Effects of omeprazole and genetic polymorphism of CYP2C19 on the clopidogrel active metabolite

Xavier Boulenc, Nassim Djebli, Juan Shi, Laurent Perrin, William Brian, Robert Van Horn, and Fabrice Hurbin

Sanofi-aventis R&D, Drug Disposition, Disposition Safety and Animal Research, Montpellier, France (X.B., N.D., L.P., F.H.); Drug Disposition, Disposition Safety and Animal Research, Great Valley, Pennsylvania, USA (J.S., W.B., R.V.H.); and Biostatistics Department, Montpellier, France (L.P.)
Running title page

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Address correspondence to:

Xavier Boulenc
Sanofi-Aventis
Drug Disposition Domain,
Disposition Safety and Animal Research,
371 rue du Professeur J. Blayac, 34184 Montpellier cedex 04, France
Tel: +33499776325
Fax: +33499776904
xavier.boulenc@sanofi-aventis.com

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ABBREVIATIONS: clopi-H4 metabolite, active metabolite of clopidogrel (H4); CYP, Cytochrome P450; DDI, drug-drug interaction; EM, extensive metabolizer; HLM, human liver microsomes; IM, intermediate metabolizer; LC, liquid chromatography; MBI, mechanism-based inhibition; MS, mass spectrometry; PM, poor metabolizer; UM, ultrarapid metabolizer
ABSTRACT:

Clopidogrel is an antiplatelet agent widely used in cardiovascular diseases and an inactive prodrug that needs to be converted to an active metabolite in two sequential metabolic steps. Several CYP450 isoforms involved in these two steps have been described, although the relative contribution in vivo of each enzyme is still under debate. CYP2C19 is considered as the major contributor to active-metabolite formation. In the current study, the net CYP2C19 contribution to the active metabolite formation was determined, from exposure of the active metabolite in two clinical studies (one phase I study with well-balanced genetic polymorphic populations, and a meta-analysis with a total of 396 healthy volunteers) at different clopidogrel doses. CYP2C19 involvements were estimated from 58 to 67% in IM, from 58 to 72% in EM and from 56 to 74% in UM, depending on the study and the dose. For this purpose, a static model was proposed in order to estimate the net contribution of a given enzyme to the secondary metabolite formation. This static model was compared with a dynamic approach (Simcyp® model) and showed good consistency. In parallel, in vitro investigations showed that omeprazole is a mechanism-based inhibitor of CYP2C19 with $K_i$ of 8.56 µM and $K_{\text{inact}}$ of 0.156 min$^{-1}$. These values were combined with the net CYP2C19 contribution to the active metabolite formation, through a static approach, in order to predict the inhibitory effect at 80 mg omeprazole doses in EM, IM and UM CYP2C19 populations, with good consistency, when compared with observed clinical values.
Introduction

The antiplatelet agent clopidogrel is a prodrug, which is metabolized in a two-step oxidative process by the hepatic cytochrome P450 (CYP) isozymes CYP1A2, CYP2B6, CYP2C19 and CYP3A4, and is converted to its active metabolite (clopi-H4) (Kazui et al., 2010). This leads to inhibition of adenosine diphosphate-induced aggregation by irreversible binding of the platelet P2Y12 receptor. An esterase-dependent step leads to an inactive carboxylic acid derivative that represents 85% of circulating plasma compounds (Lins et al., 1999).

Polymorphisms of CYP2C19 affect both the pharmacodynamic and pharmacokinetic profiles of clopi-H4 and it has been determined that this isoform is one of the major determinants of inter-individual variability in clopidogrel pharmacodynamic and pharmacokinetic responsiveness (Kim et al., 2008; Hulot et al., 2006; Mega et al., 2009; Umemura et al., 2008), while CYP3A4 has also been described as contributing to clopi-H4 pharmacokinetic variability in the clinic (Farid et al., 2007). In addition, CYP2C19 involvement in the formation of clopi-H4 was recently confirmed in a randomized cross-over study conducted in four balanced CYP2C19 phenotype-defined metabolizer groups (n=10/group) (Simon et al., 2011). The authors of this study also performed a meta-analysis on data from 396 healthy subjects and confirmed that CYP2C19 is the most important polymorphic CYP involved in clopi-H4 formation and antiplatelet response, whereas CYP1A2, CYP2C9, CYP3A5 and CYP2D6 played no significant roles.

Due to an increased risk of bleeding, antiplatelet therapy recipients are often co-prescribed proton pump inhibitors (PPIs), e.g. omeprazole. The reduced ability of clopidogrel to inhibit platelet aggregation in omeprazole recipients was documented in a randomized, double-blind trial (Gilard et al., 2008). In contrast, the PPI pantoprazole did not significantly impact the
antiplatelet activity of clopidogrel; only a small decrease (14%) in clopi-H4 exposure was observed (Angiolillo et al., 2011). Recently, a hypothesis has been proposed to interpret the phenomenon of PPI inhibition and omeprazole in particular. This hypothesis is based on the finding that clopidogrel itself is a mechanism-based inhibitor of CYP2C19 and that the amplified effect of PPIs is mainly due to the inhibition of their own metabolism by clopidogrel (Zhang et al., 2009).

This study was designed to evaluate an alternative hypothesis. In order to explain the impact of omeprazole on clopi-H4 exposure, a mechanism-based inhibition of omeprazole towards CYP2C19 was hypothesized, and a static drug-drug interaction (DDI) modeling approach was used to estimate the in vivo effect. This hypothesis is supported by the similar effect observed when omeprazole and clopidogrel were administered simultaneously or 12 hours apart (Angiolillo et al., 2011). Even if omeprazole is a well-known CYP2C19 inhibitor, it has been commonly considered as a competitive reversible inhibitor (Liu et al., 2005; Li et al. 2004). No in vitro parameters of mechanism-based inhibition (MBI) are available in the literature (University of Washington Drug interaction database; AurSCOPE ADME/DDI from Aureus sciences; for both, requests conducted on the 10 March 2011). Nevertheless, MBI of omeprazole has been previously proposed (Paris et al., 2008).

In this study, the net relative contribution (Fm) of CYP2C19 to clopi-H4 formation was estimated in three different CYP2C19 metabolizer groups, intermediate (IM), extensive (EM) and ultra (UM) metabolizers, with data obtained from a phase I clinical study with well-balanced genetic polymorphic populations, and from the meta-analysis previously described (Simon et al., 2011). A static model was proposed, and can be generalized for more metabolic steps, in order to estimate the net contribution of a given polymorphic (or total inhibition of) enzyme to the
secondary metabolite formation. A dynamic model in Simcyp software was used to compare the predictions with the two types of models. The dynamic model is based on Pysio logically-Based PharmacoKinetics (PBPK) modeling, where all the calculations are time- and concentration- dependent are taken into account, as well as organ parameters such as changes in enzymes synthesis or degradation rates, and the corresponding inter-subject variabilities. In addition, for the current analysis, a special module of the Simcyp software was used which takes into account, 2 sequential metabolic steps.

The inhibitory mechanism of omeprazole and the mechanism-based inhibition parameters towards CYP2C19 were determined in vitro in order to predict, via a static model, the inhibitory effect of several doses of this PPI on clopi-H4 plasma levels after clopidogrel loading and maintenance doses in IM, EM and UM CYP2C19 subjects. In order to support these predictions, they were compared with available clinical pharmacokinetic data (i.e. after repeated doses of 80 mg omeprazole with clopidogrel loading and maintenance doses).
Materials and Methods

Chemicals: Clopidogrel, 2-(2-chlorophenyl)-2-(2,4,5,6,7,7ahexahydrothieno[3,2c]pyridine-5yl-acetic acid methylester, 7S and active clopi-H4 metabolite, [(3Z)-a-methyl ester, 3-(carboxymethylene)-a-(2-chlorophenyl)-4 (R or S)-mercapto-1-Piperidineacetic acid] were synthesized in Sanofi-Aventis (Montpellier, France). Omeprazole, 1H-Benzimidazole, 5-methoxy-2-[[4-methoxy-3,5-dimethyl-2-pyridinyl]methyl]sulfinyl]-5-Methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]benzimidazole, and pantoprazole, 6-(Difluoromethoxy)-2-[(3,4-dimethoxypyridin-2-yl)methylsulfinyl]-1H-benzimidazole, were obtained from Sigma-Aldrich and stored in a 1:1 (v/v) mixture of methanol/water.

