a fold-change ≥ 1.5 or ≤ 0.67[37] will be qualified as CYP2C19 endogenous biomarkers. Chromatographic signals will be detected in healthy volunteers' urine, plasma and liver-derived

extracellular vesicles by liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) at each session for each volunteer.

**Secondary endpoint:**

- Correlation of significant ions measured in metabolomics with OH-OPZ/OPZ blood ratio.
- Establishment of a multivariate linear regression model to predict CYP2C19 phenotype.

### 3.3 Potential risks and benefits for the volunteers

There will be no personal benefits for the volunteers included in this study besides the possibility to know their CYP genotype and phenotype if they are willing to. Potential risks include the occurrence of adverse events related to fluvoxamine (described in detail in section 5.1.2), rifampicin (described in detail in section 5.1.3), venous and capillary blood sampling (minimal risk category: local pain, infection, haematoma) and the rare occurrence of omeprazole adverse events (described in detail in section 5.1.1).

### 3.4 Study duration for subjects and stopping rules

Apart from the screening session (visit 1), the minimal study duration will be 24 days (day 0-27±4d) including 3 half-days spent in the HUG (visit 2, 3 and 4).
Volunteers will be able to withdraw from the study at any time without any explanation or justification. The data collected up at this moment will be conserved and used unless the volunteer wants its signed informed consent withdrawn. The investigator will also have the possibility to exclude a volunteer from the study for safety reasons or lack of compliance.

### 3.5 Data collected in the eCRF

All data described in section 7.1.1. will be reported in the eCRF. A list of all participants will be kept, with the date of inclusion and the date and reason for drop-out if applicable.

## 4. STUDY POPULATION

### 4.1 Selection and recruitment procedures

Volunteers will be recruited through advertisements approved by the “commission cantonale d’éthique et de la recherche” (CCER) of Geneva and displayed in different public buildings or

transmitted by email and social media. After a first contact (either telephonic or by e-mail), potential volunteers will be invited to participate to an information session at which the study aim and design as well as possible risks will be explained. After allowing at least 72 hours for reflection, volunteers willing to participate in the study will sign the consent form. In the next step, they will be subject to a screening procedure including a review of volunteer's medical history and current health status, physical examination, hepatic and renal tests, body mass index determination, pregnancy test (for women) and genetic testing for CYP2C19.
After evaluation of the inclusion and non-inclusion criteria, eligible volunteers will be allocated to a study group (CYP2C19 PMs or NMs-RMs-UMs) and will attend sessions 1, 2 and 3.

### 4.2 Inclusion criteria

- Healthy men and women
- Age 18-65 years
- Body Mass Index (BMI) 18-27
- Understanding of French language and able to give a written inform consent
- CYP2C19 genotype: PMs, NMs, RMs or UMs
- At least one barrier method contraception during the whole study and 1 month after the end of the study (in addition of hormonal contraception if applicable)

### 4.3 Non-inclusion criteria

- CYP2C19 IMs
- Participation in any other interventional clinical study within 3 months prior to inclusion
- Pregnant or breastfeeding woman
- Any pathologies, use of drugs or food that may affect CYP activity (based on the 'drug interactions and cytochromes P450 table published by the Service of Clinical Pharmacology and Toxicology, HUG[50] and on the investigator's knowledge)
- Medical history of liver transplantation
- Regular smokers of ≥ 10 cigarettes/day
- Alcohol intake 2 days prior to session 1 and during fluvoxamine and rifampicin intake
- Alteration of hepatic tests (ASAT, ALAT, GGT, bilirubin more than 3x normal)
- Renal failure (serum urea, serum creatinine and eGFR outside the norms)
- Medical history of chronic alcoholism or abuse of psychoactive drugs
- Sensitivity to any of the drugs used

- Psychiatric disorders
- Beck Score ≥10 (question related to suicide >0)
- Contact lens wearers

### 4.4 Assignment to study groups

Each eligible participant will be assigned to a study group according to his CYP2C19 genotype. The first group will be composed of carriers of two non-functional alleles of CYP2C19 (PMs). The second group will be composed of carriers of two normally functional alleles (NMs), one normally functional allele and one increased-functional allele (RMs) or two increased-functional alleles of CYP2C19 (UMs).

IM subjects will not be included in the clinical study because of the intermediate activity of the enzyme that may not highlight significant changes in the metabolome when compared to the metabolome of PMs or NMs. In addition, NMs, RMs and UMs are grouped together because of the potentially low changes in the metabolome between them as it has been found for the endogenous DHETs blood levels and many exogenous compounds such as omeprazole, pantoprazole and lansoprazole.[8,10,11,40,51]

### 4.5 Withdrawal criteria

Volunteers are free to withdraw from the study at any time without giving any explanation. If a volunteer withdraws from the study, a new volunteer will be included. The investigators may exclude a volunteer from the study for medical reasons or when eligibility criteria are not fulfilled anymore.

## 5. STUDY INTERVENTION

### 5.1 Identity of Investigational Products

#### 5.1.1 Omeprazole

Omeprazole is the exogenous administrated probe used for CYP2C19 phenotyping. A unique intake of this drug at 10 mg will be performed at each session, for a total of 3 sessions.

