QUANTITATIVE PREDICTION AND CLINICAL OBSERVATION OF A CYP3A INHIBITOR-BASED DRUG - DRUG INTERACTIONS WITH MLN3897, A POTENT CCR1 ANTAGONIST

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Drug Metabolism and Pharmacokinetics (C.L., S.K.B., M.G.Q., S.R.P.) and Clinical Pharmacology (P.S.D., L.L.v.M.)

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Running title

PREDICTION OF CLINICAL PHARMACOKINETIC DDI

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LIST OF ABBREVIATIONS

AUC, area under the curve of plasma concentration – time plot; CCR1, C-C Chemokine Receptor-1; CYP, cytochrome P450; CYP3A4/5 is abbreviated as CYP3A; DDI, drug-drug interaction; fm, fraction of metabolism by a given enzyme; fA, fraction of activity remaining of a given enzyme in the presence of inhibitor.
ABSTRACT

A novel in vitro model was recently developed in our labs for the prediction of magnitude of clinical pharmacokinetic drug-drug interactions (DDIs), based on reversible hepatic CYP inhibition. This approach, using inhibition data from human hepatocytes incubated in human plasma, and quantitative CYP phenotyping data from hepatic microsomal incubations, successfully predicted DDIs for 15 marketed drugs with ketoconazole, a strong competitive inhibitor of CYP3A4/5 generally used to demonstrate a “worst case scenario” for CYP3A inhibition. Additionally, this approach was successfully extended to DDI predictions with the moderate competitive CYP3A inhibitor fluconazole for nine marketed drugs. In the current report, the general applicability of the model has been demonstrated by prospectively predicting the degree of inhibition, and then conducting DDI studies in the clinic for an investigational CCR1 antagonist, MLN3897 which is cleared predominantly by CYP3A. The clinical studies involved treatment of healthy volunteers (n=17-20), in a cross-over design, with ketoconazole (200 mg BID) or fluconazole (400 mg QD) while receiving MLN3897. Administration of MLN3897 and ketoconazole led to an average 8.28-fold increase in AUC of MLN3897 at steady-state, compared to the 8.33-fold increase predicted from the in vitro data. Similarly for fluconazole, an average increase of 3.90-fold in AUC was observed for MLN3897 in comparison to a predicted value of 3.26-fold. Thus, our model reliably predicted the exposure changes for MLN3897 in interaction studies with competitive CYP3A inhibitors in humans, further strengthening the utility of our in vitro model.
INTRODUCTION

Prediction of clinical DDIs using in vitro studies is one of the major challenges in the pharmaceutical industry. The main utility of such DDI predictions is to help foresee any safety issues anticipated from higher exposures, and thus help design clinical trials with better safety. In some instances, clinical DDI studies can be avoided if no significant pharmacokinetic interaction is predicted. Traditionally, DDI predictions have been based on the ratio of the inhibitor concentration [I] and the enzyme inhibition constant Ki ([I] / Ki ratio) (Rostami-Hodjegan and Tucker 2004, Ito et al., 2004; Obach et al., 2006; FDA DDI guidance draft, 2006). Although this approach had some successes in predicting DDIs, reliability of predictions still remains a challenge (Ito et al., 2004; Blanchard et al., 2004; Cook et al., 2004; Bachmann 2006; Obach et al., 2006). Difficulty remains both in estimating the physiological concentration of the inhibitor at the enzyme site and in reliably determining Ki, which often is affected by the experimental conditions used. Factors such as the protein concentration in the incubation, the substrate of choice, and the enzyme source (Thummel and Wilkinson 1998; Galetin et al., 2006; Lu et al., 2008a) have all been shown to affect the Ki determination. Since the value of [I] (the enzyme site free concentration) cannot be accurately assessed, scientists have utilized various alternative parameters to investigate what correlated well with in vivo observations for a selection of compounds. The results indicate that no uniform parameter could be used to predict interactions broadly. In our in vitro assays, we used human hepatocytes suspended in human plasma, with the plasma concentration of the inhibitors in the incubations being similar to the clinical C_{max} of the inhibitor observed with standard dosing. The free concentration at the enzyme site would be same in the two systems (Lu
et al., 2007 and 2008b). Consequently, our model eliminates the need to determine the value of \([I]\), and removes the dependency on the traditional, less desirable rule of \([I] / K_i\) ratio to predict magnitude of DDIs.