In vitro study determination of mechanism-based inhibition by omeprazole or pantoprazole. Pooled human liver microsomes (HLM) preparation from 50 donors was obtained from XenoTech (Lenexa, KS) and stored at -80°C. CYP2C19 and CYP3A activity assays and bioanalytical methods were previously validated for the quantification of marker metabolites, hydroxymephénytoin (CYP2C19), hydroxymidazolam or 6β-hydroxytestosterone (CYP3A) in HLM mixtures. To determine \( K_i \) and \( K_{\text{inact}} \), HLM reaction mixtures were pre-incubated for various time periods at 37°C with test compounds (at multiple concentrations selected to observe 10–90% inhibition or up to maximal solubility), NADPH regenerating system and 10-fold concentrated microsomal protein relative to typical conditions. At the end of the pre-incubation period, reaction mixtures were diluted 10-fold into a second set of reaction mixtures containing probe substrate (at 4- to 8-fold of \( K_m \) concentrations) and fresh NADPH regenerating systems and incubated for an additional time specified for each CYP activity assay.
as described by (Grimm et al., 2009). The amount of metabolite formed from the probe substrate was quantified using LC-MS/MS methods that were specific for each CYP activity assay.

To determine the inactivation rates ($K_{\text{obs}}$) at different test-compound concentrations, the decrease in natural logarithm of activity versus time was plotted and $K_{\text{obs}}$ values were described as the negative slope of the line for each test-compound concentration.

Apparent $K_{\text{inact}}$ and $K_I$ values were determined by non-linear regression analysis using SigmaPlot, version 10.0, (SPSS, Inc., Chicago, IL) based on the following equation 1:

$$K_{\text{obs}} = \frac{K_{\text{inact}} * I}{K_I + I}$$

where: $K_{\text{obs}}$ (min$^{-1}$) is the inactivation rate for a single test-compound concentration

$I$ (μM) is the concentration of test compound

$K_{\text{inact}}$ (min$^{-1}$) is the maximal rate of enzyme inactivation

$K_I$ (μM) is the test compound concentration that produces one-half the maximal rate of enzyme inactivation

### Static prediction models

**General static model.** The AUC of a given metabolite, after oral administration of the drug, is defined with the following equation 2 (Houston, 1986; Pang 1995). Gut metabolism is considered as negligible ($F_g = 1$); therefore, the simplified equation, derived from Pang, 1995, is proposed:

$$AUC_{M1} = \frac{Dose \times F_{m1} \times Fa \times Fh_{(M1)}}{Cl_{(M1)}}$$

With $F_{m1}$ the fraction of the drug converted into the metabolite $M1$, and $Cl_{(M1)}$ the clearance of the metabolite $M1$. $Fa$ and $Fh_{(M1)}$ are the fraction absorbed of the drug and the systemic
availability of the metabolite that can may conceived as the ratio of the amount of the metabolite leaving the liver to the amount of metabolite formed. $F_{h(M_1)}$ is dependent on its extraction ratio ($E_{h(M_1)}$), which in turn is dependent on the metabolite hepatic clearance and blood flow ($Q_h$) with: $F_{h(M_1)} = 1- \frac{C_{l(h(M_1))}}{Q_h}$. Assuming that M1 is partially metabolized into M2 as represented in Figure 1, then the AUC of the M2 metabolite can be defined as following (equation 3), also derived from Pang et al, 1995:

$$AUC_{M_2} = \frac{Dose * F_{m_1} * F_{m_2} * F_a * F_{h(M_2)}}{C_{l(M_2)}} \quad (3)$$

With $F_{m_2}$ the fraction of M1 converted into M2 and $C_{l(M_2)}$ the clearance of the metabolite M2 and $F_{h(M_2)}$, availability of the M2 metabolite.

A general equation can then be proposed (equation 4):

$$AUC_{M,q} = \prod_{i=1}^{q} \frac{F_{m_i} * Dose * F_a * F_{h(M,q)}}{C_{l(M,q)}} \quad (4)$$

With ‘q’ the metabolic steps for a given metabolite produced after ‘q’ sequential metabolic steps, and only at the last metabolic step; $C_{l(M,q)}$ and $F_{h(M,q)}$ are the clearance and the systemic availability of this metabolite.

Considering that several CYP450 isoforms are able to generate the downstream metabolites at each step, and one of these CYP450 isoforms (called CYPy) is polymorphic (or totally inhibited after co-administration of a strong inhibitor), then, in the PM population (no activity of CYPy isoform), exposure of metabolite M1 is defined by the equation 5:

$$AUC'_{M_1} = \frac{(F_{m_1} - F_{m_{1,CYPy}}) * Dose * F^'a * F^'h_{(M_1)}}{(1 - F_{m_{1,CYPy}}) * C_{l'(M_1)}} \quad (5)$$
With $F_{m1,CYPy}$ the dose fraction converted into M1 due to the genetic polymorphic enzyme CYPy, $Cl'_{(M1)}$ and $F'h_{(M1)}$ are the new clearance and availability of M1 different to $Cl_{M1}$ and $Fh_{(M1)}$ if CYPy is involved in the metabolism of M1.

$Fm1 – F_{m1,CYPy}$ represents the fraction of drug conversion into M1 that is not due to CYPy in EM.

If metabolite M1 is formed by only one additional CYP, called CYPx (in addition to CYPy in EM), then the equation is simplified to that in equation 6:

$$AUC'_{M1} = \frac{(F_{m1,CYPx}) \times Dose \times F'a \times F'h_{(M1)}}{(1 - F_{m1,CYPy}) \times Cl'_{(M1)}} \quad (6)$$

For a given metabolite produced after ‘q’ sequential metabolic steps (and only at the last metabolic step), the AUC metabolite ratio (R) in the case of total CYP inhibition or CYP genetic polymorphism comparison (i.e. PM versus EM), can be calculated as follows, Equation 7:

$$R = \frac{AUC'_{M_{-q}}}{AUC_{M_{-q}}} = \frac{\prod_{i=1}^{q} F'm_i}{\prod_{i=1}^{q} Fm_i} \times \frac{Cl_{(M_{-q})} \times F'h_{(M_{-q})} \times F'a}{Cl'_{(M_{-q})} \times Fh_{(M_{-q})} \times F'a} = \frac{\prod_{i=1}^{q} \sum_{i=1}^{k} Fm_i, CYPi - (Fm_i, CYPy)}{\prod_{i=1}^{q} Fm_i} \times \frac{Cl_{(M_{-q})} \times F'h_{(M_{-q})} \times F'a}{Cl'_{(M_{-q})} \times Fh_{(M_{-q})} \times F'a} \quad (7)$$

Or, equation 8,

$$R = \frac{AUC'_{M_{-q}}}{AUC_{M_{-q}}} = \frac{\prod_{i=1}^{q} F'm_i}{\prod_{i=1}^{q} Fm_i} \times \frac{Cl_{(M_{-q})} \times F'h_{(M_{-q})} \times F'a}{Cl'_{(M_{-q})} \times Fh_{(M_{-q})} \times F'a} = \frac{\prod_{i=1}^{q} (Fm_i - F_{m1,CYPy})}{\prod_{i=1}^{q} Fm_i} \times \frac{Cl_{(M_{-q})} \times F'h_{(M_{-q})} \times F'a}{Cl'_{(M_{-q})} \times Fh_{(M_{-q})} \times F'a} \quad (8)$$

With ‘q’ the metabolic steps and ‘k’ the CYP isoforms involved at each metabolic step in the formation of the corresponding sequential metabolite. CYPy represents the inhibited or the shut
down (i.e. genetic polymorphism CYP2D6, CYP2C19) isoform involved. \( F_m \) and \( F'_m \) are the relative contributions, at each metabolic step ‘\( l \)’, of the sequential metabolites leading to the metabolite of interest (see Figure 1) without and with inhibition (or EM versus PM) respectively. CYPy can be involved at one or more than one metabolic step. \( F_m \), CYPy is the relative contribution of a given CYPy involved in the formation of the sequential metabolite produced at the metabolic step ‘\( l \)’. \( Cl_{(M,q)} \) and \( Cl'_{(M,q)} \) are the clearance of the metabolite of interest without and with total inhibition (or in EM versus PM) respectively, produced after ‘\( q \)’ metabolic steps. \( F_{h(M,q)} \) and \( F'_{h(M,q)} \) are the systemic availabilities of the metabolite of interest without and with total inhibition (or in EM versus PM) respectively, produced after ‘\( q \)’ metabolic steps.

