REGULAR ARTICLE

blood advances

A phase 1 trial evaluating thioridazine in combination with cytarabine in patients with acute myeloid leukemia

Lili Aslostovar,[1,2,*] Allison L. Boyd,[1,*] Mohammed Almakadi,[1-3,*] Tony J. Collins,[1] Darryl P. Leong,[5] Rommel G. Tirona,[6] Richard B. Kim,[6] Jim A. Julian,[7] Anargyros Xenocostas,[8] Brian Leber,[9] Mark N. Levine,[7] Ronan Foley,[4] and Mickie Bhatia[1,2]

[1]Stem Cell and Cancer Research Institute and [2]Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, ON, Canada; [3]Division of Malignant Hematology, Department of Oncology, Juravinski Hospital, Hamilton, ON, Canada; [4]Department of Pathology and Molecular Medicine, McMaster University, Juravinski Hospital, Hamilton, ON, Canada; [5]Division of Cardiology, Department of Medicine, Population Health Research Institute, McMaster University, Hamilton, ON, Canada; [6]Division of Clinical Pharmacology, Department of Medicine, University of Western Ontario, London Health Sciences Centre, London, ON, Canada; [7]Department of Oncology, McMaster University, Juravinski Hospital, Hamilton, ON, Canada; [8]Division of Hematology, Department of Medicine, University of Western Ontario, London Health Sciences Centre, London, ON, Canada; and [9]Department of Medicine, McMaster University, Juravinski Hospital, Hamilton, ON, Canada

Key Points

- Intermediate-dose cytarabine can be safely combined with TDZ at 50 mg every 6 hours in older patients with relapsed or refractory AML.
- A 5-day monotherapy with TDZ led to reduced blast counts in 5 out of 11 patients and was associated with patient-specific DRD2 level.

We completed a phase 1 dose-escalation trial to evaluate the safety of a dopamine receptor D2 (DRD2) antagonist thioridazine (TDZ), in combination with cytarabine. Thirteen patients 55 years and older with relapsed or refractory acute myeloid leukemia (AML) were enrolled. Oral TDZ was administered at 3 dose levels: 25 mg (n = 6), 50 mg (n = 4), or 100 mg (n = 3) every 6 hours for 21 days. Intermediate-dose cytarabine was administered on days 6 to 10. Dose-limiting toxicities (DLTs) included grade 3 QTc interval prolongation in 1 patient at 25 mg TDZ and neurological events in 2 patients at 100 mg TDZ (gait disturbance, depressed consciousness, and dizziness). At the 50-mg TDZ dose, the sum of circulating DRD2 antagonist levels approached a concentration of 10 μM, a level noted to be selectively active against human AML in vitro. Eleven of 13 patients completed a 5-day lead-in with TDZ, of which 6 received TDZ with hydroxyurea and 5 received TDZ alone. During this period, 8 patients demonstrated a 19% to 55% reduction in blast levels, whereas 3 patients displayed progressive disease. The extent of blast reduction during this 5-day interval was associated with the expression of the putative TDZ target receptor DRD2 on leukemic cells. These preliminary results suggest that DRD2 represents a potential therapeutic target for AML disease. Future studies are required to corroborate these observations, including the use of modified DRD2 antagonists with improved tolerability in AML patients. This trial was registered at www.clinicaltrials.gov as #NCT02096289.

Introduction

Therapeutic options for older patients with acute myeloid leukemia (AML) remain limited because intensified chemotherapies do not improve survival and are poorly tolerated due to age-related comorbidities.[1-3] Furthermore, older patients with relapsed or refractory disease have even fewer effective treatment options, resulting in complete remission rates of 16% to 21% and median survival of 6 to 9 months.[3-5] These poor outcomes highlight the need to evaluate novel therapeutic strategies in older AML patients, especially those with relapsed or refractory disease.

In 2012, thioridazine (TDZ), a dopamine receptor D2 (DRD2) antagonist, was identified to harbor potent antileukemic properties in a large-scale chemical screen investigating drugs that alter

Submitted 2 January 2018; accepted 4 July 2018. DOI 10.1182/bloodadvances.2018015677.