MLN3897 is a potent and selective antagonist of the C-C chemokine receptor-1 (CCR1). In a preclinical reaction phenotyping study, MLN3897 was determined to be cleared mainly by CYP3A, necessitating the conduct of clinical DDI studies based on the 2006 FDA draft guidance on DDI studies. In the present study, ketoconazole was coadministered with MLN3897 in healthy volunteers to assess the extent of inhibition with a strong CYP3A inhibitor. Fluconazole was then coadministered to evaluate and compare the magnitude of interaction in the presence of a moderate CYP3A inhibitor.
Materials and Methods

Reagents. Pooled human liver microsomes from 50 donors were purchased from XenoTech LLC (Kansas City, KS). Cryopreserved human hepatocytes were purchased from In Vitro Technologies, Inc. (Baltimore, MD) and AP Sciences Inc. (Baltimore, MD). 4-Hydroxytolbutamide, 4-hydroxymephenytoin, 1’-hydroxymidazolam, (S)-(+-)(N)-(3)-benzylnirvanol (benzylnirvanol) and azamulin were purchased from BD Gentest (Woburn, MA). Phenacetin, acetaminophen, tolbutamide, dextromethorphan, dextrorphan, furafylline, sulfaphenazole, quinidine, fluconazole, midazolam, NADPH, and MgCl₂ were purchased from Sigma (St. Louis, MO). Human plasma was purchased from Bioreclamation Inc. (Hicksville, NY). S-mephenytoin was purchased from BIOMOL Research Labs., Inc. (Plymouth, PA). MLN3897 was synthesized at Millennium Pharmaceuticals, Inc. (Cambridge, MA).

CYP inhibition determination in human hepatocytes. In vitro assays, such as the reaction phenotyping to obtain the relative CYP contribution values (fm) for MLN3897, the determination of CYP activity remaining (fA) in the presence of ketoconazole or fluconazole in human hepatocytes suspended in human plasma, and the calculation of predicted AUC changes from in vitro data were described in our previous reports (Lu et al., 2007; Lu et al., 2008b). Briefly, to determine CYP enzyme activity remaining in the presence of inhibitors, the fA values, serially diluted ketoconazole or fluconazole in human plasma were added into human hepatocytes. After allowing 10 min of equilibration time, the CYP isozyme specific substrates prepared in plasma were added to start 45-min incubations for assessing the CYP activity. The reactions were stopped by
adding two volumes of acetonitrile containing 1 μM of carbutamide (internal standard). The samples were kept in a refrigerator for 30 min and then centrifuged at 3,000g for 10 min. The supernatants were analyzed by LC/MS/MS for the amount of metabolites formed from the probe substrates. The percent of metabolic activity remaining was calculated by comparing the CYP activities in samples with various concentrations of ketoconazole or fluconazole to their vehicle control. In parallel, extracellular plasma concentrations of ketoconazole or fluconazole were determined after hepatocytes were separated from the plasma using the oil layer separation technique after the 10-min equilibration (Shitara et al., 2003; Lu et al., 2008b). These in vitro extracellular plasma concentrations and their corresponding fA values were used to pair with known clinical plasma C\textsubscript{max} values to perform DDI prediction.

