Proposal of a Safe and Effective Study Design for CYP3A-Mediated Drug-Drug Interactions

Brit Silja Rohr and Gerd Mikus, MD, MSc

Abstract
Numerous drug-drug interaction (DDI) trials have to be conducted in healthy volunteers based on current regulatory guidelines. Because the worst-case scenario of strong cytochrome P450 (CYP) inhibitors has to be tested, the results and their validity have to be balanced with the risk to volunteer safety. The use of ketoconazole in clinical DDI studies has been discouraged by regulatory agencies due to an alleged risk of liver injury. In order to reduce the risk to healthy volunteers, we carried out a study with single-day exposure to each of 6 perpetrator azole fungistatic drugs. They were evaluated regarding their CYP3A inhibition using microdosed midazolam and a limited sampling strategy. Ratios of areas under the concentration-time curves ranged from 1.93 with isavuconazole to 8.42 with ketoconazole. The highest number of adverse events occurred with voriconazole, followed by ketoconazole; 2 dropouts occurred due to adverse events following itraconazole administration. Literature data on adverse events of azole fungistatic drugs in DDI trials are rare and inconclusive. Only in recent years with the newer drugs are they more precise and reliable. It can be concluded that the duration of preexposure of perpetrator drugs can be reduced to 1 hour before administration of the victim drug. This still can be sufficient to achieve the scientific objectives of the trial with the lowest possible risk.

Keywords
drug-drug interaction, azole fungistatic drugs, midazolam, CYP3A, adverse events

Knowledge about drug-drug interactions (DDI) is very important. The Food and Drug Administration (FDA) recommends in their guideline for industry for DDI studies the use of “index inhibitors” for enzymes of the cytochrome P450 system (CYP), which are usually studied under steady-state conditions of the perpetrator. In the past, ketoconazole was the established strong index inhibitor of CYP3A and P-glycoprotein (P-gp). It was used in drug interaction studies of nearly all investigational new drugs during drug development, and a vast amount of knowledge of its inhibitory effects exists. Because of safety concerns, the FDA now recommends the use of itraconazole, also classified as a strong inhibitor of CYP3A and P-gp. However, despite this recommendation, in clinical drug interaction trials, a different length of preexposure of itraconazole in comparison with ketoconazole has to be used to generate maximum inhibition, which has already been discussed in the literature. In addition, itraconazole is not as strong as ketoconazole regarding inhibition of CYP3A, with an average area under the concentration-time curve (AUC) ratio (AUCR) of 7.3 compared with an average of 11.5 for oral midazolam when ketoconazole is used.

The safety of healthy volunteers taking part in DDI trials is of paramount importance. Consequently, normal function of both liver and kidneys is required for participation. The risk of serious adverse events (AEs) in this population might be low, but there is still a lack of knowledge regarding specific risks surrounding experimental drugs. Therefore, to minimize the risk for healthy volunteers in a DDI trial evaluating CYP3A inhibition, we propose a new DDI trial design. Using this new design, we performed a clinical trial in healthy volunteers with all 6 fungistatic azoles available for systemic therapy in order to compare both safety and CYP3A inhibition properties. The CYP3A activity was assessed by the previously established limited sampling method using midazolam AUC over hours 2-4 (AUC2-4). In addition, we summarize the published evidence on AEs in DDI studies of healthy volunteers taking azole fungistatic drugs.
Methods

Clinical Trial
The phase 1, single-center, open-label, 7-part randomized clinical trial (EudraCT 2017-004453-16) was approved by the responsible Ethics Committee of the Medical Faculty of Heidelberg University and the competent authority responsible for Germany. The study was carried out in accordance with the standards of Good Clinical Practice and the specific legal requirements of German law. This study also complied with the applicable version of the Declaration of Helsinki and the International Conference for Harmonisation recommendations on Good Clinical Practice. The trial was conducted at the Clinical Research Center of the Department of Clinical Pharmacology and Pharmacoepidemiology, University Hospital Heidelberg, which is certified according to DIN EN ISO 9001:2015. All participants gave written informed consent before any trial-specific activities.