These equations allow the estimation of the metabolite AUC ratio in different situations (i.e. PM population, total inhibition of one enzyme), using the different contribution of each enzyme at each metabolic step, through a static approach. Assuming the same clearance and systemic availability for the metabolite of interest, the same fraction absorbed of the drug, it is noteworthy that the exposure ratio of the metabolite, even with one metabolic step, does not equal the contribution in the drug clearance of the polymorphic enzyme.

**Application to the clopidogrel situation.** Based on the AUC of clopi-H4 metabolite in the 4 CYP2C19 genetic polymorphic groups, CYP2C19 \( F_m \) values for UM, EM and IM (represented by EM in the following equations) were determined using the following equations:

1) In EM CYP2C19, equation 9:
\[
AUC_{H4} = \frac{Dose \times F_m \times F_a \times F_{h(H4)}}{Cl_{(H4)}}
\] (9)

2) In PM CYP2C19, equation 10:
\[
AUC'_{H4} = \frac{Dose \times F'_m \times F'_a \times F'_{h(H4)}}{Cl'_{(H4)}}
\] (10)
Definition: \( Fm \) and \( Fm' \) are the fractions of the dose converted to active metabolite in EM and PM subjects, respectively. These fractions are relatively low (no more than 0.10-0.15) because the majority of clopidogrel is converted in non active metabolites in vivo (Lins, 1999; Close, 2011) as well as in vitro (Hagihara, 2009) so that AUC\(_{H4}\) is likely to change after inhibition or induction of the CYP isofroms involved in its formation. \( Fm_{2C19} \) is the fraction of clopidogrel dose converted to clopi-H4, due to CYP2C19; \( Fmx \) is the fraction of clopidogrel dose converted to clopi-H4, not due to CYP2C19 (other CYP isofroms involved in its formation). \( Fh_h(H4) \) is the availability of the active metabolite H4.

With \( Fm = Fmx + Fm_{2C19} \) in EM and \( F'm = F'mx \) in PM subjects (since \( F'm_{2C19} = 0 \) in PM subjects).

We have, in PM, equation 11 (equivalent to equation 6):

\[
AUC'_{H4} = \frac{Dose \ast Fm_x \ast F'a \ast F'h_h(H4)}{(1 - Fm_{2C19}) \ast Cl_{H4})}
\]  

(11)

Assumptions:

1) \( Cl_{H4} \) (elimination clearance of clopi-H4) and \( Fh_{H4} \) (availability of the H4 metabolite) are considered as the same in both populations. This assumption is reasonable as the clopi-H4 metabolite is likely to be mainly eliminated through covalent binding to platelets due to the mechanism of action (i.e. irreversible modification of the platelet P2Y\(_{12}\) receptor), as stated in recent review on clopidogrel metabolic pathway (Sangkuhl et al, 2010). Therefore \( Cl_{H4} \) as well as \( Clh_{H4} \) (metabolic clearance of the clopi-H4 metabolite) and the corresponding \( Fh_{H4} \) (see above definition of \( Fh_{M} \)) are assumed to be similar in both populations. Fraction absorbed (Fa) is also considered as the same in all genetically polymorphic populations.
2) The two metabolic steps from clopidogrel to active metabolite were gathered in a global approach. Therefore equations 7 or 8 can be simplified in equation 12.

The AUC ratio of clopi-H4 can then be calculated with equation 12:

\[
R = \frac{AUC'_{H4}}{AUC_{H4}} = \frac{F'm}{Fm} = \frac{Fm - Fm_{2C19}}{Fm} = \frac{1 - Fm_{2C19}}{Fm} \tag{12}
\]

Clopidogrel is mainly converted to a non-active acid metabolite. Therefore, \(Fm_{2C19}\) can be assumed as very low relative to unity (Gurbel et al., 2009). In vitro data suggest that \(Fm_{2C19} = 0.04\)–0.02 (Hagihara et al., 2009). Thereby, equation 12 can be simplified in equation 13:

\[
R = \frac{AUC'_{H4}}{AUC_{H4}} = \frac{F'm}{Fm} = \frac{Fm_x}{(Fm_x + Fm_{2C19})} \tag{13}
\]

1-\(R\) represents the fractional decrement in total clopi-H4 “clearance” formation due to CYP2C19, (i.e.\(Fm_{H4,2C19}\)), and is defined by equation 14:

\[
R = \frac{AUC'_{H4}}{AUC_{H4}} = \frac{F'm}{Fm} = \frac{Fm_x}{(Fm_x + Fm_{2C19})} \tag{14}
\]

or:

\[
1 - R = 1 - \frac{AUC'_{H4}}{AUC_{H4}} = \frac{Fm_{2C19}}{(Fm_x + Fm_{2C19})} = \frac{Fm_{2C19}}{(Fm)} = Fm_{H4,2C19}
\]

The same calculations were used to determine \(Fm_{2C19}\) in UM and IM CYP2C19 metabolizers.

3) In this static model, as previously mentioned gut metabolism is not taken into account (\(F_g = 1\)). Generally speaking, it has been shown that clopidogrel is mainly metabolized by a specific carboxylesterase, CES1 and not CES2. In human, CES1, is highly expressed in the liver, lung, while the CES2 isozyme CE2, is mainly expressed in the small intestine (Tang et al, 2006; Imai and Ohura, 2010). In addition, in vitro investigations showed that CYP1A2, CYP2B6 and CYP2C19 contributed to clopidogrel thiolactone metabolite formation from clopidogrel in
human liver microsomes (Hagihara 2009). Therefore, CYP3A4 considered as the major isoform in the gut, does not seem to be involved in the first step of the clopidogrel metabolism, leading to the active metabolite. These results suggest that the CYP450 isoforms involved in the active metabolite formation, are only slightly expressed in the gut, generally speaking, compared with the liver; reflecting no major contribution of the gut. This hypothesis is also stated in the recent clopidogrel metabolic pathway review (Sangkuhl et al, 2010).

**DDI prediction model: predicted decrease of clopi-H4 exposure after co-administration of omeprazole.** With \( \text{AUC}'_{\text{H4}} \) and \( \text{AUC}_{\text{H4}} \) clopi-H4 exposures with and without omeprazole, respectively, equation 15 was used (adapted from Rowland and Martin, 1973; Venkatakrishnan and Obach, 2005; Grimm et al., 2009):

\[
\frac{\text{AUC}'_{\text{H4}}}{\text{AUC}_{\text{H4}}} = \frac{1}{\text{fold reduction } \text{Cl}_{\text{int}}} + \left(1 - \text{fold reduction } \text{Cl}_{\text{int}}\right)
\]

(15)

with equation 16 for MBI (from Mayhew et al., 2000):

\[
\text{Fold reduction } \text{Cl}_{\text{int}} = 1 + \frac{\text{kinact} \times [I]}{k \text{deg} \times ([\text{C}\text{maxu}]+K_{\text{iu}})}
\]

(16)

with equation 17 for reversible inhibition (not used in this study):

\[
\text{Fold reduction } \text{Cl}_{\text{int}} = 1 + \frac{[I]}{K_{i}}
\]

(17)

It has been previously shown that for MBI, the best predictions are obtained using unbound systemic \( C_{\text{max}} \) for inhibitor concentration (Fahmi et al., 2009; Boulenc et al., 2011):

\[ [I] = C_{\text{maxu}}. \]
Dynamic prediction model. To estimate the accuracy of the proposed static model, predicted ratios of the metabolite exposures in PM and EM were compared with those obtained with a dynamic model. Simcyp® algorithms (version 10.05 SE; Simcyp Ltd, Sheffield, UK) were used to predict M2 exposure in CYP2C19 PM and EM populations with several CYP2C19 involvements (F_{n2C19}) at metabolic steps 1 and 2 (see Figure 1). In the current Simcyp® version, the pharmacokinetics of only one metabolite (first step) can be addressed. Nevertheless, for modeling a secondary metabolite, a sequential metabolite of the primary metabolite was implemented as a specific module, through collaboration between Simcyp Ltd and sanofi-aventis, with the following assumptions: the secondary metabolite is only formed from a primary metabolite of the substrate; the secondary metabolite is available for metabolism and inhibition instantaneously; and the substrate is given orally and can be administered as a single dose or multiple doses. In line with the substrate primary metabolite, only a one-compartment absorption model is considered by a minimal Physiologically-Based Pharmacokinetic (minimal PBPK) model. As a result, transporter kinetic models (e.g. hepatic transporters) are not applicable. Differential equations describing the formation and elimination of metabolites in Simcyp® are described by Rowland-Yeo et al. (Rowland-Yeo et al., 2010) and are available as supplemental data.