**Reaction phenotyping of MLN3897 in human liver microsomes using CYP selective chemical inhibitors.** The relative CYP contributions to the hepatic metabolism of MLN3897 (fm) was determined using CYP selective chemical inhibitors. The concentrations of the inhibitor were previously determined in human liver microsomal incubations to produce more selective inhibition of a particular CYP, meaning high inhibition toward the target CYP but less of cross inhibition to other CYPs. CYP probe substrates were also incubated in the study to determine the partial inhibition and cross reactivity of these inhibitors. The results from CYP probe substrate incubations were then used to calculate the inhibition of MLN3897 metabolism, assuming that it is subject to same degree of partial inhibition and cross reactivity to these inhibitors as the probe substrates (Lu et al., 2007). Specifically, human liver microsomes (25 μL, final
concentration of 0.5 mg/mL in 0.1 M potassium phosphate buffer, pH 7.4) were pre-
warmed in 96-well plates for 5 min at 37°C, in duplicate, with 25 μL of MLN3897 or
probe substrates (at their respective K_m concentrations) and 25 μL of CYP selective
inhibitors prepared in the same buffer. The reactions were initiated by the addition of 25
μL of NADPH / MgCl_2 in 0.1 M potassium phosphate buffer (final concentration of 2
mM and 3 mM, respectively) and incubated for 15 min. Acetonitrile (1%, final
concentration) and DMSO (0.1%) were used as solvent to increase compound solubility,
and has minimal effect on CYP activity. The reactions were terminated by the addition
of 100 μL of acetonitrile contains 1 μM of carbutamide as the internal standard (IS).
After storing for 30 min in a refrigerator, the sample plates were centrifuged at 3,000g for
10 min and the supernatants were analyzed using LC/MS. The final concentrations of the
respective CYP selective inhibitors and probe substrates were: 100 μM furafylline and 30
μM phenacetin for CYP1A2; 5 μM sulfaphenazole and 150 μM tolbutamide for
CYP2C9; 20 μM benzyl nirvanol and 100 μM S-mephenytoin for CYP2C19; 5 μM
quinidine and 8 μM dextromethorphan for CYP2D6; and 2 μM azamulin and 50 μM
testosterone for CYP3A4. The method for calculation of AUC changes from the in vitro
data (predicted DDI) using equation 1 in the following section was described previously
(Lu et al., 2007).

**Effect of ketoconazole on steady-state pharmacokinetics of MLN3897.** For both
ketoconazole and fluconazole-MLN3897 clinical DDI studies, they were carried out in
accordance with the Good Clinical Practice (GCP) principles enunciated in the
Declaration of Helsinki (revised version of Edinburgh, Scotland, 2000, Note of

The study was approved by the institutional review board, and informed consent was obtained for each subject.

Because of the long plasma half-life of MLN3897, it was administered orally at a proposed efficacious dose of 10 mg once a day (QD) to 20 healthy subjects (10 males and 10 females), for 16 consecutive days (greater than 5 half-lives) (Figure 1) in order to reach steady-state. The mean age, height, and weight were 28 years, 171.3 cm, and 70.5 kg, respectively. The PK profile was measured on Day 16. After a wash-out period (days 17-33), ketoconazole was administered at 200 mg BID from Day 34 through Day 58. MLN3897 co-administration was started on Day 37 and continued through Day 58. The extended dosing for MLN3897 (compared to Period 1; 16 days pre-ketoconazole dosing versus 22 days with ketoconazole) was to assure achievement of a new steady-state, accounting for the anticipated increase in half-life of MLN3897 when co-administered with CYP inhibitor, ketoconazole. On Day 58, the PK of MLN3897 was measured again. The ratio of AUC of MLN3897 administrated with ketoconazole over MLN3897 alone was calculated.

**Effect of fluconazole on steady-state pharmacokinetics of MLN3897 and effect of MLN3897 on CYP3A activity.** A slightly modified study design was applied for the fluconazole DDI trial with 17 healthy subjects (11 males, 6 females) (Figure 2). The
mean age, height, and weight were 33 years, 171.7 cm, and 74.6 kg, respectively. Since MLN3897 was tolerated at higher exposure when co-administered with ketoconazole, on Day 1, a calculated, loading dose of 30 mg QD of MLN3897 was given orally followed by 25 additional doses of 10 mg QD of MLN3897. The pharmacokinetics were measured on Day 10. Fluconazole at 400 mg QD was then administered along with 10 mg of MLN3897 from Day 12 to Day 26. The PK of MLN3897 with co-administration of fluconazole was measured again on Day 26 and the AUC ratio was determined to assess the magnitude of the DDI.