Study Population
Fourteen healthy, nonsmoking volunteers (6 women) in the age range of 20 to 48 (median age 25) were included. They were mentally and physically healthy, as defined by medical history, physical examination, electrocardiogram, and routine laboratory analyses. Other than oral contraceptives for the women, no other comediations were allowed.

Study Design and Conduct
A 1-day exposure of the following oral perpetrator drugs were administered in random order (3 predefined treatment sequences with 4 volunteers each) over a period of 1 day (ie, for once daily [QD] dosing 1 dose was administered, for twice daily [BID] dosing 2 doses were administered, etc), with a washout of at least 6 days between treatments. Only solid formulations of the azoles were used to have similar conditions. Isavuconazole was always administered on the last occasion because of its long elimination half-life. Thus,

- No perpetrator drug
- 200 mgitraconazole BID (Sempera Kapseln, Jansen-Cilag GmbH, Neuss, Germany)
- 400 mg voriconazole BID (Voriconazol Zentiva, Zentiva Pharma GmbH, Frankfurt am Main, Germany)
- 400 mg fluconazole QD (Fluconazol HEXAL, Hexal AG, Holzkirchen, Germany)
- 400 mg ketoconazole QD (Ketoconazol HRA, Laboratoire HRA Pharma, Paris, France)
- 300 mg posaconazole BID (Noxafil, Merck Sharp & Dohme Ltd, Hertfordshire, UK)
- 200 mg isavuconazole 3 times a day (TID) (Cresemma, Pfizer Pharma PFE GmbH, Berlin, Germany)

One hour after administration of the perpetrator drug under fasted conditions, an oral solution of midazolam (10 μg), the victim drug, was administered as part of a microdosed cocktail. The cocktail also contained 50 μg yohimbine, 100 μg esomeprazole, 100 μg chlorzoxazone, 25 μg apixaban, 25 μg rivaroxaban, and 50 μg edoxaban. To determine the effect of the perpetrators on CYP3A activity, the previously established limited sampling method for midazolam was applied. This method demonstrated accurate determination of midazolam partial metabolic clearance using only 4 concentrations. Therefore, blood was obtained at 2, 2.5, 3, and 4 hours after midazolam dosing, and separated plasma was analyzed as described before. AUC<sub>2-4</sub> was calculated using Kinetica 5.0 (Thermo Fisher Scientific, Waltham, Massachusetts), and partial metabolic clearance (CL<sub>met</sub>) of midazolam was calculated as described previously. Log-transformed data were subjected to 1-way ANOVA with post hoc analysis to test for differences against baseline (no perpetrator) using Prism 7.0 (GraphPad, La Jolla, California).

Safety Assessments
To monitor potential liver toxicity, blood parameters (alanine transaminase, aspartate transaminase, γ-glutamyltransferase, alkaline phosphatase, and total bilirubin) were determined at the end of the day (24 hours after the first perpetrator drug administration) in each study part. Study participants were informed in detail about potential side effects of theazole fungistatic drugs before they gave written informed consent. There was no blinding in this study. During each study part, the participants were asked about AEs before each drug administration and throughout the day. In addition, spontaneous reports by participants were also documented. All AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 20.0 and were evaluated based on the clinical judgment of the same 2 investigators. The AE intensity was categorized according to National Cancer Criteria for Adverse Events, Version 4.03. The causal relationship of an AE to the study medication was classified as definitive, probable, possible, or not related.

Literature Search
To be able to compare the AE results with current knowledge, a literature search was carried out by screening PubMed for the terms “itraconazole,” “posaconazole,” “voriconazole,” “fluconazole,” or “isavuconazole” with the filter set on “human,” “clinical trials” and published between January 2009 and January 2019. The results were scanned by title and abstract for clinical studies in healthy volunteers. Studies with a therapeutic oral dose of any of the azole
Table 1. Geometric Mean [95%CI] of AUC_{2-4} and Partial Metabolic Clearance of Midazolam After Oral Administration of 10 μg Midazolam Combined With Different Perpetrators