Simcyp® models were set up using clopidogrel and its metabolites (i.e. 2-oxo metabolite for M1 and clopi-H4 for M2), with the following input data. For unchanged drug (i.e. clopidogrel): MW: 321.8; log P: 3.89; monoprotic base: pK_a = 4.6; B/P = 1, fu = 0.02; first order absorption with f_a = 0.75 and k_a = 1.38 h^{-1}; V_{ss} = 140 L/kg. For M1 (i.e. 2-oxo metabolite) metabolite: MW: 337.8; log P: 2.96; monoprotic base; pK_a = 3.41; B/P = 1, fu = 0.02; V_{ss} =
0.796 l/kg. For M2 metabolite (i.e. clopi-H4): MW: 355.8; Log P: 3.6; diprotic base: pKa_1 = 5.1, pKa_2 = 3.2; B/P = 1, fu = 0.02; V_{ss} = 3.0 l/kg, Cl_{IV} = 150 l/h (for both PM and EM populations).

This model was not validated strictly speaking with a formal comparison between observed and predicted exposure parameters. The aim of this investigation was to use the same set of Fm values, in the dynamic and static model for comparison purpose.

**Dynamic and static model comparison.** Dynamic and static models are not using the same type of parameters as input data. Fm values cannot be directly implemented as input data in Simcyp® software. Therefore, different CYP2C19 intrinsic clearance values were selected in order to simulate different sets of Fm values (see Table 4 in results). F_M values determined by Simcyp® for each subject (individual values) in the Simcyp® population (i.e. ten virtual subjects per simulation) were used in the static model, individually. Thereby, the same individual Fm values were used to run simulations in static and dynamic approaches; means and standard deviations were calculated for each simulation. In Simcyp®, simulations were conducted with the same PM and EM virtual populations of ten subjects, generated by a Monte-Carlo, regardless of the CYP2C19 metabolic status, in order to discard any other sources of variability and to have a pure comparison between the dynamic (PBPK model with differential equations) and the static models. Ratio exposures of the secondary metabolite in EMs versus PMs were calculated for each subject, with mean and standard deviations reported.

**Clinical-trial outcomes.** Details of clinical trials used in this publication have been previously described (Angiolillo et al., 2011, regarding the effect of PPI towards H4-active
metabolite; Simon et al., 2011, regarding the effect of CYP2C19 polymorphism and meta-
analysis results).

Four randomized, single-center, placebo-controlled, 2-treatment, 2-period cross-over
studies were conducted. The trial designs were as follows: clopidogrel 300-mg loading plus
75 mg/day for 4 days or placebo ± omeprazole 80 mg/day administered simultaneously (Study 1)
or 12 hours apart (Study 2); clopidogrel 600-mg loading plus 150 mg/day for 4 days or placebo ±
omeprazole 80 mg/day administered simultaneously (Study 3); and clopidogrel 300-mg loading
plus 75 mg/day for 4 days or placebo ± pantoprazole 80 mg/day administered simultaneously
(Study 4) (Angiolillo et al., 2011). Regardless of treatment sequence, omeprazole was given for 5
days before clopidogrel or placebo administration to achieve steady-state pharmacokinetic and
pharmacodynamic conditions, and pantoprazole was given for 7 days prior to clopidogrel or
placebo to reach its maximum pharmacological effect. The clopidogrel washout duration
between the 2 periods was >14 days. Healthy subjects aged 18–65 years were eligible for
enrollment if they provided written informed consent, had a body weight of 50–95 kg (40–85 kg
for women), and a body mass index (BMI) of 18–30 kg/m². The number of subjects in each
study was from 66 (Study 4, including 6 placebo subjects) to 72 (Studies 1, 2 and 3).

A clinical study was also conducted to compare clopidogrel and clopi-H4 in 4 CYP2C19-
declared metabolizers groups (Study 5). This single-center, randomized, placebo-controlled, 2-
treatment, 2-period crossover study in 4 CYP2C19-defined metabolizer groups (PM, IM, EM
and UM) was conducted to determine whether CYP2C19 polymorphisms affect the
pharmacokinetics of clopidogrel 300 mg/75 mg or 600 mg/150 mg (Simon et al., 2011). The
number of subjects was 40 (10 per group).
In addition, a pooled analysis of data obtained from healthy subjects enrolled in 7 Phase I studies (Study 6) was also performed in order to replicate the CYP2C19 genetic analysis on clopidogrel and clopi-H4 (Simon et al., 2011). The numbers of subjects for each CYP2C19 population having received loading or maintenance doses are presented in Table 1. This meta-analysis was conducted with a total of 396 subjects. Overall, the pharmacokinetic population included 388 subjects for the loading dose and 353 subjects for the maintenance dose.

**Bioanalysis and Pharmacokinetic analysis.** Clinical bioanalytical and pharmacokinetic analysis of clopi-H4 active metabolite, omeprazole and pantoprazole have been accurately described previously (Angiolillo et al., 2011; Simon et al., 2011).

Clopi-H4 analysis: For studies 1–5, plasma samples for pharmacokinetic assessment of clopidogrel and clopi-H4 were collected on Day 1 and Day 5 at T0, T0.25, T0.5, T1, T1.5, T2, T3, T4, T6, T10, T16 and T24 in both periods, except clopi-H4 was not assessed at T16 and T24 in Study 4. Clopi-H4 plasma concentrations were assayed by sanofi-aventis, (Bridgewater, NJ, USA, Malvern, PA, USA and Montpellier, France) using validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) with lower limits of quantification (LLOQ) of 0.5 ng/ml (Tuffal et al., 2011). Stability investigations covered all the plasma concentrations measurement conducted in these clinical trials. Among the pharmacokinetic parameters calculated, only the area under the plasma concentration versus time curve extrapolated to infinity (AUC) on Day 1, and area under the plasma concentration versus time curve calculated using the trapezoidal method from T0 to t_{last} (AUC_{last}) on Day 5 are used in the current analysis.

PPI analysis: In Studies 1 and 3, plasma samples for pharmacokinetic assessment of omeprazole were collected on Day -5 at T0 and T2, Day 1 at T0 and T2, and Day 5 at T0 and T2
in the period of clopidogrel or placebo + omeprazole. In Study 2, plasma samples for pharmacokinetic assessment of omeprazole were collected on Day -5 at T0 and T2 post dose of omeprazole, Day -1 at T12 post dose of omeprazole dose (pre dose of clopidogrel or placebo dose on Day 1 T0), Day 1 at T0 and T2 post omeprazole dose (T12 and T14, respectively, post dose of clopidogrel or placebo), Day 4 at T12 (pre dose of clopidogrel or placebo on Day 5) and Day 5 at T0 and T2 post dose of omeprazole (T12 and T14, respectively post dose of clopidogrel or placebo on Day 5). Omeprazole was assayed using a validated LC-MS/MS method with a LLOQ 5 ng/ml by COVANCE (Indianapolis, Indiana, USA). In study 3, plasma samples for pharmacokinetic assessment of pantoprazole were collected on Day -7 and Day 1 at T0 and T2 and on Day 5 at T0, T0.5, T1, T1.5, T2, T3, T4, T6, T10, T12 and T24 in the period of clopidogrel or placebo + pantoprazole. Pantoprazole was assayed using a validated liquid chromatography mass spectrometry method with a LLOQ of 20.0 ng/ml by Anapharm (Québec, Canada).

All pharmacokinetic parameters were calculated using non-compartmental techniques and PKDMS Version 2.0, incorporating WinNonlin Professional Version 5.2.1 (Pharsight, Mountain View, CA, USA).