Omeprazole is specifically metabolised to an inactive metabolite, OH-omeprazole (OH-OPZ), by CYP2C19.[52,53] It is a first-generation proton pump inhibitor used to treat gastroesophageal reflux disease, gastric and duodenal ulcers and other gastric disorders. Its mechanism of action consists of reducing gastric acidity by inhibiting the secretion of gastric acid. The level of individual omeprazole exposure varies depending on several factors such as the dose and

the drug PK features like absorption rate and metabolism kinetics, as well as individual characteristics like age or CYP2C19 genotype.[54] The usual posology of omeprazole is 20 mg once a day for 2 weeks. This dose is commonly increased to 40 mg and the treatment duration can be expended to 4 weeks if necessary. A dose of 10 mg, corresponding to the prophylactic dose, will be administered in this study.[45]

Omeprazole is rapidly absorbed after oral administration, with an absolute bioavailability of 30-40% at doses of 20-40 mg. The maximal plasmatic concentrations are achieved 0,5-3,5 hours after the administration. The elimination half-life of omeprazole is 0,5-1 hour in healthy CYP2C19 NMs, but can be up to three times longer in healthy CYP2C19 PMs.[46,53]

Adverse effects with standard doses of omeprazole (20-40 mg) are generally rare but headaches, nausea, diarrhoea and/or other gastro-intestinal disturbances may occur.[45] During this clinical trial, adverse effects of omeprazole are highly unlikely.

DBS sampling method using 10 µl of capillary blood obtained from a small finger prick will be performed 2 hours after omeprazole intakes. This sampling procedure has the advantage to be less invasive, more cost-effective and easier to transport and store in comparison to the standard venous blood sampling.[55]

### 5.1.2 Fluvoxamine: CYP2C19 inhibitor

Fluvoxamine is an antidepressant drug of the selective serotonin reuptake inhibitor (SSRI) class. It is used as a medical treatment in depression, as well as in anxiety disorders. The usual starting dose of fluvoxamine is 50 mg per day. The daily dose can be increased to 100 mg once a day and up to a maximum of 300 mg per day taken in divided doses (e.g. 150 mg twice a day) for non-responding patients.[47] In this study, the dose administered is 50 mg once a day for 7 days.

Following oral administration, fluvoxamine is rapidly and almost completely absorbed in the gastrointestinal tract. However, it is also subject to hepatic first-pass metabolism, resulting in an absolute bioavailability of 53%. Steady-state maximal concentrations are reached between 10 to 14 days.[47–49] Fluvoxamine is extensively metabolized in the liver by CYP2D6 and CYP1A2 to at least 11 different metabolites, of which no one is known to be pharmacologically active.[56–59] These metabolites are widely eliminated in urine, with a variable fluvoxamine elimination half-life of about 19,5 hours (range 17-22 hours) after multiple intakes.[47–49] Adverse effects of fluvoxamine are those of SSRIs, which include asthenia, tremor, somnolence, insomnia, gastro-intestinal disorders and erectile dysfunction. In patients suffering from depression, a deterioration of the depressive symptoms and/or suicidal ideation or behaviours may appear with fluvoxamine intake.[47] Consequently, subjects with any psychiatric disorders

will be excluded. Moreover, to ensure the recruitment of psychically healthy subjects, risk of depression and/or suicidal ideation will be assessed with the Beck Depression Inventory (see Section 4.6). Antidepressant discontinuation syndrome (ADS) has been described in case of abrupt cessation generating the following symptoms: dizziness, sensorial troubles, sleep disorders, agitation, anxiety, nausea, tremor, sweating, headaches, and emotional instability. In most patients, the intensity of these symptoms was minor and disappeared spontaneously.[47] Moreover, ADS is unlikely when treatments are taken less than 5-8 weeks.[60,61] In this study, a total of 7 doses of fluvoxamine 50 mg spread over 7 days will be given to volunteers, which minimizes the risk of experiencing adverse events and ADS. Fluvoxamine acts as a potent inhibitor of CYP2C19 and CYP1A2, and a moderate inhibitor of CYP2C9, CYP2D6 and CYP3A4.[15,16,47,62,63] It is mainly metabolized to fluvoxamine acid through CYP2D6, representing its major metabolic pathway, along with minor contributions of CYP1A2.[50,56–59,63]

In this clinical trial, we will administer fluvoxamine for 7 days to be able to observe a decrease in the concentration of endogenous CYP2C19 metabolites with a relatively long half-life. It is not relevant to detect metabolites with a longer half-life as it may not allow the detection of rapid changes in the phenotype. Moreover, the steady-state of fluvoxamine should be almost reached after 7 days of dosing at 50 mg.[48,49]

#### 5.1.3 Rifampicin: CYP2C19 inducer

Rifampicin is rifamycin antibiotic used to treat infections such as tuberculosis, leprosy, meningitis, and staphylococcal infections. The usual dose of rifampicin depends on the type and severity of the infection being treated, as well as the patient's age, weight, and other medical conditions. The standard adult dose ranges from 600 to 1200 mg per day.[64–66] In this study, the dose administered is 600 mg (i.e. 2 tablets of 300 mg) once a day in the evening for 10 days.