An initial part of this study had been constructed to evaluate whether MLN3897 had any effect on CYP3A activities. MLN3897 was maintained at steady-state levels, and midazolam (2 mg QD), a selective CYP3A substrate, were concurrently dosed orally on Day 0 (pre-MLN3897 dose) and Day 11, and the PK profiles were compared.

**Clinical plasma sample analysis.** Blood samples were collected at various timepoints and centrifuged at approximately 2500 rpm for approximately 15 minutes at 4°C to obtain plasma samples. Plasma samples were stored at or below -80 °C within 1 hour of collection. Samples were assayed for MLN3897 under good laboratory practice (GLP) using a validated liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS) method. Specifically, plasma samples (100 μL) were processed using a liquid-liquid extraction procedure to isolate the analyte and the internal standard (IS), MLN3897-d6, from human plasma. The extracted samples were dried, reconstituted in 100 μL of 50:50 mixture of acetonitrile and 5 mM ammonium hydroxide, and 15 μL of the
reconstituted solution was injected onto a Zorbax Extend-C\textsubscript{18} column (2.1 x 100 mm, 3.5 \(\mu\text{m}\)) for analysis. The HPLC separation was performed at a flow of 0.3 mL/min under a gradient elution mode with 5 mM ammonium hydroxide in water as mobile phase A and acetonitrile as mobile phase B. Each gradient analysis took about 4 minutes. The mass transitions used were \(m/z\) 533.4 \(\rightarrow\) 236.3 for MLN3897 and \(m/z\) 539.4 \(\rightarrow\) 236.3 for the IS. The dynamic range of the assay was 0.05 ng/mL to 50 ng/mL. Plasma concentrations below the lower limit of quantification (LLOQ) were treated as 0 ng/mL for calculation of summary statistics. Plasma concentrations above the upper limit of detection were reassayed after dilution in order for the concentrations to fall within the dynamic range of the assay.
RESULTS

The CYP3A inhibition and cross-inhibition for CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 by ketoconazole and fluconazole in human hepatocytes suspended in human plasma are presented in Tables 1 and 2 as a fraction of the activity remaining (FA). The relative contribution of the above 5 major CYPs toward the hepatic metabolism (fm) of MLN3897 is presented in Table 3. MLN3897 is mainly cleared via hepatic metabolism (i.e. fm,hep = 1) with negligible renal clearance (data not shown). The DDI prediction method described previously (Lu et al., 2007) involved two in vitro parameters fA_CYP and fm_CYP linked using following equation:

\[
\frac{AUC_I}{AUC} = \frac{CL}{CL_I} = \frac{1}{fm_{,hep}(fm_{3A4}fA_{3A4} + fm_{2C9}fA_{2C9} + \ldots) + f_{other}fA_{other}}
\]

where fm,hep is the fraction of clearance by hepatic metabolism, fm,cyp is the relative contribution of an individual CYP to the total metabolism of the compound (without inhibitor) and fA_CYP is the fraction of enzyme activity remaining in the presence of the inhibitor. ‘ f_{other}’ is the fraction of clearance by other routes such as renal or biliary excretion (fm + f_{other} = 1). Experimental or clinical information of the effects of the inhibitor on the bioavailability across gastrointestinal tract (Fg’ / Fg) are generally not available (Galetin and Houston, 2009), therefore this term was not included in our calculations. To assess the worst case scenario of interaction, the clinical maximum plasma concentration of the inhibitor (C_{max}) was used to calculate the fA values. This latter step may compensate for the former. Using this model the predicted AUC
increases of MLN3897 with ketoconazole (200 mg BID) and fluconazole (400 mg QD) at steady-state were 8.33- and 3.26-fold, respectively.

