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ARTICLE

Posaconazole and midostaurin in patients with FLT3-mutated acute myeloid leukemia: Pharmacokinetic interactions and clinical facts in a real life study

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Abstract

Midostaurin is used in combination with chemotherapy to treat patients with newly diagnosed FLT3-mutated acute myeloid leukemia. Chemotherapy-induced neutropenia exposes these patients to a significant risk of invasive fungal infections (IFIs). International guidelines recommend primary antifungal prophylaxis with posaconazole (PCZ) but nested analysis of a phase III trial showed that strong PCZ inhibition of CYP3A4 diminished midostaurin metabolism and increased midostaurin plasma levels; however, midostaurin-related adverse events (AEs) were only moderately exacerbated. We conducted a prospective multicenter real-life study to evaluate (i) how often concerns around PCZ-midostaurin interactions made the hematologist prescribe antifungals other than PCZ, (ii) how remarkably PCZ increased midostaurin plasma levels, and (iii) how significantly PCZ-midostaurin interactions influenced hematologic and safety outcomes of induction therapy. Although the hematologists were blinded to pharmacokinetic findings, as many as 16 of 35 evaluable patients were prescribed antifungal prophylaxis with micafungin, weak CYP3A4 inhibitor, in place of PCZ ($p < 0.001$ for deviation from guidelines). In the 19 patients managed as per guidelines, PCZ-midostaurin interactions were more remarkable than previously characterized, such that at the end of induction therapy midostaurin minimum plasma concentration (C_{min}) was greater than three times higher than reported; moreover, midostaurin C_{min}, maximum plasma concentration, and area under the curve were more than or equal to four times higher with PCZ than micafungin. Hematologic

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outcomes (complete remission and duration of severe neutropenia) and safety outcomes (midostaurin-related any grade or grade ≥3 AEs) were nonetheless similar for patients exposed to PCZ or micafungin, as was the number of breakthrough IFIs. In waiting for randomized phase III trials of new prophylaxis regimens, these findings show that PCZ should remain the antifungal of choice for the midostaurin-treated patient.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Midostaurine is combined with intensive chemotherapy to treat acute myeloid leukemia (AML) with FLT3 mutation (FLT3-*mut* AML). Posaconazole (PCZ) is recommended to prevent invasive fungal infections in these patients but posaconazole may also cause pharmacokinetic (PK) interactions that increase the plasma levels of midostaurin. Concerns around such interactions might lead the hematologist to use antifungals that lack PK effects on posaconazole but are not formally recommended.

WHAT QUESTION DID THIS STUDY ADDRESS?

Three questions were addressed: (i) how often would PK concerns make the hematologist replace PCZ with other antifungals, (ii) what is the actual magnitude of PCZ-midostaurin interactions, and (iii) do PK interactions introduce unacceptable toxicities?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

In a prospective, multicenter, real-life study in which the hematologist was blinded to PK findings, there was a highly significant deviation from guidelines, such that 16 out of 35 patients were treated with micafungin in place of PCZ. The magnitude of PCZ-midostaurin interaction was even higher than previously reported but this did not cause unacceptable toxicities compared to micafungin.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

This study ramificates into both clinical and translational pharmacology. On the one hand, there seems to be no justification for replacing PCZ with other antifungals. On the other hand, concerns seem to persist in everyday clinical practice, which calls for randomized clinical trials of the efficacy and safety of antifungals other than PCZ.

INTRODUCTION

Midostaurin, first-generation Fms-like tyrosine kinase 3 (FLT3) inhibitor, shows remarkable activity in patients with newly diagnosed FLT3-mutated acute myeloid leukemia (FLT3-*mut* AML) and is now approved for clinical use in combination with standard chemotherapy.[1]

As any other patient with AML, the patient with FLT3-*mut* AML is at risk of invasive fungal infections (IFIs), which is caused by chemotherapy-related profound neutropenia. The incidence of probable/proven or possible IFIs is, respectively, 10.5% and 9.7% during remission induction chemotherapy, but decreases to 2.4% and 1.8% during consolidation chemotherapy.[2] Induction chemotherapy therefore represents the time window in which the burden of IFIs is of great concern, with mortality rates being as high as 8.3%.[2]