Following oral administration, rifampicin is rapidly and almost completely absorbed. Food intake can reduce the absorption of rifampicin. Consequently, it is usually taken 1 hour before or 2 hours after a meal. Steady-state maximal concentrations are reached within 7 days.[64–67] Rifampicin is extensively metabolized in the liver to the active compound 25-O-desacetylrifampicin, which is further metabolized into inactive compounds.[64–66,68] These metabolites are primarily excreted in the bile (80%), with a small amount eliminated in the urine (4-18%). Rifampicin elimination half-life is dose-dependent and is approximately 1 to 2 hours after multiple doses of 600 mg per day.[64,69] Common adverse effects of rifampicin include gastrointestinal disturbances like nausea, vomiting and diarrhoea as well as flu-like symptoms such as fever, chills and headache. These adverse effects are generally mild and resolve once

the medication is discontinued. In addition, rifampicin is responsible of staining contact lens (in addition of urine, sweat, sputum and tears), from which contact lens wearers are excluded from the study. Because Rifampicin is contraindicated in case of severe renal impairment, renal tests (i.e. serum urea, serum creatinine and eGFR) were included during the initial screening procedure of this study in order to exclude all renal impairment cases.

Rifampicin acts as a strong inducer of CYP2C8, 2C9, 2C19 as well as 3A4/5 and a weak inducer of CYP1A2 and 2B6.[17,24,50,64,70,71] Consequently, many concomitantly administered classes of drugs such as benzodiazepines, calcium antagonists, protease inhibitors and neuroleptics become ineffective. This also includes oral contraceptives, which makes barrier contraceptive method an important requirement. Furthermore, induction does not dissipate until 2 weeks after discontinuing rifampicin, reinforcing the need to use a barrier contraceptive method after stopping treatment (i.e. male or female condom, spermicide or diaphragm).[64,70,72]

In this study, at least one barrier contraceptive method will have to be used for all the study duration and for 1 month after the last visit (session 3). During this period, women who usually take oral contraceptives can keep their intake while using a barrier contraceptive method.

It is necessary to administer rifampicin for at least 7 days to achieve the onset of full enzymatic induction. Therefore, 10 days will be applied here, which is sufficient to increase the concentration of endogenous CYP2C19 metabolites.[72]

### 5.2 Packaging, Labelling and Supply

The medications for this study will be purchased from the HUG Pharmacy. They will remain in their original primary packaging (blister packs). A pharmacist from the Division of Clinical Pharmacology and Toxicology will handle the labelling and distribution. Each drug package will be labelled with the product name, dosage form, strength, batch number, expiry date, patient number, and the warning “tenir hors de portée des enfants.” Additionally, the labels will include the protocol number and name, the statement “use only as part of a clinical trial,” and the name of sponsor and principal investigator. For fluvoxamine and rifampicin, healthy subjects will receive the exact number of tablets required for home use (7 tablets for fluvoxamine and 20 tablets for rifampicin) in a labelled ziplock bag.

### 5.3 Storage conditions

The storage of the drugs used in this study will be ensured by a pharmacist of the Division of Clinical Pharmacology and Toxicology. Drug packages used in this study will be labelled with the mention “store at room temperature”.

### 5.4 Compliance with study intervention

Fluvoxamine and rifampicin intakes will be monitored by taking back empty blisters at session 2 and 3 respectively. Fluvoxamine and rifampicin plasma concentrations will also be quantified using a validated LC-MS/MS method.

### 5.5 Data Collection and Follow-up for withdrawn participants

In the event of withdrawal from the study, the data collected up to this moment will still be conserved and analysed. In such a case and if needed, the investigator will provide all necessary information and medical care (including adequate medical referrals) to the volunteer.

### 5.6 Trial specific preventive measure

Healthy subjects will be asked to inform the investigator or another collaborator of the study of all concomitant medication they might take during the study.
Women will be asked to use at least one barrier contraceptive method (i.e. male or female condom, spermicide or diaphragm) throughout all the study duration and for 1 month after the last visit (session 3). Pregnancy test will also be performed during each session to ensure absence of pregnancy.
Participants will be asked to stop driving and using dangerous machinery while fluvoxamine is administrated.
During the inclusion session, the Beck Depression Inventory will be used to exclude any potential symptoms of depression as well as suicidal ideation and behaviours. It is a 21-question multiple choice self-rated scale. The maximum score is 63 and indicates severe depression. Total score of 0-9 represents minimal range.[73] Therefore, subjects with score ≥10 will be excluded from the study. Furthermore, only a score of 0 for the question related to suicide will be accepted.

### 5.7 Drug accountability

Batch numbers and expiry dates for all drugs used in the study will be indicated in a study drugs accountability log. The date, quantity, volunteer number as well as the batch number for every administered drug will be noted in a study drug accountability form. A final count will be made after drug administration to the last volunteer.

### 5.8 Destruction of Study Drug

Unused drugs will be returned to the pharmacy of HUG for destruction.

# 6. STUDY ASSESSMENTS

The study flowchart and study plan on pages 12 and 13 and section 2.1 provide a summary of the various procedures, measurements and data collections that will be carried out during the study. The following paragraphs complete section 2.1.