| Treatment          | AUC_{2-4} (h*nmol/L per mg) | CL_{met} (mL/min) |
|--------------------|-----------------------------|-------------------|
| Baseline           | 11.9 [9.3-15.2]             | 476.3 [372.8-608.5] |
| Ketoconazole       | 100.2 [88.6-113.3]          | 56.6 [50.0-64.0]   |
| Voriconazole       | 62.9 [56.5-70.0]            | 90.1 [81.0-100.2]  |
| Fluconazole        | 52.2 [43.4-62.9]            | 108.6 [90.2-130.7] |
| Itraconazole       | 44.9 [39.3-51.3]            | 126.3 [110.6-144.2] |
| Posaconazole       | 29.9 [25.6-34.9]            | 189.8 [162.6-221.7] |
| Isavuconazole      | 23.0' [18.5-28.5]           | 247.0' [189.9-306.7] |

AUC_{2-4} indicates area under the concentration-time curve over hours 2-4 after dose; CL_{met}, partial metabolic clearance of midazolam.

*P < .05 tested against baseline

antifungal drugs were selected. Only publications of pharmacokinetic DDI studies with theazole antifungal drugs were included (including microdosed drugs as victim drugs). Information on AEs and safety aspects were extracted from the publications.

Results

Clinical Trial

Twelve volunteers with a median age of 24.5 years (range 20-48 years) and median body mass index at screening of 24.5 kg/m^2 (range 21.9-29.9 kg/m^2) completed all 7 study parts. The geometric mean of the AUC_{2-4} of midazolam alone (baseline) was 11.9 (h*nmol/L)/mg (95%CI 9.3-15.3 [h*nmol/L]/mg), and the calculated CL_{met} was 476.3 mL/min (95%CI 372.8-698.5 mL/min). Each azole drug caused a significant \( P < .05 \) increase of AUC_{2-4} and, consequently, a decrease of the partial metabolic clearance of midazolam (Table 1). Midazolam AUC_{2-4} was increased by ketoconazole 8.42-fold, voriconazole 5.29-fold, fluconazole 4.39-fold, and itraconazole 3.77-fold and decreased CL_{met} to 11.9%, 18.9%, 22.8%, and 26.5%, respectively (Figure 1). The weakest inhibition was observed for isavuconazole and posaconazole.

Isavuconazole increased midazolam AUC_{2-4} 1.93-fold and decreased CL_{met} to 51.9%; posaconazole increased AUC_{2-4} 2.51-fold and decreased CL_{met} to 39.9%.

Safety

Fourteen participants (6 women) were included in the drug interaction study. Two volunteers (1 woman) dropped out, both during the itraconazole part of the study, which was their first study part. One participant finished the study part with itraconazole and suffered from headache, nausea, and cold sweats (all drug-related, mild in intensity, and occurring within 6 hours after the first drug intake). He did not turn up for the next study part and was lost to follow-up. The second participant only received the first dose of itraconazole and suffered from vertigo (mild, within 6 hours) and from hypertension, vertigo (mild), headache, and nausea (moderate) within 6-12 hours after the first drug intake (all drug-related). This participant was withdrawn from further study treatments for safety reasons.

For the 12 participants who completed the study, a total of 42 AEs occurred; 38 AEs were classified as mild and 4 as moderate in intensity. No serious AEs occurred. All AEs resolved completely. Details of the AEs are listed for each participant and study part in Table 2. Seven AEs were not related to the study drug. A higher frequency of AEs was observed in the 5 women (24 AEs) in comparison with the 7 male participants (18 AEs). One participant received no second dose of voriconazole after suffering from nausea, headache (both moderate), and photophobia (mild). Most AEs occurred under voriconazole, followed by ketoconazole, itraconazole, fluconazole, posaconazole, and isavuconazole (Table 2). Except for the known visual disorders caused by voriconazole (n = 16), no specific pattern of AEs was observed.

Regarding liver parameters, which were measured at the end of each azole treatment day, no increased γ-glutamyltransferase, alanine transaminase, and alkaline phosphatase values were observed. Aspartate transaminase was increased once under the treatment of posaconazole (44 U/L; upper limit of normal <37 U/L). Total bilirubin values ranged from 0.4 to 1.1 mg/dL during screening, with increased variability during the study (range 0.3 to 1.5 mg/mL; no obvious pattern observed), and a bilirubin range from 0.2 to 1.3 mg/dL after the study.