Posaconazole (PCZ), a triazolic antifungal agent, is formally recommended for primary prophylaxis of IFIs during induction chemotherapy for AML[3,4] but its use is complicated by pharmacokinetic (PK) interactions with midostaurin. PCZ strongly inhibits CYP3A4, thereby diminishing midostaurin metabolism and exposing the patient to unnecessarily high and potentially toxic

circulating levels of midostaurin. This has been referred to as the midostaurin-PCZ dilemma.[5] There is scant information on how the midostaurin-PCZ dilemma would be managed in real-life settings. Concerns about PK interactions might lead the hematologist to replace PCZ with other antifungals, with such a decision representing a significant deviation from guidelines. On a different note, the actual magnitude of PK interactions remains only partially characterized at this point in time. PK interactions were characterized in retrospective analyses of subgroups of patients of the RATIFY phase III trial that advanced midostaurin to approval for use in FLT3-*mut* AML,[6] yet only midostaurin minimum plasma concentration (C_{min}) was measured. Moreover, correlations between PCZ and midostaurin levels were not disentangled from potential effects of other CYP3A4 inhibitors.[7]

Keeping the aforesaid premises in mind we conducted a prospective proof-of-concept study that addressed the following questions: (i) how often would the hematologist replace PCZ with other antifungals in everyday clinical practice, (ii) what is the actual magnitude of PCZ-midostaurin interactions when the role of PCZ is evaluated in isolation and midostaurin is measured both before and after dosing, and (iii) do PK interactions introduce unacceptable toxicities that justify a decision to use antifungals other than PCZ?

PATIENTS AND METHODS

Study design

This was a prospective, multicenter, proof-of-concept, no profit study with observational and interventional objectives, conducted from March 2021 to September 2022 in nine hematology units belonging to the Italian network of Sorveglianza Epidemiologica InFezioni nelle EMopatie (SEIFEM). The study recruited adult patients (≥18 years of age), newly diagnosed with FLT3-*mut* AML and deemed fit for remission induction chemotherapy by "7 + 3" scheme (200 mg of cytarabine/m^2 on days 1–7, 60 mg of daunorubicin/m^2 on days 1–3) or "5 + 2" scheme (200 mg of cytarabine/m^2 on days 1–5, 60 mg of daunorubicin/m^2 on days 1–2). Midostaurin (50 mg b.i.d./p.o.) was administered from day +8 to day +21. Antifungal prophylaxis was started on day 1 of chemotherapy and the choice of antifungals was left at the investigator's discretion. A decision to prescribe antifungals during subsequent consolidation therapy was also left at the investigator's discretion. The study was coordinated by the Hematology Unit of Azienda Socio-Sanitaria Territoriale-Spedali Civili di Brescia, and was approved by Institutional Review Boards of all participating centers. Signed informed consent was obtained from all patients.

Study objectives

This study had observational and interventional objectives. PCZ prevalence in antifungal prophylaxis during induction chemotherapy was the primary observational objective, whereas hematologic outcomes (complete remission [CR] and duration of severe neutropenia) and incidence of midostaurin-related adverse events (AEs) were secondary observational objectives. Midostaurin and PCZ plasma levels during induction chemotherapy were the interventional objectives. Hematologists were blinded to PK findings until study termination; therefore, clinical decisions, such as midostaurin withdrawal and/or dose adjustments solely reflected an investigator's assessment of patient clinical conditions. AEs were evaluated by local investigators and judged as probably or possibly midostaurin-related. AE grading was in accordance with Common Toxicity Criteria from the National Cancer Institute, version 5.0.[8]

Midostaurin and PCZ assays

Blood samples were drawn at day +8 and +21 of induction chemotherapy (first and last day of midostaurin administration), before and 3 and 12 h after midostaurin administration. The 3 h timepoint was chosen in view of the reported 1–3 h time to maximum plasma concentration (C_{max}) of midostaurin.[9] Midostaurin and its metabolites CGP62221 and CGP52421 were measured by a validated liquid chromatography tandem mass spectrometry method with a limit of quantitation of 25 ng/mL.[10] Quantification was obtained against seven-point calibration curves (from 0 to 2500 ng/mL), built with authentic standards. Intra-day accuracy and precision were checked by using three levels of freshly prepared quality controls (QClow 75 ng/mL, QCmedium 750 ng/mL, and QChigh 1500 ng/mL). Plasma samples with midostaurin levels greater than 2500 ng/mL were diluted as appropriate and retested. Where indicated, midostaurin was measured also in a subgroup of 12 patients undergoing consolidation therapy with high dose cytarabine (days 1-3-5) and midostaurin (from day +8 to day +21 at the same dose as that of induction therapy).