Figure 3 shows the steady-state mean plasma concentration increase of MLN3897 in both male and female subjects (total n = 20) before and after multiple days of treatment with 200 mg BID of ketoconazole. There was little difference between the concentration-time profiles in male and female subjects, in agreement with the literature that gender differences in humans are minimal (Greenblatt DJ and von Moltke LL, 2008). However, an over 8-fold increase in MLN3897 AUC was observed in both groups, relative to their MLN3897 given alone groups. Figure 4 presents the daily trough concentration of MLN3897 with and without co-administration of ketoconazole. The data show MLN3897 reaches steady-state concentrations in 10-14 days, and longer time is required after ketoconazole co-administration. Table 4 summarizes the main pharmacokinetic parameters. Upon co-administration with ketoconazole, increases in AUC, C_{max}, and C_{min} at the steady-state were 8.28-, 7.39-, and 9.27-fold, respectively. Figure 5 presents the individual AUC increases upon co-administration for the 20 subjects. The AUC change ranged from 4.5 to 13.0-fold.

A summary of the main pharmacokinetics parameters for the MLN3897-fluconazole DDI study (n=17) is shown in Table 5. The mean MLN3897 AUC change upon co-administration of fluconazole was 3.93-fold with individual variation from 2.5- to 7.3-fold. The increase of C_{max} and C_{min} at the steady-state was at the similar level (3.51- and
4.98-fold, respectively). The PK profile of MLN3897 at steady-state before and after co-administration of fluconazole is shown in Figure 6.

Part of this clinical DDI study is designed to access whether repeated dosing of MLN3897 would affect the CYP3A activity. The PK profiles (data not shown) of midazolam pre- and post- MLN897 treatment showed no CYP3A activity change, as expected. Since the evaluation of MLN3897 as a perpetrator of CYP3A-mediated DDI is not the focus of this manuscript, no further discussion is included.
DISCUSSION

The primary objectives of this study were to investigate the potential CYP3A inhibitor-mediated DDI risk for an investigational CCR1 antagonist and CYP3A substrate, MLN3897, in healthy volunteers, and demonstrate an in vitro – in vivo correlation (IVIVC) using a recently proposed novel in vitro prediction model (Lu et al., 2007; Lu et al., 2008a,b). In our DDI prediction model, inhibitors such as ketoconazole or fluconazole were incubated with human hepatocytes in 100% human plasma to construct concentration-CYP inhibition titration curves under conditions closely resembling those in vivo. At equilibrium, the extracellular (plasma) concentrations of the inhibitor were measured after separating the plasma from hepatocytes. By matching the extracellular plasma concentration in vitro with the plasma Cmax found in in vivo study then provided the extent of inhibition of various CYPs from the titration curves. This avoided the need to use the [I] / Ki ratio approach for DDI predictions. Essentially, the free concentrations at the enzyme site in vitro and in vivo would be comparable, and so would the extent of inhibition. This model has been validated with 15 marketed drugs with ketoconazole ($r^2 = 0.97$ for the in vitro – in vivo correlation, Lu et al., 2008b). A similar approach was applied to fluconazole with 9 marketed drugs, where a good DDI prediction was demonstrated with $r^2 = 0.71$ (Lu et al., 2008a).

Three parameters used in this model are $f_{m, hep}$, $f_{m, CYP}$, and $f_{A, CYP}$. The ‘$f_{m, hep}$’ is considered to be unity based on the human ADME study, where MLN3897 was cleared mainly through metabolism. The $f_{m, CYP}$ was determined by quantitative CYP phenotyping. As previously discussed (Lu et al., 2007), $f_A$ was calculated by
intrapolation of the two closely measured fA values, covering the in vivo C_{\text{max}} value of
the inhibitor. For the model, at the reported C_{\text{max}} of 10 \mu M ketoconazole after 200 mg
BID (Pelkonen et al., 1998), the fA values for CYP1A2, 2C9, 2C19 and 3A4 were
calculated to be 0.864, 0.739, 0.604, and 0.006, respectively. CYP2D6 was not inhibited
by ketoconazole at physiologically relevant concentrations. For fluconazole, at the
reported C_{\text{max}} of 69.2 \mu M at 400 mg QD dose (Brunton et al., 2006), the fA values were
calculated to be 0.308, 0.133, and 0.203 for CYP2C9, CYP2C19, and CYP3A4,
respectively. CYP1A2 and 2D6 were not inhibited by fluconazole.