## 6.1 Urine collection

At each session, urine will be collected for 24 hours before omeprazole intake. The total urinary volume will be recorded, aliquoted and stored at -80°C until analysis. These samples will serve for the targeted and untargeted metabolomics analysis that will be carried out by the Laboratory of Forensic Toxicology and Chemistry at the University Centre of Legal Medicine and the Laboratory of Clinical Pharmacology and Toxicology of Geneva University.

## 6.2 Plasma collection

Six tubes of 6 ml venous blood sample (6 x 6 ml) will be drawn during the three sessions and collected in EDTA plasma collection tubes[74] at the same time as the omeprazole is administrated. Plasma will be obtained after centrifugation of blood samples at 2500 rcf for 10 minutes and aliquots will be conserved at -80°C until analysis. These samples will serve for the untargeted metabolomics analysis of direct plasma and liver-derived extracellular vesicles (liver-specific liquid biopsy) that will be performed by the Laboratory of Forensic Toxicology and Chemistry at the University Centre of Legal Medicine and the Laboratory of Clinical Pharmacology and Toxicology of Geneva University. Fluvoxamine and rifampicin will also be quantified in the Laboratory of Clinical Pharmacology and Toxicology using validated methods.

### 6.2.1 Liver-derived extracellular vesicles preparation and characterization

Liquid biopsy can be defined as an indirect analysis of a specific organ using information (DNA, RNA, protein, metabolites) shaded from the organ to the circulation. This approach is widely used in oncology as a non-invasive diagnostic method for early detection of mutations and cancer biomarkers. These circulating markers are present in extracellular vesicles originated from a specific organ. We recently published a proof-of-concept study evaluating the potential use of liquid biopsies as a valid indicator of liver cytochromes activities.[75] This study shows a good correlation between liver EVs cytochrome expression levels with patients' cytochrome activity as measured by the Geneva phenotyping cocktail.

Liver specific liquid biopsies will be prepared according to the general recommendations of the International Society for Extracellular Vesicles from plasma samples at each session and following the general flowchart presented in Habtemariam and Guchelaar study[76]. It requires to have a sufficient plasma volume for characterization and analysis[77]. Hence, 36 ml of whole blood will be available in this study at each session from each volunteer, from which 30 will be used for EVs while the rest will be used for direct metabolomic analysis in plasma. A preliminary EVs cleaning and enrichment step will be performed by centrifugation to obtain platelet deprived samples. Specific liver derived EVs will then be isolated through immuno-affinity precipitation using magnetic-bead coupled commercial antibodies directed against hepatocyte specific proteins known to be packaged to the surface of EVs. Anti-ASGR1 antibody coupled to Dynabeads (Thermo Fisher Scientific, Waltham, USA) have already been successfully used for the isolation of liver specific EVs and will be our starting method of choice.[78] The development of new EVs isolation methods is a rapidly evolving field and other isolation techniques might come under consideration depending on the accomplished progress.[76,79]

Isolated EVs characterization will be performed using a combination of flow cytometry and electron-microscopy (EM) techniques.[80,81] Vesicle size and homogeneity of the final preparations will be assessed by transmission electron microscopy (TEM) at the EM platform of the University of Geneva. EVs purity and cell specificity will be followed by flow cytometry using a combination of antibodies directed both against liver EVs membrane surface cargos and undesired components (such as platelet EVs markers). During the method development stages, flow cytometry will be used to follow EVs purity as well as subtype specificity and enrichment efficiency at each step of the isolation procedure comparing enriched fractions versus left-overs. Once the experimental pipeline is entirely defined, flow cytometry will remain the technique of choice for testing the quality of each extract but will be limited to key isolation steps and a selection of highly informative antibodies. Antibodies target will include both general EV markers as well as liver specific surface proteins and frequently encountered protein contaminants (such as markers of EVs of a different origin). It was previously demonstrated that normalization of EVs with respect of their number and proportion of cells of hepatic origin is an important step to account for inter-individual variability in EVs production. A shedding factor was therefore developed based on a pool of 13 mRNA markers that will be measured in our study.[82]

As with urine and plasma samples, EVs will be submitted to untargeted metabolomics after a sample preparation method adapted from recent protocols.[77]

#### 6.2.2 CYP2C19 mRNA and protein measurements

CYP2C19 expression levels in liver biopsies will be measured using conventional techniques. After extraction of messenger RNA using commercial extraction kits and reverse transcription (iScript™ cDNA Synthesis kit, Bio-Rad Laboratories, Inc.), relative quantification of the resulting cDNA will be performed by RT-PCR using validated TaqMan® Expression probes (ThermoFisher Scientific, Waltham, USA) in a StepOne™ apparatus (ThermoFisher Scientific, Waltham, USA).

### 6.3 Capillary blood collection

2 hours after omeprazole intake at each session, the capillary blood will be sampled by pricking the fingertip using a conventional lancet. A blood drop of 10 µL will be collected by the medical device HemaXis DB 10. In total, 4 x 10 µL will be collected. After 30 minutes drying time at room temperature, DBS samples will be packed in sealable plastic bags containing a desiccant and stored at -20°C until analysis. The extraction of DBS samples will be performed using an automated system, allowing an online desorption of DBS directly into the HPLC-MS/MS device without sample pretreatment. Finally, the quantification of omeprazole and OH-omeprazole will be performed in order to determine the phenotype of CYP2C19 in the Laboratory of Clinical Pharmacology and Toxicology through a validated analytical method.