PCZ was quantified by CE-IVD diagnostic KIT (Eureka Lab Division s.r.l.), using a five-point calibration curve (from 0.1 to 1.8 μg/mL). For intra-day accuracy and precision two freshly prepared PCZ quality controls were used (QClow 0.7 μg/mL and QChigh 1500 μg/mL).

Other details

We estimated to recruit 40 patients, which was based on the prespecified 18 months duration of the study and the incidence of FLT3-*mut* AML cases in the participating centers (one case in approximately every 4 months at each participating center). Moreover, the prospected sample size was comparable to that adopted to quantify significant PK differences among patient cohorts in midostaurin dose escalation studies.[11]

One sample proportion test[12] with null hypothesis at 20% was used to evaluate whether there was a significant deviation from guideline-recommended PCZ in favor of other antifungals. CR after induction chemotherapy was defined as less than or equal to 5% blasts in the bone marrow, recovery of neutrophils and platelets, and absence of extramedullary disease on days 28–31 after chemotherapy initiation.[13] Categorical variables were analyzed by two-tailed Fisher's exact test. Unless otherwise indicated, continuous variables were expressed as geometric means with 95% confidence intervals (CIs). Differences between means were analyzed by two-tailed paired or unpaired *t*-test as appropriate, with statistically significant difference being set at $p < 0.05$. All analyses were carried out by Prism 5, version 5.01 (GraphPad Software Inc.).

RESULTS

Patient characteristics and treatments

This study recruited 37 patients but two of them died early during induction chemotherapy from IFI-unrelated causes and were excluded from analyses. There were therefore 35 evaluable patients, corresponding to 88% of the target sample size of 40 patients. Demographic and clinical characteristics of the evaluable patients are reported in Table 1. Median age was greater than 60 years, consistent with the reported age of patients at risk for FLT3-*mut* AML.[6] FLT3 ITD mutation was largely prevalent over TKD mutation, and concomitant NPM1 mutation was detected in half of the patients. All such figures were consistent with the pathobiology of FLT3-*mut* AML.[6] Thirty-four patients received the "7 + 3" induction chemotherapy scheme, with just one patient receiving the "5 + 2" scheme.

All patients were treated with midostaurin from days +8 to +21 of induction therapy. All patients also received primary antifungal prophylaxis; interestingly, however, 19 patients received PCZ (loading dose of 300 mg b.i.d./p.o., followed by 300 mg/day), whereas 16 received the echinocandin agent, micafungin (50 mg/day i.v.).

TABLE 1 Demographic and clinical characteristics of 35 patients with FLT3-*mut* AML.

Clinical and biological features	Patients ($n = 35$)
Males	16
Females	19
Age (median, range)	62 (35–73)
FLT3 mutation	
ITD	31 (89%)
TKD	4 (11%)
NPM1 mutation	18 (51%)
Induction regimen	
"7 + 3"	34 (97%)
"5 + 2"	1 (3%)
Midostaurin	35 (100%)
Primary antifungal prophylaxis	
PCZ	19 (54%)
Micafungin	16 (46%)

Note: "7 + 3", 200 mg of cytarabine/m^2 on days 1–7, 60 mg of daunorubicin/m^2 on days 1–3; "5 + 2", 200 mg of cytarabine/m^2 on days 1–5, 60 mg of daunorubicin/m^2 days 1–2.

Abbreviations: AML, acute myeloid leukemia; ITD, internal tandem duplication; NPM1, nucleophosmin 1 mutations; PCZ, posaconazole; TKD, tyrosine kinase domain.