*L.A., A.L.B., and M.A. contributed equally to this study.

The full-text version of this article contains a data supplement.

cancer stem cell behavior.[6] Preclinical studies with TDZ led to a marked suppression of leukemic clonogenicity and leukemia initiation capacity.[6] Moreover, in vitro combination of TDZ with the standard chemotherapy agent cytarabine resulted in an even more dramatic suppression of leukemic clonogenicity compared with TDZ alone, highlighting a potentially synergistic effect between TDZ and cytarabine.[6] Importantly, healthy primitive cell output was not affected after exposure to TDZ at optimal concentrations in vitro, indicating TDZ's selectivity to suppress leukemic vs healthy hematopoietic functions.[6]

Based on the prescribed dosage and recognized potential for adverse effects of TDZ when used as an antipsychotic,[7-10] we designed a phase 1 clinical trial to evaluate the safety of oral TDZ at 25 to 100 mg every 6 hours alone, and in combination with intermediate-dose cytarabine (1 g/m^2)[11] in patients 55 years and older with relapsed or refractory AML or advanced stage myelodysplastic syndrome. In addition to the primary outcome measure of safety, we also investigated circulating DRD2 antagonist levels in relation to TDZ dose. Finally, we report preliminary observations that suggest an association between leukemic blast reduction and the level of DRD2 expression in AML patient cells during a window of TDZ monotherapy.

Methods

Patient population

Study patients were 55 years of age or older with relapsed or refractory AML or high-risk myelodysplastic syndrome who progressed to AML after hypomethylator therapy. Relapsed AML eligibility was defined by the presence of ≥5% leukemic marrow blasts after 3 months from receiving up to 3 prior induction regimens. Refractory disease was defined by the presence of ≥5% leukemic marrow blasts in patients failing to achieve a complete remission (CR) or complete remission with incomplete count recovery (CRi) following not >1 prior induction regimen. Exclusion criteria included: (i) concomitant use of any other standard or other investigational anti-leukemic therapies; (ii) >3 prior lines of chemotherapy for AML; (iii) chemotherapy within the previous 4 weeks, except for hydroxyurea; (iv) impaired renal function (estimated glomerular filtration rate <60 mL/min/1.73 m^2) or abnormal liver function (serum bilirubin >1.5× upper limit of normal; aspartate transaminase, alanine transaminase, and alkaline phosphatase >2.5× upper limit of normal); (v) acute or chronic graft versus host disease; or (vi) on-going uncontrolled systemic infection. Other specific exclusion criteria included a left ventricular ejection fraction <45%, uncontrolled cardiac arrhythmias, diagnosis of prolonged QTc interval at ≥470 ms for men and ≥480 ms for women, or presence of conditions or medications that prolong the QT interval, known severe hypotensive or hypertensive heart disease.

All patients provided written informed consent, and the trial protocol was approved by the institutional research ethics board and Health Canada. The trial was conducted at a single institution, Hamilton Health Sciences/Juravinski Hospital and Cancer Centre in Hamilton Ontario. Patients were registered centrally by the Ontario Clinical Oncology Group in Hamilton Ontario, who was responsible for data management and adverse event reporting.

Study design and treatment schema

Trial design was a traditional 3+3 dose escalation phase 1 study[12] with 3 dose levels of oral TDZ: 25 mg every 6 hours (dose level/cohort I), 50 mg every 6 hours (dose level/cohort II), and 100 mg every 6 hours (dose level/cohort III). TDZ was administered for 21 consecutive days spanning days 1 to 22, and cytarabine was administered at an intermediate dose (1 g/m^2)[11] for 5 consecutive days, on days 6 to 10 of the trial. Hydroxyurea was considered optional and was permitted in the first 5 days of the study, prior to the planned administration of cytarabine (Figure 1A).