## 7. ADVERSE EVENTS MANAGEMENT

### 7.1 Definitions

An adverse event (AE) is defined as any adverse change from the subject's baseline condition. An adverse event does not necessarily have a causal relationship with the study treatment.

AE monitoring begins when participants receive omeprazole during the second visit (session 1) and continues through subsequent visits, with follow-up phone calls two days after each visit, including a final safety follow-up after the fourth visit.

The Common Terminology Criteria for Adverse Events (CTCAE) displays Grade 1 to 5 with unique clinical description of severity for each AE based on this general guideline:

**Grade 1**: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

**Grade 2:** Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental Activities of Daily Living (ADL) (i.e. preparing meals, shopping for groceries or clothes…).

**Grade 3:** Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL (i.e. bathing, dressing and undressing…).

**Grade 4:** Life-threatening consequence; urgent intervention indicated.

**Grade 5:** Death related to AE.

A serious adverse event (SAE) as defined by ICH is any untoward medical occurrence that at any dose meets any of the following conditions:

- Results in death
- Is life-threatening (the subject was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it was more severe)
- Requires or prolongs hospital stay significantly
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is an important medical event

Important medical events are those that may not be immediately life threatening but are clearly of major clinical relevance. They may jeopardize the subject and may require an intervention to prevent one of the other serious outcomes noted above.

SAEs should be followed until resolution or stabilisation. Participants with ongoing SAEs at study termination (including the final safety follow-up) will be further followed up until recovery or until stabilisation of the disease after termination.

An “unexpected” adverse drug reaction (ADR) is an adverse reaction of which the nature of severity is not consistent with the official product information for drugs used in this study.

The investigator makes a causality assessment of the event to the study drug based on the criteria listed in the ICH E2A guidelines:

| Relationship | Description |
|---|---|
| Definitively | Temporal relationship<br>Improvement after dechallenge*<br>Recurrence after rechallenge<br>(or other proof of drug cause) |
| Probably | Temporal relationship<br>Improvement after dechallenge<br>No other cause evident |
| Possibly | Temporal relationship |

| | |
|---|---|
| | Other cause possible |
| Unlikely | Any assessable reaction that does not fulfil the above conditions |
| Not related | Causal relationship can be ruled out |
| *Improvement after dechallenge only taken into consideration if applicable to reaction | |

A suspected unexpected serious adverse reaction (SUSAR) is defined as a serious adverse event that is not expected (not described before or known with a lower frequency) and which the causation with the study product is established with an imputability of at least possible.

### 7.2 Adverse events collection and grading

Drugs used in this study are approved in Switzerland and their security profile is well known. Therefore, AEs of grade ≥ 2, SAEs and SUSARs are unlikely. If that were to happen, only SAEs that result in death and SUSARs would be reported in the eCRF.

AE monitoring and filling out of the eCRF for each participant begins when they receive omeprazole during the second visit (session 1). This monitoring will continue throughout the two-hour duration of the visit and the same procedure will be followed for the third visit (session 2) and the fourth visit (session 3). To ensure comprehensive safety monitoring, a follow-up phone call will be conducted two days after each visit, including the fourth one (final safety follow-up). This allows for the early detection of any potential AEs related to fluvoxamine and rifampicin, as well as adverse events that may appear after the study. Participants will be encouraged to contact study investigators at any time if they experience an AE, even if it occurs after the study has ended. This is particularly important if the participant feels they need to discontinue the medication during the study or if they require medical assistance.

The eCRF report will document the reasons for notification (e.g. death, life-threatening event), the date of first occurrence, and a description of SAEs/SUSARs. The report will also assess the nature of the SAE (expected or unexpected) and its relationship to the study drug (imputability) to determine if it qualifies as a SUSAR. Additionally, information on any potentially responsible medications will be recorded.

All AEs, SAEs and/or SUSARs will be followed up until the event is resolved. The SAEs that are not reported in the eCRF and AEs of grade ≥ 2 will be recorded in the electronic medical records of HUG for each study subject by the investigator.

### 7.3 Safety reporting

<u>Reporting of AEs</u>

Only adverse events of special interest (AESI) will be reported by the promotor and/or the investigator to the regional centre of pharmacovigilance of Geneva as it is the case under usual conditions of these drugs.

Reporting of SAEs

Only SAEs resulting in death must be reported immediately and within a maximum of 24 hours to the Sponsor-Investigator of the study and to the CCER within 7 days.

Reporting of SUSARs

A SUSAR needs to be reported to the CCER and to Swissmedic for category B and C studies within 7 days, if the event is fatal, or within 15 days (all other events).

Reporting of safety signals

All suspected new risks and relevant new aspects of known adverse reactions that require safety related measures, i.e. safety signals, must be reported to the Sponsor-Investigator within 24 hours. The Sponsor-Investigator must report the safety signals within 7 days to CCER and Swissmedic in case of a category B or C study.

Reporting of pregnancy

Pregnant participants must immediately be withdrawn from the clinical study. Any pregnancy during the treatment phase of the study and within 30 days after discontinuation of study medication will be reported to the Sponsor-Investigator within 24 hours. The course and outcome of the pregnancy should be followed up carefully, and any abnormal outcome regarding the mother or the child should be documented and reported.