Based on the one sample proportion test, the percentage of patients exposed to micafungin was high enough (46%) to denote a significant deviation from guideline-recommended usage of PCZ ($p < 0.0001$, 95% CI 29–64). Micafungin shows little or no effect on CYP3A4[14]; therefore, a decision to replace PCZ with micafungin clearly reflected the hematologist's concerns around PCZ inhibiting CYP3A4 and altering midostaurin PK. Patients exposed to PCZ or micafungin were balanced with respect to main demographic and clinical characteristics (Table 2).

Midostaurin plasma levels during induction therapy

At day +8 of induction therapy (i.e., 1 week after antifungal prophylaxis and chemotherapy were started), patients exposed to PCZ showed an approximately twofold higher C_{max} than patients exposed to micafungin. More remarkable changes were observed at day +21, a timepoint when C_{min}, C_{max}, and C_{12h} were approximately four to five times higher in patients receiving PCZ compared to micafungin (Figure 1, left panel). PCZ levels always exceeded the plasma level required for effective prophylaxis (0.7 μg/mL)[15]; moreover, there was no PCZ accumulation from day +8 to day +21 (μg/mL: 1.4 (95% CI 1.1–1.9) at

Clinical and biological features	Patients with PCZ ($n=19$)	Patients with micafungin ($n=16$)	p
Males	9	7	1.000
Females	10	9	
Age (median, range)	62 (25–69)	62 (35–73)	0.907
FLT3 mutation			
ITD	17	14	1.000
TKD	2	2	
NPM1 mutation (%)	8 (42)	10 (62)	0.315
Induction regimen			
"7+3"	18	16	1.000
"5+2"	1	0	

TABLE 2 Demographic and clinical characteristics of 35 patients with FLT3-*mut* AML exposed to PCZ or micafungin.

Note: "7+3", 200 mg of cytarabine/m^2 on days 1–7, 60 mg of daunorubicin/m^2 on days 1–3; "5+2", 200 mg of cytarabine/m^2 on days 1–5, 60 mg of daunorubicin/m^2 days 1–2;

Abbreviations: AML, acute myeloid leukemia; ITD, internal tandem duplication; NPM1, nucleophosmin 1 mutations; PCZ, posaconazole; TKD, tyrosine kinase domain.

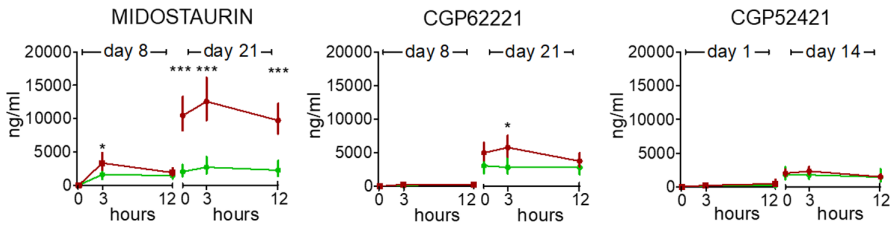

FIGURE 1 Plasma levels of midostaurin and its metabolites at days +8 and +21 of induction therapy. The left panel shows that in comparison with micafungin, PCZ caused higher midostaurin C_{max} at day +8 of induction therapy (*$p<0.05$) and higher C_{min}, C_{max}, and C_{12h} at day +21 (***$p<0.0001$). The central panel shows that PCZ increased the C_{max} of CGP62221 at day +21 ($p<0.05$). The right panel shows that PCZ lacked effects on CGP52421 levels at either day +8 or day +21. All values were geometric means with 95% CI. PCZ, red lines; micafungin, green lines. CI, confidence interval; C_{max}, maximum plasma concentration; C_{min}, minimum plasma concentration; PCZ, posaconazole.

days +8 and 1.2 (95% CI 0.9–1.6) at day +21 ($p=0.133$). Midostaurin accumulation at day +21 therefore reflected continued strong inhibition of CYP3A4 by optimal steady-state plasma levels of PCZ.