Literature Data on Safety

Published DDI studies in healthy volunteers using azoles as perpetrators are limited (Figure 2) with the exception of ketoconazole, for which a large number
Table 2. Adverse Events in a Clinical DDI Trial Using 6 Different Azole Fungistatics Administered Over a 24-Hour Treatment Period

| Subject No. | No Azole | Itraconazole | Voriconazole | Fluconazole | Ketoconazole | Posaconazole | Isavuconazole |
|-------------|----------|--------------|--------------|-------------|--------------|--------------|--------------|
| 2           | 0        | 0            | 0            | 0           | 0            | 0            | 0            |
| 3           | [Tooth infection]b | 0            | 0            | 0           | 0            | 0            | 0            |
| 4           | 0        | 0            | 0            | 0           | 0            | 0            | 0            |
| 5           | 0        | 0            | 2x Xanthopsiaa | [Headache]b | 0            | 0            | 0            |
| 6           | [Headache]b | Headache    | 0            | 0           | 0            | 0            | 0            |
| 7           | 0        | 0            | 0            | 0           | 0            | 0            | 0            |
| 8           | 0        | 0            | 0            | 0           | 0            | 0            | 0            |
| 9           | 0        | 0            | 0            | 0           | 0            | 0            | 0            |
| 11          | 0        | 0            | 2x Xanthopsiaa | [Headache]b | 0            | 0            | 0            |
| 12          | 0        | 0            | Xanthopsia, Blurred vision | [Viral urt infection]b | 0            | 0            | 0            |
| 13          | 0        | Headache*, Nausea, Vomiting | Xanthopsia | 0            | 0            | 0            | 0            |
| 14          | 0        | 0            | Xanthopsia, Photophobia | 0            | Muscle tightness | 0            | 0            |

| Overall | 2 | 4 | 20 | 4 | 9 | 2 | 1 |
| ~6 hc | 0 | 1 | 5 | 0 | 2 | 0 | 1 |
| 6-12 h | 0 | 0 | 3 | 0 | 1 | 1 | 0 |
| 12-25 h | 0 | 3 | 12 | 1 | 5 | 1 | 0 |
| Mild | 2 | 4 | 18 | 4 | 7 | 2 | 1 |
| Moderate | 0 | 0 | 2 | 0 | 2 | 0 | 0 |

AST† indicates increased aspartate transaminase; DDI, drug-drug interaction; urt, upper respiratory tract.
[...] indicates adverse event is not related to study drug; lowercase, mild intensity; CAPITAL LETTERS, moderate intensity. Subjects 1 and 10 were dropouts.
aOccurred within 6 hours after first drug intake.
bOccurred later than 25 hours after first drug intake.
cTime of initial adverse event occurrence after first drug intake.

Of DDI studies exist due to the previous regulatory requirements of CYP3A inhibition studies.2,8 The quality of safety reports on AEs in the literature is heterogeneous. The publications on the newer azoles (posaconazole and isavuconazole) report AEs in more detail than studies using the older drugs (itraconazole, voriconazole, and fluconazole). Some publications report about AEs in detail in tabular form, others not at all, and others have only 1 sentence stating that no serious AEs occurred. Furthermore, the dose and duration of azole administration vary considerably among studies. The safety extracts from all relevant DDI studies regarding azoles as perpetrator drugs are given in Supplementary Tables S1-S5.

Although ketoconazole used to be administered for 4 to 7 days before the victim drug, the frequency of AEs reported was very low regarding liver enzyme elevation.2,8 Of the 29 publications with a cumulative itraconazole dose range of 100 to 4800 mg, one third did not report any side effects, and about 17% contained a clear statement that no AEs occurred at all (mostly studies with the lowest cumulative doses). About 43% contained incomplete, unspecific, or ambiguous AE descriptions or listings. Only 3 studies reported AEs adequately. There was not a single AE that was reported more frequently than others. The cumulative fluconazole dose ranged from 50 to 8400 mg in 18 studies, of which 40% did not report side effects or stated that no AEs had occurred at all (studies with the lowest cumulative doses). Similar to itraconazole, incomplete, unspecific, or ambiguous AEs were reported in about 44% of fluconazole studies. Only 3 studies contained...
useful AE data. Voriconazole cumulative doses ranged from 200 to 6800 mg in 14 studies, with 11 not reporting extensive AE data. The most prominent and often reported AE for voriconazole was visual impairment, which was always transient. Most of the studies with isavuconazole extensively reported AEs, mainly in tabular form, with frequencies. The cumulative dose ranged from 200 to 4600 mg. Regarding posaconazole, with a cumulative dose range of 300 to 12 800 mg, only 1 study did not report on side effects; the majority reported the most frequent side effects or those that occurred above a certain frequency in the study population.