MLN3897 is being investigated for chronic oral administration to treat inflammatory
diseases. In order to assess the maximal effect of CYP3A inhibition on MLN3897,
administration of MLN3897 until steady-state was reached was necessary. Since
MLN3897 has a half-life of approximately 3–4 days, the steady-state was expected in 16
days (4–5 half lives). In the presence of an inhibitor, the half-life of MLN3897 was
expected to be prolonged, and therefore the co-administration of MLN3897 and
ketoconazole was extended out to 21 days to ensure achievement of steady state. A
similar time frame was applied for a washout period.

Co-administration of the CYP3A substrate MLN3897 with a strong CYP3A inhibitor
ketoconazole resulted in the predicted elevation (8.33-fold; as predicted from the in vitro
model) of steady-state MLN3897 AUC, and represents the most extreme inhibition likely
to be seen with clinically used CYP3A inhibitors. Fluconazole is a moderate CYP3A
inhibitor and also inhibits other CYPs, such as CYP2C9 and 2C19 (Venkatakrishnan,
The DDI study using fluconazole, illustrates what would be expected when a moderate CYP3A inhibitor is used with MLN3897 (which has no CYP2C9 or 2C19 contributions to its clearance). Since the observed greater than 8-fold elevation of exposure to MLN3897 was well tolerated in healthy subjects, a loading dose design was applied in the MLN3897-fluconazole DDI study to reduce the study time needed to reach the steady-state. The pharmacokinetic parameters of MLN3897 determined after administration of a 30 mg loading dose of MLN3897, followed by 10 mg QD administration of MLN3897 over 10 days were comparable to those observed in the ketoconazole study. The AUC increase of MLN3897 with fluconazole was 3.90-fold, an increase closely predicted (3.26-fold) by our in vitro model.

In summary, the clinical drug interaction studies of MLN3897 with ketoconazole and fluconazole yielded exposure increases for MLN3897, at steady-state, which were predicted closely by our in vitro model. The model is not dependent on the traditional, ambiguous, and unreliable [I] / Ki method. The DDI study for fluconazole was an 'adaptive' design based on ketoconazole data, and allowed subjects to reach the steady-state faster through the safe use of a loading dose. The predicted power of this simple in vitro model is further validated by the current studies. Such dependable predictions are expected to benefit clinical trials since any increased pharmacodynamic or side effects which are linked to pharmacokinetics can be anticipated. If necessary, doses can be adjusted to stay within a desired plasma range.
REFERENCES


Galetin A and Houston JB (2009) Inhibition of drug metabolism enzymes in gastrointestinal tract and its influence on the drug-drug interaction prediction, in Enzyme


Legends for Figures

Figure 1  MLN3897 and ketoconazole clinical drug-drug interaction study dosing schedule. MLN3897 PK profiles were measured on Days 16 and 58.

Figure 2  MLN3897 and fluconazole clinical drug-drug interaction study dosing schedule. Thirty milligram of loading dose was given on Day one. MLN3897 PK profiles were measured on Days 10 and 26.

Figure 3  Mean Plasma Concentration versus Time Profile following 10 mg Dose of MLN3897 and MLN3897 + Ketoconazole.

Figure 4  Mean Trough Plasma Concentration versus Time Profile following 10 mg Dose of MLN3897 and MLN3897 + Ketoconazole.

Figure 5  AUC\text{0-24h} increase following co-administration of MLN3897 and ketoconazole among 20 healthy volunteers.

Figure 6  Mean Trough Plasma Concentration versus Time Profile following 10 mg Dose of MLN3897 and MLN3897 + fluconazole.
Table 1  CYP activity remaining in human hepatocytes in the presence of ketoconazole in human plasma

<table>
<thead>
<tr>
<th>Keto in incubation (μM)</th>
<th>Keto extracellular (μM)</th>
<th>CYP1A2</th>
<th>CYP2C9</th>
<th>CYP2C19</th>
<th>CYP2D6</th>
<th>CYP3A4</th>
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a: fA(%) – fraction of enzyme activity remaining in the presence of ketoconazole inhibition.

Ketoconazole was equilibrated with human hepatocytes in human plasma to allow non-specific binding. After equilibration, the extracellular concentration of ketoconazole was measured. The remaining CYP activities in hepatocytes (fA) were also determined using the probe substrates.