**Statistical Analyses.** Effect of omeprazole on clopidogrel at Days 1 (loading dose) and 5 (maintenance dose) were assessed on AUC (Day 1) and AUC_last (Day 5) for clopidogrel and clopi-H4 compounds based on data from studies 1, 2 and 3. A linear mixed-effects model was used with fixed terms for treatment, sequence, period, sex and with an unstructured R matrix of treatment (i,j) variance and covariance, grouped by treatment for subject within sequence blocks, using SAS® PROC MIXED. Estimates and 95% CIs for the ratios of clopidogrel + omeprazole
80 mg versus clopidogrel alone at Day 1 and Day 5 were obtained by computing estimates and 95% CIs for the differences between treatment means within the linear mixed-effects model framework, and then converting to ratios by the antilog transformation. In all models, 2-sided P-values with a threshold of <0.05 were used to test for significance.

For the pharmacogenetic study (Study 5), Days 1 (loading dose) and 5 (maintenance dose) estimates of clopi-H4 AUC (Day 1), and AUC_{last} (Day 5) were obtained separately for each dose regimen by computing estimates and 95% CIs for the ratios of PM versus IM, EM and UM within a linear fixed-effects model framework (with fixed terms phenotype, period and sex) on log-transformed data and converting to ratios by antilog transformation.

For the pooled analysis (Study 6), log-transformed AUC (Day 1) and AUC_{last} (Day 5) of clopi-H4 were analyzed by using a linear fixed-effects model for CYP2C19 with fixed terms for study, dose, sex, and phenotype, plus covariates for age and weight. Estimates and 95% CIs for the ratios of PM versus IM, EM and UM were obtained by computing estimates and 95% CIs for the differences between phenotype means within the linear mixed-effects model framework, and then converting to ratios by the antilog transformation.

A two-sample t-test for unequal variances or the two-sample t-test for equal variances was used in order to conclude if the mean plasma omeprazole concentrations of the clopidogrel+omeprazole treatment was significantly different or not from the mean plasma omeprazole concentrations of the placebo+omeprazole treatment.
Results

PPI with and without clopidogrel. Omeprazole and pantoprazole plasma concentrations after co-administration with clopidogrel or placebo are presented and compared in Tables 2 and 3, respectively.

Clopidogrel did not have a pronounced effect on omeprazole or pantoprazole plasma concentrations after co-administration. In Table 2, even if the comparison between the group receiving placebo and clopidogrel is unbalanced, there is no trend suggesting an increase of omeprazole plasma concentration when co-administered with clopidogrel, supported by the lack of statistical difference when the two treatments are compared. Clopidogrel co-administration had minimal effect on omeprazole pharmacokinetic time dependency. Moreover, an increase of omeprazole was observed after repeated administration as already described, suggesting a clearance decrease of the compound (Shi and Klotz, 2008). These results are discussed in the discussion part.

After repeated co-administration of pantoprazole and clopidogrel, a 35% increase in pantoprazole C\text{max} and a small concomitant increase in AUC (8%) was observed, suggesting no clear impact of clopidogrel towards pantoprazole clearance in our conditions, with no clear statistical significance even if the effect towards C\text{max} is nearly significant.

Determination of Kinetic Parameters for mechanism-based Inhibition

Omeprazole. mechanism-based inhibition of CYP2C19 and CYP3A4 activities by omeprazole was observed, based on a shift in IC\text{50} values in the HLM reaction mixtures after pre-incubation. Irreversible inhibition of CYP2C19 metabolic activity by omeprazole was characterized by determining the enzyme kinetic constants, apparent K\text{inact} and K\text{i}, in HLM
reaction mixtures using non-linear regression analysis (Figures 2 and 3). Experiments were also conducted in reaction mixtures containing HLM to determine kinetic constants for irreversible inhibition of CYP3A4 by omeprazole, but the kinetic constants could not be determined since omeprazole caused minor mechanism-based inhibition of CYP3A4 activity at concentrations up to 500 μM (maximal solubility).

_Pantoprazole._ No irreversible inhibition of CYP2C19 activity by pantoprazole was observed in HLM reaction mixtures after pre-incubation. A minor increase in the inhibition of CYP3A4 activity by pantoprazole was observed in HLM reaction mixtures after pre-incubation with either midazolam or testosterone as probe substrates, but kinetic constants could not be determined due to the limited solubility of pantoprazole (tested up to 500 μM).

All results obtained with positive and negative controls, investigated with MBI and reversible inhibitors, with or without co-factor (NADPH) were as expected.
Static and dynamic comparison of metabolite exposure ratios in PM and EM populations. A static model was proposed (see Materials and Methods section) to calculate the metabolite exposure ratios with the Fm values at each metabolic step. Considering two metabolic steps (Figure 1), M2 exposure ratios were estimated with the static model and compared with values obtained through a dynamic model as implemented in Simcyp®. The predicted ratios were assessed with the two models as described under Materials and Methods and reported in Table 4 and Figure 4. Regardless of the Fm values and relative CYP2C19 involvement used in the formation of M1 and M2, very good consistency was found between the two models, with a ratio slightly lower when calculated with the dynamic model (see discussion).

Determination of CYP2C19 involvement in clopi-H4 exposure. CYP2C19 Fm values in the clopi-H4 formation were determined in the polymorphic genetic populations from study 5 (well-balanced populations; 10 subjects per group) and from study 6 (meta-analysis) in which several phase I outputs were combined. In Study 6, these investigations were conducted for both loading- (300 or 600 mg) and maintenance-doses (75 or 150 mg). CYP2C19 Fm values for each population were determined with AUC ratios of clopi-H4, using equation 14, as reported in Table 5.

Analysis of Study 5 revealed similar clopi-H4 exposure ratios with clopidogrel dose across the populations (UM, EM and IM; Table 5). However, in Study 6, an increase of CYP2C19 Fm value was observed in UMs and EMs compared with IMs while only a trend was observed between UMs and EMs,. This last study reflects a higher CYP2C19 contribution in UMs and EMs vs IMs, whereas a slight tendency observed between UMs vs EMs The lack of consistency between studies 5 and 6 may be due to the lower number of subjects in Study 5, compared with
Study 6 (meta-analysis). For both, loading and maintenance doses in study 6, Fm values were ranked as follows: UM > EM >> IM; reflecting an increase of the CYP2C19 isoform during clopi-H4 formation (Table 5). CYP2C19 contributions were higher at maintenance compared with loading doses, suggesting a possible saturation effect at the loading doses.

Because of the large inter-subject variability of the clopi-H4 metabolite exposure variability, overlaps of the 95% confidence intervals are observed between the populations as well as between the doses, showing that even if some Fm estimates are consistent with what is expected from a theoretical point of view (e.g. increase of CYP2C19 Fm values from IM to UM, in study 6), the power of these data set is not sufficient to clearly ascertain these differences.

**Prediction of omeprazole effect on clopi-H4 formation.** Using equations 15 and 16, the MBI parameters determined for omeprazole (Figure 3), clopi-H4 metabolite AUC ratios were predicted at several omeprazole doses in IM, EM and UM populations based CYP2C19 Fm values calculated from Studies 5 and 6 (Tables 6 and 7). For Study 5, because the CYP2C19 Fm values were similar regardless of the clopidogrel dose, a mean value was used to estimate the omeprazole effect (0.60 and 0.64 for IM and EM, respectively). For Study 6, interaction ratios were calculated from loading and maintenance doses in IM, EM and UM populations. For 80 mg omeprazole, the calculated values were similar to those previously obtained in the clinical interaction trials (pooled analysis of studies 1, 2 and 3). In particular, for both predicted and observed values, a slight but consistent ratio decrease was observed in UM compared with IM, reflecting a higher CYP2C19 involvement in clopidogrel active metabolite formation for UM (Table 7).
Discussion

This is the first study proposing a static model to determine the comparative net involvement of CYP2C19 in clopidogrel active metabolite (clopi-H4 metabolite) formation in populations with genetically different activity of this enzyme. An approach gathering the two metabolic steps (clopidogrel to 2-oxo-clopidogrel and 2-oxo-clopidogrel to H4-active metabolite) was performed, to determine the net CYP2C19 contribution to the active metabolite formation using pharmacokinetic data from two clinical studies: one pharmacokinetic study with well-balanced genetic polymorphic populations (CYP2C19 PMs, IMs, EMs and UM) and a meta-analysis of about 400 healthy subjects. A static model was developed to determine the contribution of a given enzyme to the secondary metabolite formation. The relative involvement of CYP2C19 in clopi-H4 formation was predicted to be between 56 and 64% in the different phenotypic populations (IM, EM and UM). The relative contribution of CYP2C19 increased from IM to UM, in particular in study 6, as expected based on the theoretical quantities of CYP2C19 in these populations. The CYP2C19 contribution tends to be higher at maintenance-compared with loading-doses, suggesting a possible saturation effect of CYP2C19 at high clopidogrel doses. Nevertheless, these differences (between the populations as well as the doses) are not statistically significant, with overlaps of the 95% confidence intervals of the AUC ratios, reflecting the high inter-subject variability of clopi-H4 metabolite exposure.