**Discussion**

The current regulatory guidelines on DDI studies are very specific. The FDA states “When evaluating the investigational drug as a substrate in a DDI, clinical DDI studies should start with a strong index inhibitor and a strong index inducer” and specifically recommends itraconazole and clarithromycin as strong index inhibitors of CYP3A. The guideline continues: “These index inhibitors and inducers are preferred because there is a large body of information about (1) their defined effects on specific CYP pathways; (2) their appropriate dosing regimens; (3) their safety profiles; and (4) their anticipated effects on their respective sensitive substrates.” The European Medicines Agency guideline, which is currently under revision, is more detailed with “If cytochrome P450 enzymes are identified as candidate enzymes involved in the main elimination pathways of the drug (or in major formation or elimination pathways of clinically relevant active metabolites), evaluation of the pharmacokinetics of the investigational drug with and without concomitant administration of a strong enzyme inhibitor ... is recommended to verify and quantify the involvement of a specific enzyme in the investigational drug elimination” and recommends 4 strong inhibitors of CYP3A: itraconazole, ketoconazole, ritonavir, and clarithromycin. The guideline also gives information regarding dose, formulation, and time of administration of the perpetrator drug. When itraconazole is used as a perpetrator drug, different treatment schemes have been applied ranging from 5 consecutive days of 200-mg QD dosing, with the victim drug on day 4, to 11 days of 200-mg BID dosing, with the victim drug on day 8. Although no systematic data are available on the frequency of AEs depending on treatment duration (range 1 to 28 days) and/or the cumulative dose in DDI studies, it can be anticipated that a longer exposure will at least increase the risk for AEs. To balance the required need for maximal effect of a perpetrator with the desired reduction of risk for AEs in healthy subjects, the goal for an ideal DDI design would be to elicit the maximum effect using the shortest time period of exposure to the perpetrator. It has al-
Table 3. Published Literature on Perpetrator Properties of Ketoconazole Regarding CYP3A Inhibition Using Oral Midazolam as a Probe Drug