Midostaurin metabolites CGP62221 (O-desmethyl-midostaurin) and CGP52421 ((S)-3-hydroxy-midostaurin) were also measured. Both metabolites increased from day +8 to day +21. Compared to micafungin, PCZ caused further augmentation of CGP62221 C_{max} at day +21 but did not cause any effect on CGP52421 at either day +8 or day +21 (Figure 1, central and right panels, respectively). The observation that midostaurin accumulation was accompanied by increased levels of CGP62221 and CGP52421, with PCZ further increasing the C_{max} of CGP62221 at day +21, had to be reconciled with an anticipated effect of PCZ on increasing midostaurin while also decreasing metabolites. We surmised that concomitant elevations of midostaurin and its metabolites could be due to multiple factors, such as competition between high midostaurin levels and PCZ for CYP3A4, midostaurin auto-induction of CYP3A4, and effects of CGP62221 and CGP52421 on inducing/inhibiting CYP3A4.[10,16] To obtain more evidence that PCZ increased midostaurin levels by inhibiting its metabolization through CYP3A4, net levels of each metabolite were normalized to total midostaurin availability, expressed as the sum of unmodified midostaurin with CGP62221 and CGP52421.[10] This gave a fractional conversion that, by normalizing metabolite formation to substrate availability, could more accurately reflect the efficiency with which midostaurin was converted to one metabolite or the other. Patients exposed to PCZ or micafungin showed comparable fractional conversion to CGP62221 or CGP52421 at day +8 but not at day +21, a timepoint when patients receiving PCZ showed significantly lower fractional conversion to both CGP62221 and CGP52421 (Figure 2).

Role of PCZ

We could not exclude that midostaurin accumulation was at least in part contributed by an under-reported co-administration of other drugs that inhibit CYP3A4. To

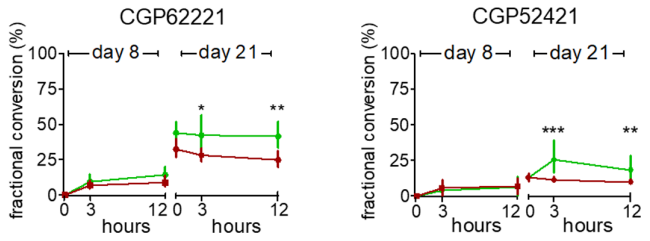

FIGURE 2 Fractional conversion of midostaurin to CGP62221 and CGP52421 at days +8 and +21 of induction therapy. Net levels of CGP62221 or CGP52421 were normalized to total midostaurin availability to obtain midostaurin fractional conversions to each metabolite, as described in Results. At day +21, PCZ reduced midostaurin fractional conversion to CGP62221 (left panel, $*p<0.05$ at 3 h and $**p<0.01$ at 12 h), and CGP52421 (right panel, $***p<0.001$ at 3 h and $**p<0.01$ at 12 h). All values were geometric means with 95% CI. PCZ, red lines; micafungin, green lines. CI, confidence interval; PCZ, posaconazole.

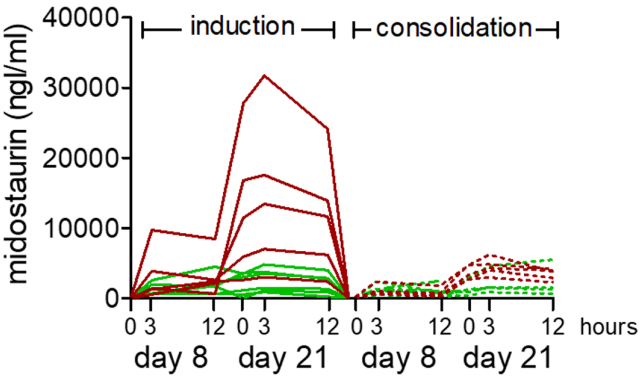

FIGURE 3 Midostaurin plasma levels during induction therapy with antifungal prophylaxis and consolidation therapy without antifungal prophylaxis. A total of 12 patients were monitored for midostaurin levels during induction therapy and antifungal prophylaxis with micafungin ($n=7$, green lines) or PCZ ($n=5$, red lines). At day +21, PCZ caused higher midostaurin levels compared to micafungin. Antifungals were discontinued and consolidation therapy was started after greater than or equal to 4 weeks. At day +21 of consolidation therapy, patients with prior PCZ (red dotted lines) did not develop midostaurin accumulation compared to patients with prior micafungin (green dotted lines). PCZ, posaconazole.