In addition, a dynamic model in Simcyp® was set up for comparison purpose with the static model. A specific secondary metabolite module, to cope the clopidogrel active metabolite situation, was implemented in Simcyp. Virtual Fm values were selected to test the consistency between the two models, in two virtual populations of ten PM and EM subjects. Predicted exposure metabolite ratios with the two approaches were similar, with slightly higher (about 5%)
values, when calculated with the static model. One of the limitations of the static approach is that it does not take into account for CYP isoforms at the gut level, yielding a slight underestimation of CYP2C19 involvement in the formation of M2. This is reflected by the slightly higher M2 exposure PM/EM ratio observed with the static model (see Figure 4).

The mechanism of CYP inhibition by omeprazole was also investigated. An MBI toward CYP2C19 was clearly identified and the corresponding in vitro parameters ($K_I$ and $K_{inact}$) calculated (8.56 µM and 0.0156 min$^{-1}$, respectively). These values are in line with those previously reported (Paris et al., 2008): 9.10 µM and 0.0457 min$^{-1}$, respectively. This mechanism of inhibition is supported by clinical studies showing similar effects after co-administration of omeprazole and clopidogrel, simultaneously or 12 hours apart (Angiolillo et al., 2011). In addition, an increase of omeprazole plasma concentration after repeated once-daily administration was observed in our clinical conditions (see table 2) and has also been previously described, despite a very short terminal half-life (1 hour) of the compound in EMs (Shi and Klotz, 2008), which would suggest minimal accumulation. It has been hypothesized that omeprazole increases its own bioavailability after repeated dosing either by decreased first-pass elimination and/or by reduced degradation in the stomach secondary to the profound decrease in intragastric acidity caused by omeprazole (Anderson et al., 1990). The presented results suggest that the increase of omeprazole plasma concentration is likely to stem, at least partially, from MBI, where its metabolic clearance is reduced after repeated administration. An interesting comparison of omeprazole clearance after single versus repeated dosing in PMs (in which metabolic-based hypotheses are not relevant) and EMs, suggests that these two mechanisms (increased absorption and reduced clearance) co-exist clinically in non PM subjects (Shirai et al., 2001). Although it was not the primary objective of this study, the authors observed a non-
statistically significant increase (~20%) of omeprazole AUC after repeated dosing in PMs compared with a single dose, and a statistically significant increase (~50%) in EMs. These data, presented by Shirai et al. (Shirai et al., 2001), suggest that the metabolic-based mechanism is the primary cause of the reduced oral clearance following repeated administration of omeprazole in EM subjects even if absorption increase cannot be ruled out, based on the results obtained in PM subjects. Omeprazole MBI also supports the inhibition of moclobemide clearance by omeprazole, as this effect was more pronounced after 1 week repeated administration of omeprazole compared with a single dose (Yu et al., 2001). So, these clinical data clearly support our findings, regarding the mechanism of inhibition of omeprazole.

A static model in combination with net CYPC219 contribution as determined from clinical outcomes, was used to predict omeprazole inhibition of active metabolite formation in CYP2C19 IMs, EMs and UMs. The results are consistent with those previously observed in clinical studies following repeated administration of 80 mg omeprazole.

The consistency between predicted and observed data is in agreement with the observed lack of class effect and in particular the lack of pH effect, since these predictions are only metabolic-based. Pantoprazole is a weak reversible inhibitor of CYP2C19 and showed only a slight effect on the active metabolite plasma concentration and pharmacological response (Angiolillo et al., 2011; Cuisset et al., 2009). In our condition, no clear increases in omeprazole and pantoprazole plasma concentration were observed after co-administration of clopidogrel, in our conditions, in contrast to the hypothesis proposed by Zhang et al. 2009. However, a weak inhibition of omeprazole clearance after co-administration with clopidogrel in CYP2C19 EM subjects has been described by Chen et al., 2009. This apparent inconsistency may be due to the different study populations included in our interaction studies (IM, EM and UM). Even if no omeprazole or pantoprazole plasma concentration
increases were observed after co-administration of clopidogrel, supported by the lack of statistical significance in our analysis, it must be emphasized that the studies were not primarily designed to investigate a clinical interaction between clopidogrel and PPIs. Therefore, further investigations are required.

It is also unlikely that the weak omeprazole plasma level increases observed previously (28%) (Chen et al., 2009) were sufficient to support the hypothesis proposed by Zhang et al., 2009. As also hypothesized by these authors, the effect of omeprazole towards the AUC of the active clopidogrel metabolite is likely to involve a MBI mechanism, as shown in the current study. It is also noteworthy that these two hypotheses are not mutually exclusive and could co-exist to some extent.

This is the first study to simulate PPI (omeprazole) involvement in clopidogrel active metabolite formation. A general static model, for metabolite AUC ratio calculation, was developed to account for total inhibition and genetic polymorphism. To our knowledge, this is the first report of such a static model, enabling to estimate the contribution of a given polymorphic enzyme or its inhibition in the formation of a secondary metabolite, although metabolite kinetics has been previously described (Pang, 1995). However, because only net CYP2C19 involvement in the formation of the active metabolite has been estimated from clinical data (and not CYP2C19 contribution in each metabolic step), the general static model has been simplified, assuming only one virtual metabolic step (net effect). The relative involvement of each CYP isoform in clopidogrel metabolism will therefore need to be considered in the future. Moreover, this approach cannot mix several sources of variability (e.g. effect of omeprazole in elderly population), provide a concentration time profile and reflect the effect of an inhibitor on the active clopidogrel metabolite pharmacokinetic parameters other than clearance, as C\text{max} for example. Static model cannot reflect impact of CYP2C19 in special population (e.g. hepatic...
impaired subject) and is limited to co-administration of victim (clopidogrel) and perpetrator (omeprazole). Any study designs, with no co-administration of clopidogrel with omeprazole like dose staggering that can mitigate the effect of the inhibitors (reversible inhibitor in particular) cannot be simulated. Owing to these limitations of the static model, a full PBPK model will need to be developed to allow for inter-individual variability, full PK profile and to provide improved estimates of any DDI, different populations and clinical conditions toward the pharmacokinetics of the clopidogrel active metabolite.
Acknowledgements. Xavier Benain, Franck Poitiers and Laure Siraudin for their respective implication in the interaction studies as statisticians. Olivier Nicolas, Céline Ollier and Christine Farenc for their respective implication in the interaction studies as pharmacokineticists.
Authorship Contributions.

Participated in research design: Boulenc, Djebli, Hurbin

Conducted in vitro experiments: Shi, Brian, Van Horm

Contributed new reagents or analytic tools: -

Performed data analysis: Boulenc, Perrin

Wrote or contributed to the writing of the manuscript: Boulenc, Hurbin
References


Footnotes:

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b) Editorial support was funded by Sanofi-Aventis and provided by Alpha-Plus Medical Communications Ltd.
Legends for Figures:

Fig. 1. Schematic representation of sequential metabolic steps up to the production of the metabolite of interest (M2 in the Figure). In this illustration, M2 is produced only from metabolite M1 through CYP,Y and CYP,Z isoforms and is eliminated through a non-CYP mechanism. The CYP,Y isoform is involved in the first and second metabolic steps and can be considered to be CYP2C19 in the clopidogrel situation. Ma and Mb represent the metabolites at the first and second metabolic steps, respectively; these are not involved in the formation of the metabolite of interest. Fm refers to the fraction of drug or metabolite that is eliminated by the pathway shown with an arrow. In this diagram, only two metabolic steps are represented.

Fig. 2. Determination of $K_{obs}$ in HLM with increasing concentrations of omeprazole.

Fig. 3. Determination of $K_{inact}$ and $K_I$ for irreversible inhibition of CYP2C19 by omeprazole in HLM.