Periodic reporting of safety

An annual safety report (ASR) is submitted once a year to the CCER and to Swissmedic by the Sponsor-Investigator for category B clinical trials.

### 7.4 Follow-up of AE

In case of detected AEs during the safety follow-up included in sessions' visits or phone calls, or if reported by the participant at any time (even after the study has concluded), the volunteer will be treated as appropriate. This may include discontinuation of study medication if necessary or provision of medical assistance. The event will be documented in the eCRF, and

study investigators will closely monitor the situation to ensure participant safety. If the required treatment for the adverse event is not compatible with the study protocol, the subject will be excluded from the study.

# 8. QUALITY CONTROL AND ASSURANCE

## 8.1 Data handling and record keeping/archiving

### 8.1.1 eCRF

The electronic Case Report Form (eCRF) is an investigator file, written for each study subject, which contains data necessary to the progress and the analysis of the study, including safety data.

In this study, the following data will be entered in the eCRF using the browser-based software REDcap :

- Review of inclusion and non-inclusion criteria (check boxes) *
- Demography: gender*, age*
- Concomitant medication(s)*
- weight*, size* and BMI*
- CYP2C19 genotype and phenotype
- For women: date of last menstrual cycle, contraception methods and pregnancy test result at each visit*
- Time of drug intakes*
- Time of venous and capillary blood samplings*
- Time of urine collections*
- SAE resulting in death and SUSARs*

All items followed by an asterisk will be entered directly in the eCRF and will be considered source documents.

Each page will include the subject's and visit's number as well as all the modifications dated and signed by the investigator.

### 8.1.2 Source data

eCRF will be considered as a source document for some data collected during the clinical trial (see section 7.1.1.). All phenotyping, genotyping and metabolomics data (including raw files)

will be available in an electronic version considered as a source document and stored on the secured server of HUG.

The section of the eCRF dedicated to the collection of medical data will also be integrated in the electronic medical records of HUG for each study subject to record all medical information, which could be of medical interest in case of further hospital admission out of the frame of this clinical trial. Other medical data (e.g. concomitant illness, medical history, known drug allergies, blood pressure and cardiac frequency) will be reported only in the electronic medical records of HUG for each study subject in order to evaluate their eligibility.

In case of monitoring, audit or inspection, the principal investigator will allow free access to these source data.

#### 8.1.3 Documents storage and keeping

Data obtained during the study will be collected in the eCRF and/or in an electronic form (i.e. metabolomics and genetic data) and will be coded. The access will be secured and limited to the investigator and co-investigators or people authorized by the principal investigator to ensure the best confidentiality. Data of the study will be kept for 20 years after termination in a locked cabinet and/or a secured server with restricted access.

Subject's identification list will be kept for a sufficient time to allow the necessary medical follow-up when appropriate.

In the inform consent, the volunteers will be able to choose whether they want to know their genotypes/phenotypes or not. In case of particular (poor or ultrarapid) metabolism detection, volunteers will be informed on its potential impact in any future treatments.

### 8.2 Data Management

#### 8.2.1 Data management system

All research subjects as well as biological samples will be coded. Data management will be performed on the secured server of HUG. The necessary precautions will be taken in order to ensure safety as described in section 7.2.

All the collected data are the property of the sponsor institution (HUG) and under the direct responsibility of the sponsor.

### 8.2.2 Data security, access and backup

Physical access to the data centres is logged and limited to authorized personnel using badge authentication. On a regular basis, vulnerability testing is performed to reduce potential exposure. Remote access to servers is limited to authorized personnel and connections are encrypted using SSH.

The internal HUG network is protected by multiple firewalls, proxy, reverse-proxy and antivirus solutions. Web servers operate under SSL (HTTPS) certifications, ensuring that Web connections are encrypted and secure.

Only people part of the investigation team, the sponsor team, the affiliated reviewers or auditors, as well as inspection authorities (Swissmedic) are given access to data.

Identification is made by a personnel ID and a password. Failure to provide the correct password after a limited number of attempts automatically disables the faulty account (protection against non-authorized attacks).

HUG infrastructure is under the responsibility of DSI (“Direction des Systèmes d’Information”). All exploitation, monitoring and backup operations are performed by DSI. Frequent backups are carried out using the best backup methods at HUG and are physically stored in a fire-proof safe.

### 8.2.3 Electronic and central data validation

Validation/review will be performed in parallel by appropriate people designated by the sponsor (co-investigators or other collaborators of the study). Up-to-date graphical and text reports, as well as descriptive statistics, will be made available to monitor data in real time.

## 8.3 Monitoring

Throughout the study, a monitoring will be realised to check the good application of the study protocol (see monitoring plan document).

## 8.4 Audit and inspection

In case of an audit or inspection by the regulatory authorities, the investigator will allow the authorized people to:

- visit the study local, installations and used material;
- meet all the people involved from near or far in the study;
- have a direct access to all the study documents and source data;
- see all the documents related to the study.

### 8.5 Confidentiality and data protection

Individual research subjects' data cannot legally nor ethically be made available to non-authorized people. Only the sponsor and other members of the study team, reviewers, auditors as well as inspection authorities are entitled to access such data.