confirm or exclude this potential bias we took advantage of data from five patients who received PCZ during induction therapy and then underwent consolidation therapy without PCZ. These patients were compared to seven patients who received micafungin during induction but not consolidation therapy. Because antifungals were discontinued on day +21 of induction therapy, and consolidation therapy was started greater than or equal to 4 weeks later, midostaurin could be measured when PCZ was no longer measurable in plasma ("PCZ washout"). As shown in Figure 3, patients who received PCZ and developed midostaurin accumulation at day +21 of induction therapy did not show significant midostaurin accumulation at day +21 of consolidation (mean C_{max} values with PCZ and micafungin [ng/mL]: 10972 [95% CI 3585–33583] vs. 1994 [95% CI 1069–3718] at day +21 of induction therapy, $p=0.018$, and 4326 [95% CI 3081–6075] vs. 1800 [95% CI 953–3399] at day +21 of consolidation, $p=0.073$). For the five patients who received PCZ during induction therapy, we also observed that mean PCZ washout (0.9 µg/mL at day +21 of induction therapy vs. not detectable at day 1 of consolidation therapy) correlated significantly with Δ midostaurin from day +21 of induction therapy to day +21 of consolidation therapy ($p=0.039$ for Δ C_{min}, $p=0.031$ for Δ C_{max}, $p=0.018$ for Δ C_{12h}). These results showed that PCZ was the main perpetrator of midostaurin accumulation.

PCZ-midostaurin interactions and clinical outcomes

Midostaurin accumulation during induction therapy, expressed as day +21/day +8 ratios for C_{max} and area under the curve (AUC), averaged 4.1 and 6.5 in patients exposed to PCZ but only 1.2–1.6 in patients exposed to micafungin.

In spite of such remarkable differences, the two groups were similar with respect to hematologic outcomes, such as CR and duration of severe neutropenia (Table 3).

As far as safety outcomes were concerned, there was no difference between PCZ and micafungin groups in terms of any grade AE. There were four grade greater than or equal to 3 AEs in the PCZ group compared to one in the micafungin group but the difference was not statistically significant within the sample size of this study. In the PCZ group, grade greater than or equal to 3 AEs were QTc prolongation, hyperbilirubinemia, skin rash, and gastrointestinal toxicity, all attributed to midostaurin. In the micafungin group, the grade greater than or equal to 3 AE was QTc prolongation, also attributed to midostaurin. Patients receiving PCZ or micafungin were also similar for IFIs incidence and midostaurin withdrawal or dose reductions (see also Table 3).

DISCUSSION

The dilemma of midostaurin and PCZ

In a multicenter phase III, double blind, randomized clinical trial, PCZ was more effective than other triazolic antifungals (fluconazole and itraconazole) in preventing IFIs during remission induction chemotherapy for AML.[17] PCZ is therefore recommended for primary antifungal prophylaxis in this special population (grade A-I evidence).[4] Clinical use of PCZ is nonetheless complicated by AEs, such as liver toxicity, gastrointestinal intolerance,

Event	Patients with PCZ	Patients with micafungin	*p*
Midostaurin accumulation[a]			
C_{max}	4.1 (2.9–5.8)	1.2 (0.7–1.9)	<0.001
AUC	6.5 (4.3–9.8)	1.6 (1.2–2.1)	<0.0001
CR	16 (84%)	13 (81%)	1.000
Duration of ANC <500 mmc (days, median with ranges)	25 (15–43)	25 (17–40)	0.974
Any grade adverse events	4 (21%)	4 (25%)	1.000
Grade ≥3 adverse events	4 (21%)	1 (6%)	0.347
Midostaurin withdrawal	2 (11%)	2 (13%)	1.000
Midostaurin dose reduction	1	2	1.000
IFIs	1 (5%)	2 (17%)	0.582

Abbreviations: ANC, absolute neutrophil count; AUC, area under the curve; C_{max}, maximum plasma concentration; CR, complete remission; IFIs, invasive fungal infections; PCZ, posaconazole.

[a]Geometric means with 95% confidence interval.