Fig. 4. Comparison of the M2 exposure ratios in CYP2C19 PMs versus EMs, as predicted using static and dynamic models. Data are presented as means and standard deviations.
### TABLE 1

*Cytochrome P450 2C19 alleles assessed as part of the meta-analysis (Study 6) and their corresponding genotypes, predicted metabolizer phenotypes, and distribution*

<table>
<thead>
<tr>
<th>Gene</th>
<th>Predicted</th>
<th>Genotype</th>
<th>LD Population</th>
<th>MD Population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n)a</td>
<td>(n)a</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>UM</td>
<td>*1/*17</td>
<td>88 (23.7)</td>
<td>82 (24.4)</td>
</tr>
<tr>
<td>(370/335)</td>
<td>(99/91)</td>
<td>*17/*17</td>
<td>11 (2.9)</td>
<td>9 (2.6)</td>
</tr>
<tr>
<td></td>
<td>EM</td>
<td>*1/*1</td>
<td>173 (46.7)</td>
<td>156 (46.5)</td>
</tr>
<tr>
<td>(173/156)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IM</td>
<td>*1/*2</td>
<td>75 (20.2)</td>
<td>68 (20.2)</td>
</tr>
<tr>
<td>(82/75)</td>
<td></td>
<td>*1/*3</td>
<td>3 (0.8)</td>
<td>3 (0.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*1/*8</td>
<td>2 (0.5)</td>
<td>2 (0.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*1/*6</td>
<td>1 (0.2)</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*1/*4</td>
<td>1 (0.2)</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td></td>
<td>PM</td>
<td>*2/*2</td>
<td>12 (3.2)</td>
<td>11 (3.2)</td>
</tr>
<tr>
<td>(13/12)</td>
<td></td>
<td>*2/*3</td>
<td>1 (0.2)</td>
<td>1 (0.2)</td>
</tr>
</tbody>
</table>

*Presented as loading dose population/maintenance dose population.

CYP indicates cytochrome P450; EM, extensive metabolizer; IM, intermediate metabolizer; LD, loading dose; MD, maintenance dose; NC, not classified; PM, poor metabolizer; UM, ultrarapid metabolizer.
### TABLE 2

**Arithmetic mean (standard deviation) omeprazole plasma concentrations (ng/ml) co-administered with placebo or clopidogrel in Studies 1, 2 and 3 (see Materials and Methods)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day -5</th>
<th>T2h ²</th>
<th>Day 1, T2h ³</th>
<th>Day 4, T12h</th>
<th>Day 5, T2h</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Omeprazole + clopidogrel (n = 64)</td>
<td>1220 (1080)</td>
<td>2790 (1380)</td>
<td>-</td>
<td>2890 (1330)</td>
<td></td>
</tr>
<tr>
<td>Omeprazole + placebo (n = 5)</td>
<td>1490 (809)</td>
<td>2290 (2080)</td>
<td>-</td>
<td>3210 (2120)</td>
<td></td>
</tr>
<tr>
<td>Ratio (P-value)</td>
<td>-</td>
<td>1.22</td>
<td>(p=0.449)</td>
<td>0.90 (p=0.619)</td>
<td></td>
</tr>
<tr>
<td><strong>Study 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Omeprazole + clopidogrel (n = 66)</td>
<td>768 (977)</td>
<td>388 (615)</td>
<td>319 (304)</td>
<td>997 (1140)</td>
<td></td>
</tr>
<tr>
<td>Omeprazole + placebo (n = 6)</td>
<td>337 (274)</td>
<td>326 (444)</td>
<td>274 (235)</td>
<td>1640 (1170)</td>
<td></td>
</tr>
<tr>
<td>Ratio (P-value)</td>
<td>-</td>
<td>1.19</td>
<td>(p=0.812)</td>
<td>1.16 (p=0.727)</td>
<td>0.61 (p=0.189)</td>
</tr>
<tr>
<td><strong>Study 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Omeprazole + clopidogrel (n = 66)</td>
<td>1320 (966)</td>
<td>2290 (1080)</td>
<td>-</td>
<td>2460 (1020)</td>
<td></td>
</tr>
<tr>
<td>Omeprazole + placebo (n = 6)</td>
<td>1740 (1040)</td>
<td>2650 (1350)</td>
<td>-</td>
<td>3080 (763)</td>
<td></td>
</tr>
<tr>
<td>Ratio (P-value)</td>
<td>0.86</td>
<td>0.80</td>
<td>(p=0.449)</td>
<td></td>
<td>(p=0.152)</td>
</tr>
</tbody>
</table>

²Omeprazole alone, first day of administration
³First day of clopidogrel administration, co-administered with omeprazole.
⁴Ratio of arithmetic means.
⁵In study 2, concentrations reported at 2 hours post-dose of omeprazole were lower than those reported for omeprazole administered in a fasted state (studies 1 and 3). This is likely due to the administration in a non-fasted state, as dinner was provided commencing 2 hours prior to omeprazole administration, and food appears to increase t<sub>max</sub> (up to 4 hours) and decrease C<sub>max</sub>. In this study, variability observed between D1 and D5 is likely to stem from food effect.

Testing the Equality of Means between two treatments.
TABLE 3

**Arithmetic mean (standard deviation) pantoprazole exposures after co-administration with clopidogrel or placebo in Study 4, on Day 5 (i.e. after administration of pantoprazole 80 mg/day for 12 days, see Materials and Methods section)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$C_{\text{max}}$ (ng/ml)</th>
<th>$\text{AUC}_{0-24h}$ (ng.h/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pantoprazole + clopidogrel</td>
<td>3830 (1660)</td>
<td>9460 (6630)</td>
</tr>
<tr>
<td>(n = 56)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pantoprazole + placebo</td>
<td>2840 (631)</td>
<td>8750 (5490)</td>
</tr>
<tr>
<td>(n = 6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ratio$^a$</td>
<td>1.35</td>
<td>1.08</td>
</tr>
<tr>
<td>(P-value$^b$)</td>
<td>(p=0.011)</td>
<td>(p=0.802)</td>
</tr>
</tbody>
</table>

$^a$Ratio of arithmetic means.

$^b$Testing the Equality of Means between two treatments
TABLE 4

Predicted M2 (i.e. clopidogrel active metabolite H4) exposure ratios in CYP2C19 PMs versus EMs using static and dynamic models (see Fig. 1)

Compared with the general situation schematized in Fig. 1, CYP2C19 was considered to be involved in the two metabolic steps to mimic the clopidogrel situation. Using the static model, M2 exposure ratios were determined using static model with equations 7 or 8 and using Simcyp® as described under Materials and Methods. In the clopidogrel situation, F_{M,X}, F_{M,Z} and F_{M,2C19} represent CYP450-dependent pathways; F_{M,a} and F_{M,b} represent the undefined clearance (esterase for F_{M,a}). Several studies have shown that F_{M,a} (esterase) is high compared with CYP450-dependent pathways at the first metabolic step and F_{M,b} would be equivalent at the second metabolic step (Kazui et al., 2010; Gurbel et al., 2009). In the six simulations, F_M values were selected to mimic this situation.