Toxicity evaluation

Toxicity criteria were assessed according to NCI-CTCAE version 4.03. Dose-limiting toxicity (DLT) was defined as the presence of any toxicity that was possibly, probably, or definitely related to TDZ, including ≥grade 2 neurosensory toxicity-vision, ≥grade 3 gastrointestinal toxicity, ≥grade 3 neurosensory toxicity, ≥grade 2 renal toxicity, ≥grade 2 hepatic toxicity, ≥grade 2 pulmonary toxicity, ≥grade 2 neurological toxicity (other than neurosensory toxicity), and ≥grade 2 cardiac toxicity. A 12-lead electrocardiogram (ECG) was performed daily. QTc prolongation to >500 ms on at least 2 separate recordings was considered a DLT (grade 3 as per the NCI-CTCAE version 4.03). A second ECG was completed within 1 hour from the initial ECG showing QTc prolongation. If a patient experienced a DLT, the study drug was permanently discontinued. Drugs known to prolong the QTc, including specific anti-emetics and anti-fungal agents, were not permitted.

Response evaluation

Bone marrow (20 mL) and peripheral blood (50 mL) specimens were collected prior to the start of TDZ treatment (day 1), after treatment with TDZ as a monotherapy and prior to the start of cytarabine (day 5), on the last day of TDZ treatment (day 22) and on the last day of the trial (day 36), which was 14 days after the last dose of TDZ and 26 days after the last cytarabine dose. Previously defined criteria for complete remission, complete remission with incomplete count recovery, partial remission, treatment failure, or not evaluable were used to evaluate leukemic response.[3]

Pharmacokinetic analysis

Plasma trough concentration of TDZ and its 4 metabolites,[13] including TDZ 2-sulfoxide (mesoridazine), thioridazine 2-sulfone (sulforidazine), thioridazine 5-sulfoxide, and *N*-desmethyl-thioridazine (northioridazine), were measured in samples collected on days 1 (prior to the first dose and ~5 hours after the first dose of TDZ) as well as days 5, 8, 10, 17, 22, 29, and 36. Plasma concentrations of TDZ and metabolites were determined using liquid chromatography-tandem mass spectrometry. Briefly, plasma was spiked with internal standard (d3-thioridazine; Toronto Research Chemicals) and proteins precipitated by addition of acetonitrile. Solutes were separated from the resulting plasma extracts by reverse-phase chromatography (Hypersil Gold C18 column 3 × 50 mm, 5 μm; ThermoFisher Scientific; Agilent 1100 chromatograph) using gradient elution with 0.1% formic acid in water and acetonitrile. Compounds were detected after electrospray ionization and detection in positive mode on a triple quadrupole mass spectrometer (Thermo Vantage) with multiple reaction monitoring (TDZ *m/z* 371.2 → 126.2, d3-TDZ *m/z* 374.2 → 129.2, Northioridazine *m/z* 357.1 → 112.2, mesoridazine and 5-sulfoxide *m/z* 387.2 → 98.1, Sulforidazine *m/z* 403.2 → 98.1). The lower limits of quantitation were 3.5 nmol/L for TDZ, 15 nmol/L