TABLE 3 Midostaurin accumulation versus clinical outcomes in patients receiving PCZ or micafungin during induction chemotherapy.

and risk of prolonged cardiac repolarization (long QTc), especially when patients present at treatment with co-existing risk factors or PCZ engages in PK interactions with CYP3A4 substrates that prolong QTc.[18] Micafungin shows a better safety profile than PCZ and was effective in preventing IFIs in at-risk hematologic patients; however, the evidence derived from small single-center retrospective or observational studies,[19,20] or prospective single arm interventional studies.[21] Guidelines do not therefore recommend micafungin for primary antifungal prophylaxis in patients with AML (grade C-II evidence).[4]

The midostaurin-PCZ dilemma made this scenario even more complex. Similar to PCZ, midostaurin can cause liver and gastrointestinal toxicity; moreover, midostaurin is known to introduce a significant risk of QTc prolongation.[22] To avoid PCZ-induced midostaurin overexposure, and summation of toxicities from the two drugs, the hematologist might consider declining PCZ in favor of an antifungal, like micafungin, that is not formally recommended in these settings. Here, we have shown that in real-life clinical practice there is in fact a highly significant deviation from guidelines, with many hematologists using micafungin in place of PCZ. To the best of our knowledge, this is the first multicenter prospective study to demonstrate how significant this deviation can be when antifungals are prescribed according to the clinician's decision rather than protocol-guided patient randomization to one treatment or the other.

Magnitude and metabolic consequences of PCZ-midostaurin interactions

Our results show that the magnitude of PCZ-midostaurin interactions is greater than previously reported. In earlier studies serial measurements of midostaurin C_{min} showed that PCZ caused midostaurin to accumulate by a factor of 1.44.[7] In our study, the ratio of C_{min} with PCZ to C_{min} with micafungin at day +21 of induction therapy was as high as 5.1 (95% CI 4–6.6). Differences between our study and previous reports warrant considerations. PK analyses of patients recruited in the RATIFY trial were confounded by co-administration of a number of CYP3A4 substrates/inhibitors but details on the schedule and steady-state levels of confounders at the time of midostaurin measurements were not available. Competition between confounders and PCZ for CYP3A4 could not therefore be excluded, precluding firm conclusions on the magnitude of PCZ-midostaurin interactions in isolation. Moreover, determinations of midostaurin levels were not accompanied by concomitant measurements of PCZ levels.[7] We considered that also in our study there could have been an under-reported co-administration of confounders but we obtained data that helped to elucidate the role of PCZ as the main perpetrator of midostaurin accumulation. In fact, there was no significant midostaurin accumulation in patients receiving consolidation therapy without antifungal prophylaxis, and decreases of midostaurin C_{min}, C_{max}, and C_{12h} during consolidation therapy correlated with the amount of PCZ that was cleared from plasma over the time interval between induction and consolidation therapy.

There are further differences between previous studies and our present investigation. In PK analyses of the RATIFY trial, there was no significant effect of PCZ or other CYP3A4 inhibitors on midostaurin metabolites, CGP62221 and CGP52421.[7] Here, we confirmed that PCZ did not reduce the circulating levels of CGP62221

and CGP52421. This would appear as a counterintuitive finding if one considers that CYP3A4 catalyzes the two independent pathways of midostaurin O-demethylation or hydroxylation to CGP62221 and CGP52421, respectively.[23] However, significantly impaired metabolite formation at day +21 of induction therapy was characterized in terms of fractional conversions, a metabolic index that normalized metabolite formation to the increased levels of midostaurin. Of note, fractional conversions decreased regardless of well-known differences in the half-life of CGP62221 and CGP52421 (33.4 vs. 495 h, respectively).[9]

PCZ, midostaurin overexposure and clinical outcomes

Continued inhibition of CYP3A4, and consequent midostaurin accumulation both before and after daily dosing, were evidenced by high day +21/day +8 ratios for midostaurin C_{max} and AUC in patients exposed to PCZ but not in patients exposed to micafungin. Hematologic outcomes, such as CR and duration of severe neutropenia, were nonetheless similar in the two patient groups as also was the apparent incidence of IFIs.