<table>
<thead>
<tr>
<th>F_M values</th>
<th>Step 1</th>
<th>Step 2</th>
<th>M2 exposure ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F_{M,a}</td>
<td>F_{M1,X}</td>
<td>F_{M1,2C19}</td>
</tr>
<tr>
<td>Simulation 1</td>
<td>0.819 (0.0908)</td>
<td>0.163 (0.0837)</td>
<td>0.0175 (0.0173)</td>
</tr>
<tr>
<td>Simulation 2</td>
<td>0.819 (0.0908)</td>
<td>0.163 (0.0837)</td>
<td>0.0175 (0.0173)</td>
</tr>
<tr>
<td>Simulation 3</td>
<td>0.819 (0.0914)</td>
<td>0.146 (0.757)</td>
<td>0.0354 (0.0341)</td>
</tr>
<tr>
<td>Simulation 4</td>
<td>0.819 (0.0914)</td>
<td>0.146 (0.575)</td>
<td>0.0354 (0.0341)</td>
</tr>
<tr>
<td>Simulation 5</td>
<td>0.862 (0.0807)</td>
<td>0.0761 (0.0433)</td>
<td>0.0615 (0.0582)</td>
</tr>
<tr>
<td>Simulation 6</td>
<td>0.827 (0.125)</td>
<td>0.0203 (0.0125)</td>
<td>0.152 (0.123)</td>
</tr>
</tbody>
</table>
TABLE 5

calculated ratios of clopidogrel active metabolite H4 (clopi-H4) AUC\textsubscript{last} (75 or 150 mg maintenance doses) or AU, calculated with extrapolation lower than 20% (300 or 600 mg loading doses) between PM versus the other phenotypic groups from studies 5 and 6 and CYP2C19 involvement in the clopidogrel active metabolite H4 formation in each CYP2C19 genetic polymorphic population

<table>
<thead>
<tr>
<th>Clopidogrel Dose (mg)</th>
<th>Clopi-H4 exposure ratio [95% CI]</th>
<th>Estimated CYP2C19 Fm in clopi-H4 formation clearance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PM/IM\textsuperscript{a}</td>
<td>PM/EM\textsuperscript{a}</td>
</tr>
<tr>
<td>Study 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>75 (Day 5)</td>
<td>0.33\textsuperscript{b*} [0.23-0.47]</td>
<td>0.29\textsuperscript{b*} [0.21-0.42]</td>
</tr>
<tr>
<td>150 (Day 5)</td>
<td>0.42\textsuperscript{*} [0.31-0.57]</td>
<td>0.36\textsuperscript{*} [0.27-0.49]</td>
</tr>
<tr>
<td>300 (Day 1)</td>
<td>0.46\textsuperscript{*} [0.31-0.70]</td>
<td>0.42\textsuperscript{*} [0.29-0.63]</td>
</tr>
<tr>
<td>600 (Day 1)</td>
<td>0.45</td>
<td>0.38</td>
</tr>
<tr>
<td>Study 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loading doses (300; 600)</td>
<td>0.47\textsuperscript{*} [0.38-0.58]</td>
<td>0.36\textsuperscript{*} [0.30-0.45]</td>
</tr>
<tr>
<td>Maintenance doses (75; 150)</td>
<td>0.39\textsuperscript{*} [0.31-0.47]</td>
<td>0.28\textsuperscript{*} [0.22-0.34]</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Estimate and 95% CI
\textsuperscript{b}Fm values might be overestimated, because of underestimation of AUC in PMs at this dose (high lower limit of quantification).
* $P < 0.001$
TABLE 6

Predicted clopidogrel active metabolite H4 (clopi-H4) AUC ratios after clopidogrel and omeprazole co-administration at steady-state

Clopidogrel H4 metabolite AUC ratios were calculated from study 5, with equations 15 and 16 and observed AUC ratios (AUClast and AUC for maintenance and loading dose, respectively). K_i was corrected with a fumic function (fumic: 0.796), and prediction was done with Aureus DDI predict (Aureus Sciences) (fumic: 0.796), using a Hallifax model (Hallifax and Houston, 2006); f_u = 0.05 was obtained from Voltano et al. (Votano et al., 2006); k_{deg} = 0.00044 min^{-1} was obtained from Yang et al. (Yang et al., 2008). For observed values, ratios of geometric means (clopidogrel + omeprazole versus clopidogrel alone) and 95% confidence intervals are presented (with P < 0.001 for all ratios), coming from pooled studies 1, 2 and 3 with about at least 40 subjects per metabolizer group; loading doses (LD): 300 and 600 mg; maintenance doses (MD): 75 and 150 mg. AUC ratios for UMs are not reported as they are similar to those calculated for EMs. For IM and EMs, because the CYP2C19 Fm values are similar regardless of clopidogrel dose (see Table 5), a mean value was considered in order to estimate the omeprazole effect. The value obtained with clopidogrel 75 mg was not taken into account (see Table 5).

<table>
<thead>
<tr>
<th>Omeprazole dose (mg)</th>
<th>C_max for omeprazole at steady state (µM)</th>
<th>Clopi-H4 AUC (LD) and AUC_{last} (MD) ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IM (F_m = 0.60)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EM (F_m = 0.64)</td>
</tr>
<tr>
<td>20</td>
<td>2.1\textsuperscript{a}</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.78</td>
</tr>
<tr>
<td>40</td>
<td>4.7\textsuperscript{a}</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.66</td>
</tr>
<tr>
<td>60</td>
<td>6.0</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.62</td>
</tr>
<tr>
<td>80</td>
<td>8.1\textsuperscript{b}</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.58</td>
</tr>
<tr>
<td>80</td>
<td>(Observed values\textsuperscript{c})</td>
<td>LD: 0.55 [0.49-0.61]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LD: 0.51 [0.48-0.55]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MD: 0.57 [0.51-0.64]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MD: 0.54 [0.51-0.58]</td>
</tr>
</tbody>
</table>

\textsuperscript{a}From US prescribing information for omeprazole (Nexium\textsuperscript{®}).
\textsuperscript{b}C_{2\text{h}} observed in Study 5 (see Table 2) (might be under-estimated compared with an actual C_{max}).
\textsuperscript{c}Ratios of geometric means (clopidogrel + omeprazole versus clopidogrel alone) and 95% confidence intervals.
### TABLE 7

**Predicted clopidogrel active metabolite H4 (clopi-H4) AUC ratios after clopidogrel and omeprazole co-administration at steady-state**

Predicted clopi-H4 AUC ratios were calculated from study 6 from loading and maintenance doses with equations 15 and 16 and observed AUC ratios (AUC\text{last} and AUC for maintenance and loading doses, respectively). For $fu_{mic}$, $fu_p$ and $k_{deg}$ values, and observed values, see legend of Table 6.

<table>
<thead>
<tr>
<th>Omeprazole dose (mg)</th>
<th>C\text{max} at steady state (µM)</th>
<th>Loading dose (AUC)</th>
<th>Maintenance dose (AUC\text{last})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IM $(F_M=0.53)$</td>
<td>EM $(F_M=0.64)$</td>
<td>UM $(F_M=0.67)$</td>
</tr>
<tr>
<td>20</td>
<td>2.1$^a$ (Observed values$^c$)</td>
<td>0.82</td>
<td>0.78</td>
</tr>
<tr>
<td>40</td>
<td>4.7$^a$</td>
<td>0.71</td>
<td>0.66</td>
</tr>
<tr>
<td>60</td>
<td>6.0</td>
<td>0.68</td>
<td>0.62</td>
</tr>
<tr>
<td>80</td>
<td>8.1$^b$</td>
<td>0.65</td>
<td>0.58</td>
</tr>
<tr>
<td>80 (Observed values$^c$)</td>
<td>-</td>
<td>[0.49-0.61]</td>
<td>[0.48-0.55]</td>
</tr>
</tbody>
</table>

$^a$From US prescribing information for omeprazole

$^b$C\text{2h} observed in study 2 (see Table 2) (C\text{2h} might be under-estimated compared with an actual C\text{max})

$^c$Ratios of geometric means (clopidogrel + omeprazole versus clopidogrel alone) and 95% confidence intervals coming from pooled studies 1, 2 and 3
Figure 1

\[ \text{Drug} \]

\[ F_{Ma}, F_{MI.X}, F_{MI.Y} \]

\[ \text{Ma} \quad \text{M1} \quad \text{M1} \]

\[ \text{Elimination} \]

\[ F_{Mb}, F_{M2.Y}, F_{M2.Z} \]

\[ \text{Second metabolic step: } F_{Mb} + F_{M2.Y} + F_{M2.Z} = 1 \]

\[ \text{M1} \quad \text{M2} \quad \text{M2} \]

\[ \text{Clearance of M2 (CL}_{M2}) \]

\[ \text{Elimination} \]

\[ \text{Elimination} \]
Figure 2

The figure shows a graph with the y-axis labeled as Ln % of Control and the x-axis labeled as Pre-incubation Time (min). The graph includes multiple lines, each representing different concentrations of Omeprazole: 0 μM, 5 μM, 10 μM, 15 μM, 25 μM, 50 μM, and 100 μM. The lines are marked with different symbols for each concentration, allowing for easy identification of the data points at various time intervals.
Figure 3

$k_{\text{obs}}$ (min$^{-1}$) vs. Omeprazole Conc (uM)

$k_{\text{inact}} = 0.0156$ min$^{-1}$

$K_I = 8.56$ µM
Figure 4

Increased contribution of CYP2C19 towards metabolic reactions of Step 1 and 2