| Ketoconazole Dose | Midazolam Dose | Time Point | AUC Ratio | Error | Reference |
|-------------------|----------------|------------|-----------|-------|-----------|
| Current study: 400 mg SD | 10 μg[a] | 1 h[b] | 8.42[c] | 6.31-11.23[d] | ... |
| 100 mg SD | 5 mg | at once | 2.3[d] | 0.7[f] | 25 |
| 200 mg SD | 5 mg | at once | 2.7[f] | 1.1[f] | 25 |
| 200 mg SD | 2 mg | 2 h before | 4.95[h] | 3.92-6.25[n] | 26 |
| 200 mg SD | 2 mg | simultaneous | 6.45[k] | 3.4-12.22 | 26 |
| 400 mg SD | 5 mg | 4 h before | 1.2[g] | 0.3[f] | 25 |
| 400 mg SD | 5 mg | 2 h before | 2.0[f] | 0.6[f] | 25 |
| 400 mg SD | 5 mg | simultaneous | 4.2[f] | 1.7[f] | 25 |
| 400 mg SD | 5 mg | simultaneous | 5.4[f] | 1.0[f] | 25 |
| 400 mg SD | 2 mg | 5 min[f] | 10.28[g] | 8.68-12.19[d] | 27 |
| 400 mg SD | 5 mg | 12 h[f] | 3.9[f] | 1.1[f] | 25 |
| 400 mg SD | 5 mg | 2 h[f] | 4.9[f] | 1.0[f] | 25 |
| 200 mg BID | 6 mg | with 2nd dose | 16.0[f] | 6.1[f] | 28 |
| 200 mg BID | 2 mg | 1 h after 2nd dose | 8.1 | ... | 29 |
| 200 mg BID 4 d | 75 μg | day 3 | 6.5 | ... | 30 |
| 400 mg QD 2 d | 2 mg | day 2 | 13.14[t] | 11.08-15.58[d] | 27 |
| 400 mg QD 2 d | 10 μg | day 2 | 9.77[c] | 7.85-12.14[b] | 31 |
| 400 mg QD 4 d | 7.5 mg | day 4 | 15.9[h] | ... | 32 |
| 400 mg QD 5 d | 2 mg | day 5 | 13.96[f] | 11.79-16.53[d] | 27 |
| 400 mg QD 5 d | 2 mg | day 4 | 16.95[h] | 15.49-18.55[h] | 33 |
| 200 mg BID 5 d | 2 mg | day 4 | 15.34[f] | 13.47-17.47[h] | 33 |
| 200 mg QD 12 d | 10 mg | day 12 | 7.72[f] | 5.96[f] | 34 |
| 400 mg QD 7 d | 2 mg | day 6 | 7.52[f] | 6.75-8.38|h | 18 |
| 400 mg QD 10 d | 0.075 mg/kg | day 6 or 9 | 9.5[f] | ... | 35,36 |
| 400 mg QD 14 d | 2 mg | day 13 | 11.53[f] | 9.94-13.38[b] | 37 |
| 400 mg QD 15 d | 100 ng | day 2 | 19.5[h] | 10.3-36.8[h] | 38 |
| 400 mg QD 15 d | 300 mg | day 2 | 18.8[h] | 15.5-24.4[h] | 39 |
| 400 mg QD 15 d | 1 mg | day 8 | 10.9[h] | 7.8-15.1[h] | 40 |
| 400 mg QD 15 d | 3 mg | day 8 | 13.6[k] | 10.9-16.9[h] | 40 |
| 400 mg (200-400) | | | REVIEW | | 4 |
| 3 d (0-14) preexposure | | | 11.5 | | |

AUC indicates area under the concentration-time curve; BID, twice a day; CYP, cytochrome P450; QD, once a day; SD, single dose.
The studies that were already analyzed in a recent review (last 2 lines) are highlighted in gray.
aAdditional drugs at once.
bAfter last dose of fungistatic drug.
cBased on AUC_{2-4} geometric mean ratio.
d95%CI.
eBased on AUC_{∞} mean ratio.
fStandard deviation.
gBased on AUC_{∞} geometric mean ratio.
h90%CI.
iBased on percentage ratio estimates of AUC_{∞}.
jBased on percentage ratio estimates of AUC_{last}.
kBased on AUC_{last} geometric mean ratio.
lBased on AUC_{0-12} geometric mean ratio.

Regarding the published data for the 6 perpetrators used together with midazolam as the victim drug (Table 3 and Supplementary Table S6), it should be noted that multiple different treatment durations of preexposure to the perpetrators were used. Ketoconazole was the most widely used potent CYP3A inhibitor in DDI studies and was usually administered for 4 to 7 days at 400 mg QD, although different regimens were also applied (Table 3). A comprehensive review of the data was published recently showing that, in 15 studies using ketoconazole as a perpetrator, daily doses ranged from 200 to 400 mg for 0 to 14 days.4 The average AUCR was 11.5, which was

ready been suggested that DDI studies be designed with the duration of preexposure of the perpetrator drug to be minimal but sufficient to achieve the scientific objectives of the studies with the lowest possible risk.12