No personal data or data that may easily identify subjects will be provided, with respect to the Swiss law on human research (Federal Act on Research involving Human Beings (HRA)) and its applicable ordinance OClin.

Along with the study number, each participant's biological sample and results data will be coded as the following : Patient's number as per the order of entrance in information session of the study + S (for session) + number corresponding to the session + the initial letter of the name of biological sample (urine, plasma, EV, or DBS), e.g. 12S3P, 3S2U, 23SEV, 7S1D. Venous blood samples relative to genotyping will be coded as the following : Patient's number as per the order of entrance in information session of the study + G (for genotyping), e.g. 12G, 3G, 7G.

At the end of the project, the entire database will be archived in a reusable format, including the subject's identification list. Archives encompass all raw data, transformed data, version history, and audit trails. Redeployment of the entire database is therefore possible whenever needed. An electronic copy of the archive will be stored in a coded form within the DSI infrastructure for a theoretical infinite period of time while all the measures necessary to maintain confidentiality are fully implemented. Data will be provided to authorized third parties as much as possible in non-proprietary formats (text, CSV, XML, PDF). However, some material will remain proprietary due to technical restrictions.

### 8.6 Storage of biological material and related health data

If participants sign the consent form that allows the reuse of data and biological samples, coded genetic data (blood) and other biological samples (plasma, DBS card and urine) will be stored. In that case, the 'biobank' storage procedure will be applied (see the file "règlement de la biobanque de l'étude biomarqueurs endogènes CYP2C19"). As mentioned earlier, an individual numerical code will be attributed to the biological samples from each volunteer, along with the study number. The coded samples will be stored in HUG at -80°C (except DBS cards, which are at -20°C) for an indeterminate period. If the blood and other biological samples were to be used for a study distinct than the present one, the investigator will ask the authorisation of the CCER by submitting a new protocol. Volunteers will be informed that they can withdraw their consent at any time and in that case, the samples will be destroyed.

# 9. STATISTICS

## 9.1 Number of subjects

The data from Bosilkovska et al.[24], Magliocco et al.[37], Zhou et al.[11] and Akasaka et al.[40] were used as a basis to measure the effect size required for our clinical study and the number of subjects required for each study group.

The software G*Power (version 3.1.9.7) was used to calculate the sample size for a two-sided paired samples t-test with a power (1-β) of 80% for the part 1 of the study. The Bonferroni correction was applied to adjust the p-value (α = type 1 error) in order to correct for multiple tests as it is the case in metabolomics analyses. Therefore, p-value is divided by the number of expected features, which was defined as 5000 according to the number of metabolic features obtained after data processing in the Magliocco et al. clinical study, of which metabolomics were used to prospect on CYP2D6 endogenous biomarkers[37]. Consequently, the p-value used for the power calculation will then be $1 \cdot 10^{-5}$. Moreover, the effect size obtained for the statistical analysis and based on the Bosilkovska et al. trial is 1.156. After entering all these parameters, a sample size of 30 is measured, which implies that 30 healthy volunteers NMs-RMs-UMs are required to observe a difference between control session, inhibition session and induction session in the part 1 of the study.

For the second part of the study (validation of identified features in the first part), a semi-quantitative targeted analysis will be performed. In Zhou et al.[11] study, 14% of the total number of participants were PM individuals among all the study participants and were enrolled to confirm a statistical difference between CYP2C19 PMs and non-PMs in the pharmacokinetic of a single 20 mg dose of omeprazole. Moreover, 35% of the total number of patients were PMs in the study of Akasaka et al.[40] where PMs and non-PMs were compared to have a significant difference between serum EETs levels (potential endogenous biomarker of CYP2C19).

Following these data, 10 PM individuals i.e. 10 subjects, which is 25% of the total number of our subjects (mean proportion of Zhou et al. and Akasaka et al. studies when combined), will be recruited. Therefore, already genotyped healthy volunteers known from our database or new ones will be contacted and if achievable, 10 PMs and 30 NMs-RMs-UMs (40 in total) subjects will be recruited and compared to each other at baseline.

## 9.2 Data analysis

For metabolomics analysis, peak extraction and grouping as well as retention time correction and alignment will be performed on XCMS Online.[83] Then, multivariate statistical analysis as

well as univariate will be done. First of all, regarding multivariate approaches, a principal component analysis will be applied to verify the analytical variability. Partial least squares-discriminant analysis (PLS-DA), the supervised version of PCA, will also be used to maximise the separation between different groups (i.e. control session versus inhibition session versus induction session). Then, the variable importance in projection (VIP) score calculated from the PLS-DA model may be applied to identify potential biomarkers.

In univariate analysis, a volcano plot will be performed to detect any significant changes in signal intensities before and after inhibition or induction as well as between PMs and NMs-RMs-UMs. The raw p-values will be adjusted by false discovery rate method to correct for multiple testing in the first case.

All the metabolomics analysis will be performed on the online platform Metaboanalyst.[84]

The possible correlation between the blood MR OH-OPZ/OPZ and significant ions identified will be evaluated using Spearman's rank correlation. Values of $p < 0.05$ will be considered significant.

### 9.3 Dropout and missing data management

Withdrawal volunteer will be replaced by a new volunteer. A subject screening log will be kept in the TMF and regularly updated. This document will be accessible for monitoring and inspection in order to analyze rates and reasons for non-inclusions and withdrawals.