(Table adapted from Lu et al., 2007)
Table 2  Effect of fluconazole on CYP activity remaining in human hepatocytes suspended in human plasma

<table>
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<tr>
<th>Total fluconazole in incubation (μM)</th>
<th>Fluconazole extracellular (μM)</th>
<th>CYP1A2</th>
<th>CYP2C9</th>
<th>CYP2C19 fA (%)\textsuperscript{a}</th>
<th>CYP2D6</th>
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\textsuperscript{a}: fA(%) – fraction of enzyme activity remaining in the presence of fluconazole inhibition.

Fluconazole was equilibrated with human hepatocytes in human plasma to allow non-specific binding. After equilibration, the extracellular concentration of fluconazole was measured. The remaining CYP activities in hepatocytes (fA) were also determined using the probe substrates.

(Table adapted from Lu et al., 2008a)
Table 3  Relative contributions of CYP1A2, 2C9, 2C19, 2D6, and 3A4 to the metabolism of MLN3897 in human liver microsomes

<table>
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<th>CYP</th>
<th>1A2</th>
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<th>2C19</th>
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<td>fm (%)</td>
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<td>0</td>
<td>21</td>
<td>87</td>
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a: fm(%) – fractional contribution of specific CYPs to the metabolism of MLN3897.
Table 4  Summary of the plasma pharmacokinetic parameters of MLN3897 for treatments with and without ketoconazole

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters</th>
<th>MLN3897 + Ketoconazole</th>
<th>MLN3897</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{AUC}_{0-24} ) (ngxhr/mL)</td>
<td>830.6 ± 236.3</td>
<td>100.3 ± 60.5</td>
<td>8.28</td>
</tr>
<tr>
<td>( T_{\text{max}} ) (hr)</td>
<td>7.99</td>
<td>6.01</td>
<td>1.33</td>
</tr>
<tr>
<td>( C_{\text{max,ss}} ) (ng/mL)</td>
<td>40.8 ± 11.7</td>
<td>5.52 ± 3.17</td>
<td>7.39</td>
</tr>
<tr>
<td>( C_{\text{min,ss}} ) (ng/mL)</td>
<td>29.4 ± 8.9</td>
<td>3.17 ± 2.14</td>
<td>9.27</td>
</tr>
</tbody>
</table>
Table 5  Summary of the plasma pharmacokinetic parameters of MLN3897 for treatments with and without fluconazole

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters</th>
<th>MLN3897 + fluconazole Mean ± SD</th>
<th>MLN3897 Mean ± SD</th>
<th>Change Fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC_{0-24} (ngxhr/mL)</td>
<td>327.3 ± 115.3</td>
<td>83.3 ± 35.2</td>
<td>3.93</td>
</tr>
<tr>
<td>T_{max} (hr)</td>
<td>8.00</td>
<td>6.55</td>
<td>1.22</td>
</tr>
<tr>
<td>C_{max,ss} (ng/mL)</td>
<td>16.3 ± 6.1</td>
<td>4.64 ± 1.97</td>
<td>3.51</td>
</tr>
<tr>
<td>C_{min,ss} (ng/mL)</td>
<td>11.0 ± 4.2</td>
<td>2.21 ± 1.02</td>
<td>4.98</td>
</tr>
</tbody>
</table>
Figure 2

Days 0

Midazolam dosing
2 mg QD

MLN3897 dosing
30 mg QD

Days 1

MLN3897 dosing
10 mg QD

Days 2 – 26

Day 11

Midazolam dosing
2 mg QD

Fluconazole dosing
400 mg QD

Days 12 – 26
Figure 4

Mean Trough MLN3897 Plasma Concentration (ng/mL) vs. Time (Day)

- Dotted line with circles: Mean MLN3897
- Dotted line with triangles: Mean MLN3897 Female
- Dotted line with squares: Mean MLN3897 Male
Figure 6

Mean MLN3897 plasma concentration (ng/mL)

- Mean MLN3897
- Mean MLN3897+Fluconazole

Time (h)