With the study design used with the administration of the perpetrator drug 1 hour before administration of the victim drug, it was possible to generate valid DDI data with respect to CYP3A inhibition. We used a limited sampling strategy with midazolam, which uses 4 blood samples between 2 and 4 hours after midazolam administration.5 Therefore, the results presented from this trial represent the magnitude of CYP3A inhibition after a single oral dose of the perpetrators used.
almost achieved (AUCR 8.42) by the 400-mg single dose administered 1 hour before midazolam in our study. This was the strongest inhibition observed in comparison with the other 5 azoles used. However, as shown in Table 3, AUCR varies substantially between 1.2 and 19.5, depending on the dose and the duration of the pretreatment period or other reasons. Thus, we are confident that a single oral dose of ketoconazole administered 1 hour before any victim drug (in our case midazolam) is sufficient to elicit a substantial (strong) CYP3A inhibition. The recently recommended strong CYP3A perpetrator itraconazole was also reviewed by Greenblatt and Harmatz, and the 5 studies with midazolam resulted in an average AUCR of 7.3 with a daily dose from 100 to 400 mg on 0.17 to 5 preexposure days. The same magnitude of AUCR is observed using a single oral dose of 400 mg ketoconazole without needing long preexposure periods. One hour after a single oral dose of 200 mg itraconazole, we administered midazolam, and an AUCR of 3.77 resulted. This is much lower than previously observed, but this reflects the need for accumulation of a metabolite that contributes significantly to CYP3A inhibition for maximal inhibition to be achieved. The lower AUCR is in accordance with results obtained using a single dose of 200 mg oral solution of itraconazole followed by administration of midazolam 4 hours later, which resulted in a 4.7-fold increase in midazolam AUC. It is also in good agreement with another study in which midazolam was administered 2 hours after a single 200-mg dose of itraconazole, and an AUCR of 3.5 resulted. If this single-dose approach is used, the required strong CYP3A inhibition cannot be attained with itraconazole.

The time course of CYP3A inhibition by voriconazole has previously been evaluated. When midazolam was administered 10 minutes after the first 400-mg voriconazole dose, a midazolam AUCR of 4.38 resulted. A similar magnitude of inhibition was observed when midazolam was administered 30 minutes after 400 mg voriconazole (AUCR 6.95). This is consistent with the AUCR of 5.29 in this study, with a time delay between perpetrator and victim administrations of 1 hour. However, using the recommended dosing schedule of the manufacturer (first day 400 mg BID; second and further days 200 mg BID), a stronger CYP3A inhibition ranging between 7- and 12-fold AUC increase is seen.

Following a single oral dose of 400 mg fluconazole, a more than 4-fold increase in midazolam exposure was observed. This is in line with the degree of CYP3A inhibition after single and multiple doses of fluconazole, with AUCRs ranging from 3.5 to 5.3. Fluconazole is regarded as a moderate CYP3A inhibitor and can consistently elicit this CYP3A-inhibiting effect, even with a single oral dose of 400 mg.

Regarding the interaction between posaconazole or isavuconazole and midazolam, there are only a few data published. After 7 days of either 200 or 400 mg posaconazole BID, a 4.6- or 5.0-fold increase of oral midazolam AUC was observed. Another study used 50, 100, or 200 mg posaconazole QD for 8 days and found an increase in midazolam exposure of 3.1, 4.0, or 5.7, respectively. We used 300 mg posaconazole as gastroresistant tablets 1 hour before midazolam, with a resulting AUCR of 2.51. For the tablet formulation, it is known that time to peak concentration ranges between 4 and 5 hours after administration; therefore, this weak CYP3A inhibition is not surprising and does not match with posaconazole being classified as a strong CYP3A inhibitor. This weak inhibition was also observed in patients during posaconazole treatment.

Isavuconazole is a CYP3A-sensitive substrate, with its exposure increased 4.2-fold by ketoconazole. It is also a CYP3A inhibitor, as shown by a 2-fold increase of midazolam exposure after 2 days of isavuconazole loading doses of 600 mg and subsequent 200-mg doses for 8 days. We observed CYP3A inhibition in the same range (1.93-fold increase of midazolam AUC), which was obtained after the first 200-mg isavuconazole dose. Isavuconazole can be regarded as a weak to moderate CYP3A inhibitor; however, a single oral dose of 200 mg is sufficient to achieve this degree of inhibition.