## 10. ETHICAL AND REGULATORY ASPECTS

### 10.1 Study registration

After obtaining the agreement by the CCER and Swissmedic, this clinical trial will be registered on the international register of clinical trials (www.clinicaltrial.gov), in accordance with the requirements of the international committee of medical reviews editors. In addition, the clinical trial will also be registered in the supplementary federal database (the SNCTP).

### 10.2 Categorisation of study

The present clinical trial is categorised under Category B according to the OClin, art. 19. All therapeutic products are authorized in Switzerland but do not have any indication for healthy volunteers.

### 10.3 Ethical Committee and Regulatory Authority

The study protocol, the documents for the subjects and the investigator's list will be submitted to the CCER of Geneva canton and to the Swiss Agency for Therapeutic Products (Swissmedic). The study will only start after the formal authorisation of both the CCER and Swissmedic. During the study, the investigator and the sponsor will have the obligation to submit to the CCER and Swissmedic, respectively, the study progression, any modification to a document and any new important information about the investigational product. No changes can be made to the protocol without prior Sponsor and CCER approval, except when it is necessary to eliminate apparent immediate hazards to the study participants.
Premature study end or interruption of the study is reported within 15 days. The regular end ("last patient last visit") of the study is reported to the CCER and Swissmedic within 90 days and the final study report shall be submitted within one year after study end. Amendments are reported according to chapter 9.9.

### 10.4 Ethical Conduct of the Study

This study will be conducted in accordance with the ethical principles of the Declaration of Helsinki, the ICH guidelines for the Good Clinical Practices (GCP), the human research act (LRH) and the ordinance on clinical trials on research involving humans (OClin). The investigators will be responsible for the strict enforcement of the protocol during the study. The CCER and Swissmedic will receive annual safety reports and be informed about study stop/end in accordance with local requirements.

### 10.5 Declaration of Interest

The investigators of this clinical trial declare no conflict of interest.

### 10.6 Information and consent

The investigators or delegated person will explain to each participant the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits and any discomfort it may entails in words easy to understand. All participants will be informed that their participation in the study is voluntary and that they may withdraw at any time without consequence or explanation. Participants must be informed that their medical records may be examined by authorized individuals other than their treating physician. All study volunteers will receive a participant information sheet and a consent form describing the study and providing

sufficient information to make an informed decision about their participation in the study. A minimum of 72 hours will be given to the volunteers to decide whether they participate or not. The volunteer's information sheet and the consent form will be submitted to the CCER to be reviewed and approved. The formal consent of a participant, using the approved consent form, must be obtained before the participant is submitted to any study procedure. The participant should read and consider all the statements before signing and dating the informed consent form, and should be given a copy of the signed document. The consent form must also be signed and dated by the investigator (or delegated person) as well as retained as part of the study records.

Participants fulfilling eligibility criteria will receive a compensation of 200 Swiss francs for each session corresponding to a total of 600 Swiss francs. Non-eligible participants will receive a compensation of 20 Swiss francs for attending the first visit.

### 10.7 Participant privacy and confidentiality

The investigator affirms and upholds the principle of the participant's right to privacy and that they shall comply with applicable privacy laws. Furthermore, anonymity of the participants shall be guaranteed when presenting the data at scientific meetings or publishing them in scientific journals. Individual subject medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited. There will not be any commercial exploitation of the study subjects' medical data and biological samples.

Subject confidentiality will be further ensured through subject identification code numbers to correspond to treatment data in the computer files. For data verification purposes, authorized representatives of HUG, Swissmedic or Geneva CCER may require direct access to parts of the medical records relevant to the study, including participants' medical history.

The results obtained from genotyping and phenotyping will be communicated to the volunteers if they wish to. They will also decide in the consent form if they agree that their reversibly anonymized genetic data and biological samples (plasma and urine) will be kept for a potential further use.

Volunteers can contact the investigators at any moment if they have questions concerning the meaning of the genotyping and phenotyping results.

### 10.8 Early termination of the study

The Sponsor-Investigator may terminate the study prematurely according to some circumstances, for example:

- ethical concerns
- insufficient participant recruitment
- when the safety of the participants is doubtful or at risk, respectively
- alterations in accepted clinical practice that make the continuation of a clinical trial unwise
- early evidence of benefit or harm of the experimental intervention

### 10.9 Protocol amendments

Substantial amendments are only implemented after approval of the CCER and Swissmedic respectively. Under emergency circumstances, deviations from the protocol to protect the rights, safety and wellbeing of human subjects may proceed without prior approval of the CCER or Swissmedic. Such deviations shall be documented and reported as soon as possible to the CCER and Swissmedic. All non-substantial amendments are communicated to Swissmedic as soon as possible if applicable and to the CCER within the Annual Safety Report (ASR).

## 11. PUBLICATION POLICY

Data obtained from this study will be subject to publication in a peer reviewed journal.

## 12. FINANCING AND INSURANCE

### 12.1 Funds

This study is funded by the Swiss National Science Foundation (SNSF).

### 12.2 Insurance

The Geneva University Hospitals have subscribed liability insurance with Bâloise Assurance SA to cover health damage that may have been attributed to the therapeutic products or procedures used in the study.

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