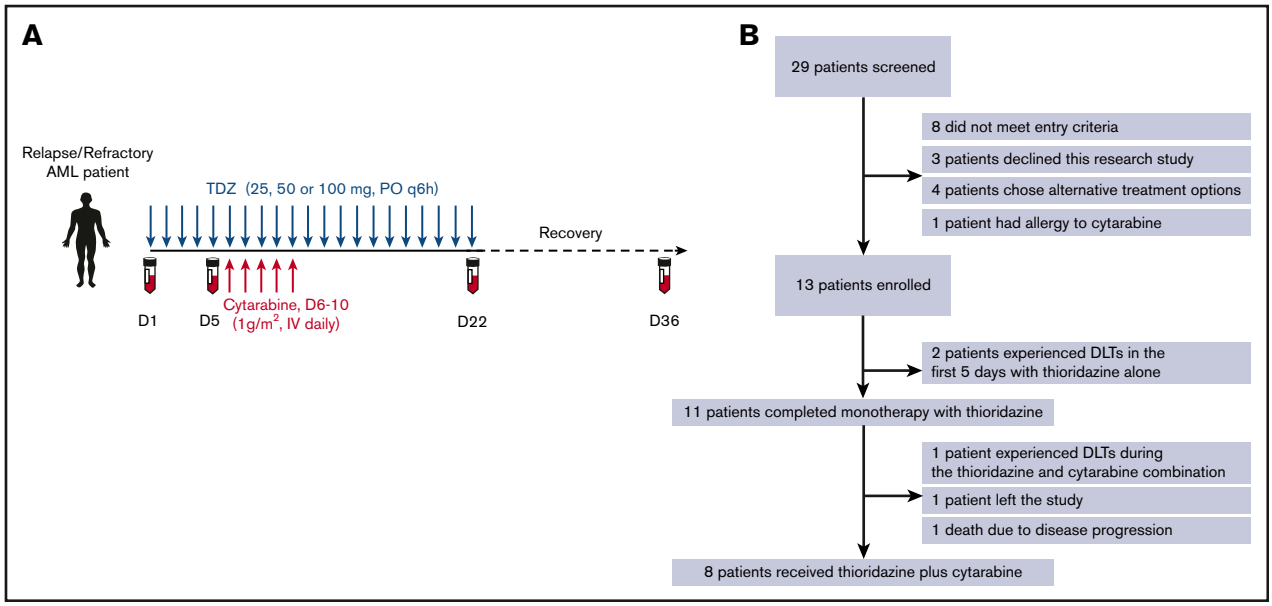

Figure 1. Study design. (A) Patients received TDZ daily at 3 dose levels including 25 mg every 6 hours (dose level I), 50 mg every 6 hours (dose level II), and 100 mg every 6 hours (dose level III) for 21 consecutive days, spanning days 1 to 22 of the study. Intermediate-dose cytarabine at 1 g/m^2 was administered as a 2-hour infusion for 5 consecutive days between days 6 and 10 of the study. (B) Summary of screened and enrolled patients. PO, orally.

for mesoridazine, 7 nmol/L for 5-sulfoxide, 0.2 nmol/L for northioridazine, and 7.5 nmol/L for sulforidazine.

Pharmacogenetic analysis

Genomic DNA was isolated from blood sample preparations with the Gentra Puregene extraction kit (Qiagen) and quantified by Quant-iT PicoGreen double-stranded DNA (Invitrogen). *CYP2D6* genotyping was performed by TaqMan Drug Metabolism Genotyping and Copy Number Assays (Applied Biosystems), using a 7500 Real-time polymerase chain reaction system (Applied Biosystems). The following inactive or reduced function alleles were analyzed: *CYP2D6*4* (g.1846G>A; rs3892097), *CYP2D6*3* (g.2549A>del; rs1057910), *CYP2D6*9* (g.2613_2615delAGA; rs5030656), *CYP2D6*10* (g.100C>T; rs1065852), and *CYP2D6*41* (g.2988G>A; rs28371725). *CYP2D6* (Hs04502391_cn) copy number variation, deletion, or multiplications of the gene were also assessed.

Based on *CYP2D6* genotypes, patients were categorized into the following phenotypes[14]: poor metabolizers if carrying 2 inactive alleles; intermediate metabolizers if carrying 2 decreased function alleles or 1 active/decreased function allele and 1 inactive allele; extensive metabolizers if they were carrying 2 wild-type alleles; or ultrarapid metabolizers if carrying a gene duplication in the absence of inactive or decreased function alleles.

Primary patient sample processing and flow cytometry

Mononuclear cells (MNCs) were prepared from clinical bone marrow and peripheral blood samples at baseline by density gradient centrifugation (Ficoll-Paque Premium, GE Healthcare), followed by red blood cell lysis using ammonium chloride solution (Stemcell Technologies).

Cell surface immunophenotyping for DRD2 was performed using mouse anti-human DRD2 (clone B-10; Santa Cruz Biotech) with donkey anti-mouse immunoglobulin G Alexa-Fluor-647 (Life Technologies) as the secondary antibody. Samples were blocked with donkey serum (Jackson ImmunoResearch Laboratories) plus human FC block (eBioscience). Fluorescence-minus-one controls were used for optimized gating. 7-Aminoactinomycin D (Beckman Coulter) was used for live/dead cell discrimination.