There are only limited AE data for the “older”azole antifungal agents in DDI studies with healthy volunteers. Ketoconazole, which was required by the regulatory authorities to be used in DDI studies concerning CYP3A inhibition, was previously evaluated regarding safety and AEs. It was clearly stated that ketoconazole carries minimal risk to healthy volunteers in DDI studies because exposure duration is limited. From the literature published on the safety of the otherazole fungistatic drugs when used in DDI studies, it can be concluded that there is no concern for using these azoles in healthy volunteer DDI studies. However, safety reporting is worse the older the publications are. For the newer drugs, there are at least some excellent tabular safety reports within the publication, which are helpful to address the potential risk when using the specific drug in a DDI study. When voriconazole is used, visual disturbances are quite common, and volunteers must be informed about this during the informed consent.

A clear limitation of DDI studies regarding AE reporting is the small number of study participants; rare AEs will not be picked up regularly. If certain AEs occur frequently in such a small study population, this must be reported in a sufficient way. Each
study has to undergo a risk/benefit assessment before study initiation. Therefore, it is important to collect all available information that has been obtained in similar studies. Solely relying on the summary of product characteristics of the registered drug to be used might not be sufficient. We suggest addressing this issue by adding the following information as a supplement to every future DDI study:

1. Listings of participants with any AE during the study, broken down to each study part (at least study part with victim drug only and study with victim and perpetrator drug and duration of treatments), with description of AE, severity, and relationship to study drug.
2. MedDRA coding for AE.
3. AE severity (mild, moderate, severe).
4. Relationship to study drug (related versus unrelated).

If there are no AEs, there should be an unmistakable statement. Statements such as “there were no discontinuations due to AE and no severe AEs” can only be used in addition to the AE reporting. The AE reporting for our study follows this suggestion (Table 1).

In our study there were 2 dropouts. Interestingly, these were both receiving itraconazole, the perpetrator recommended by the FDA to be used in DDI studies as a strong CYP3A inhibitor. In our study we only used 400 mg divided into 2 doses of 200 mg, 12 hours apart. In DDI studies using itraconazole, a 3-day pretreatment period is applied before administration of the victim drug; on the first day, an itraconazole loading dose of 400 mg (200 mg BID) is given, followed by 200 mg QD on the subsequent days.²² Itraconazole has a long half-life, and metabolites contributing to the CYP3A inhibitory effect cause a delay of the onset and offset of inhibition.²² Therefore, the question still remains if a negligible risk (ketoconazole in DDI studies²,⁸) can be improved on by the use of another azole (itraconazole) where there are far fewer data on safety risk in healthy volunteers (see Supplementary Table S1).

Limitations
Regarding AE documentation, there is a large variability in the assessment, which is mainly investigator dependent and, therefore, somewhat subjective. It is also volunteer dependent, which might result from a more or less extensive study information process. Everyone should be aware of these factors as these assessments cannot be completely uniform.

The proposed study design in which the perpetrator drug is administered 1 hour before the victim drug could be used for all possible victim drugs; however, depending on the anticipated elimination half-life of the victim, there might be additional doses of the perpetrator required. In the case of midazolam with the limited sampling methodology, a single dose of a perpetrator drug was sufficient, and, thus, the 1-week washout period between the perpetrator treatments used here should also have been sufficient. However, for perpetrators given over a longer time period, it has to be considered that a longer washout period might be needed, because it has been demonstrated for posaconazole that, even after 14 days, a perpetrator effect is observed.²³,²⁴ If the half-life of a victim drug is intrinsically long, the duration of exposure to the perpetrator has to be extended, thereby theoretically increasing the risk of AEs, although this is not the case for ketoconazole.²,⁸

Conclusions
In order to minimize the potential risks coming from a longer preexposure to perpetrator drugs, a reduction from several days to 1 hour before administration of the victim drug is possible while still maintaining the achievement of the scientific objectives of the trial, at least for ketoconazole. Therefore, it seems justified to reinstate ketoconazole as an index inhibitor for CYP3A when given only 1 hour before the victim drug, thereby reducing ketoconazole exposure and hence reducing the risk for AEs.

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Data Sharing
The midazolam data can be requested from the corresponding author (gerd.mikus@med.uni-heidelberg.de).

Conflicts of Interest
Part of the data were presented as a poster at the 53rd Scientific Conference of the German speaking Mycological Society eV, September 5-7, 2019, Mannheim, Germany.

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**Supplemental Information**

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