In the midostaurin-PCZ group, both skin rash and gastrointestinal and liver toxicity (hyperbilirubinemia) were well consistent with midostaurin AEs reported in regulatory documents[24] and clinical trials[6,7] and were therefore attributed to midostaurin; however, an overlap of gastrointestinal and liver toxicities from both midostaurin and PCZ cannot be excluded a priori. On a different note, grade greater than or equal to 3 QT_C prolongation was equally represented in PCZ and micafungin groups. It thus seems that toxicity did not increase significantly when PCZ made midostaurin C_{max} and AUC increase four- or six-fold, respectively. It is worth noting that in phase I studies of single agent midostaurin, the majority of grade 3 AEs occurred when doses were escalated from the currently approved regimen of 50 mg b.i.d. to greater than 200 t.i.d., with midostaurin C_{max} and AUC consistently increasing greater than six-fold or greater than 10-fold, respectively.[25] These facts show that the therapeutic window of midostaurin is wide enough to tolerate PK interferences from PCZ.

Study limitations and strengths

AML is a rare disease, accounting for 25%–30% of all leukemia cases in Italy.[26] FLT3 mutation occurs in no greater than 30% of all AML cases.[27] The sample size of our study therefore reflected rates of diagnosis and treatment of a rare disease in Italian hematology centers. The sample size was robust enough to characterize PCZ-midostaurin interactions in detail but probably remained too small to firmly conclude that PCZ and micafungin caused a comparable risk of midostaurin-related AEs. This having been acknowledged, we note that clinical outcomes in PCZ and micafungin groups provided a small-scale replication of what the RATIFY trial demonstrated in large cohorts of midostaurin-treated patients. In that trial, PCZ and other CYP3A4 inhibitors only caused a shorter time to AEs and a trend toward more grade greater than or equal to 3 AEs,[6,7] which were manageable in the majority of cases. It is in keeping with this picture that overall indexes of midostaurin tolerability and manageability, such as midostaurin withdrawal or dose reductions, were similar in PCZ-midostaurin and micafungin-midostaurin groups described in our study. We therefore suggest that PCZ-midostaurin interactions, as strong as they may be, would not per se justify the hematologist's decision to replace PCZ with micafungin.

CONCLUSIONS AND PERSPECTIVES

There is an unmet need for prophylaxis regimens that relieve the hematologist from concerns around PK interactions with midostaurin. Here, medical needs intersect with research issues. An open label, parallel groups study of PCZ or micafungin prophylaxis, started at the end of intensive induction chemotherapy, showed that prophylaxis failures occurred more often with PCZ than micafungin; however, PCZ failures were mostly due to PCZ-related AEs, whereas micafungin failures were due to breakthrough IFIs.[28] We have shown that IFI incidence was similar in PCZ and micafungin groups, which in principle should reassure about the appropriateness of replacing PCZ with micafungin; however, absent robust studies in the settings of primary prophylaxis, these findings need to be considered with due caution. A phase II single arm study probed isavuconazole,[29] a broad spectrum triazole that causes only mild–moderate inhibition of CYP3A4[30] and shows a good safety profile in terms of QTc prolongation and other AEs commonly associated with triazoles.[31] In the latter study, which also included patients with FLT3-*mut* AML, breakthrough IFIs were slightly more prevalent compared to historical cohorts of patients exposed to PCZ.[29]

It goes without saying that none of the available studies can advance micafungin or isavuconazole to frontline drugs for IFIs prevention in patients with AML. Phase III randomized clinical trials, whether promoted by scientific societies or drug companies, remain the only setting in which an antifungal can be reliably compared to PCZ in terms of efficacy and safety.

At this point in time, concerns around PCZ-midostaurin interactions should not be taken to replace PCZ with another antifungal, unless patient-related risk factors pose an absolute contraindication to PCZ.

AUTHOR CONTRIBUTIONS

P.M., F.M., C.C., A.C., M.D.L., G.N., A.V., C.P., S.P., L.V., D.A., M.D.P., N.F., E.S., S.L., I.T., B.A., G.M., and L.P. wrote the manuscript. P.M., F.M., C.C., and G.M. designed the research. P.M., F.M., C.C., A.C., M.D.L., G.N., A.V., C.P., S.P., L.V., D.A., M.D.P., N.F., E.S., S.L., I.T., B.A., G.M., and L.P. performed the research. P.M., F.M., C.C., A.C., M.D.L., G.N., A.V., C.P., S.P., L.V., D.A., M.D.P., N.F., E.S., S.L., I.T., B.A., G.M., and L.P. analyzed the data. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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