In vitro drug treatment with TDZ

Patient-derived MNCs were exposed to 0.1% dimethyl sulfoxide control or TDZ at doses ranging from 0.69 μM to 40 μM for 24 hours in vitro, followed by analysis of leukemia cell viability by 7-aminoactinomycin D exclusion using a LSRII flow cytometer (BD). Culture conditions included Stemspan medium (Stemcell Technologies) supplemented with 100 ng/mL stem cell factor, 100 ng/mL FMS-related tyrosine kinase 3 ligand, and 20 ng/mL thrombopoietin (all sourced from R&D Systems).

Statistical analysis

Continuous data were described using means and 95% confidence intervals (CI), and dichotomous data were summarized as percentages. Group comparisons were performed using 2-tailed Student *t* test for continuous measures and Fisher's exact test for dichotomous outcomes. Square root transformation was used prior to statistical analysis when data failed to meet parametric requirements and data set included values equal to 0. Prism software (version 5.0a; GraphPad) was used for all statistical analyses. $P \leq .05$ was considered to be statistically significant.

Results

Trial overview

Between July 2014 and September 2016, a total of 29 AML patients were screened. Of these, 13 AML patients aged 55 years or older with relapsed or refractory disease (n = 9) or AML with

myelodysplastic-related changes (AML-MRC; n = 4) met the eligibility criteria and were enrolled (Figure 1B). Baseline patient characteristics are summarized in Table 1. Patient 3 in dose level I experienced grade 3 QTc interval prolongation to 535 ms on day 7. This DLT led to discontinuation of TDZ for this patient, and the patient was removed from the study after completion of cytarabine on day 10. As a result of this DLT, 3 additional patients were enrolled in dose level I according to the study protocol.[12] At dose level II, patient 8 left the study on day 9 on personal request. For patient 11 in dose level III, the dose of TDZ was temporarily deescalated from 100 mg every 6 hours to 50 mg every 6 hours on day 10 after this patient experienced grade 2 QTc prolongation events. This patient later developed severe sepsis on day 16, which was considered unrelated to TDZ but led to permanent discontinuation of TDZ because the patient required intensive care. Patient 12 at dose level III experienced a grade 3 depressed level of consciousness on day 3. Patient 13 at dose level III suffered from grade 2 dizziness and gait disturbance on day 3. These neurological symptoms were considered DLTs and subsequently defined the maximum tolerated dose of 50 mg TDZ every 6 hours in this patient population. Thus, in total, 2 patients (15%) did not complete the study; 3 participants (23%) developed DLTs, and 8 patients (61%) received the treatment regimen that included TDZ treatment in combination with intermediate-dose cytarabine (Figure 1B). Among these 8, patient 11 received cytarabine on days 6 to 10 and TDZ up to day 16. The remaining 7 patients received the full course of TDZ therapy between days 1 to 22 in addition to cytarabine on days 6 to 10.

Table 1. Patient characteristics at baseline

	Dose level I (n = 6)	Dose level II (n = 4)	Dose level III (n = 3)
Median age (range), y	66 (58-75)	71.5 (67-79)	71 (58-75)
Sex, male:female	4:2	1:3	2:1
ECOG performance, n			
0	2	0	2
1	4	4	1
2	0	0	0
Diagnosis, n			
Relapsed/refractory AML	4	3	2
AML-MRC	2	1	1
Cytogenetics risk group, n			
Favorable	0	0	0
intermediate	4	2	2
Adverse/high	2	2	1
No. of prior chemotherapy regimens			
≤3	5	1	0
≥4	1	3	2
NA	0	0	1
% BM blast at baseline, mean ± SEM	60.3 ± 10.0	71 ± 10.6	50 ± 14.9
ABC × 10^9/L at baseline, mean ± SEM	9.2 ± 3.9	29.4 ± 26.7	4.0 ± 3.9

ABC, absolute blast count; BM, bone marrow; ECOG, Eastern Cooperative Oncology Group; SEM, standard error of the mean.

Safety profile

Major toxicities are summarized in Table 2. Consistent with previous reports, QTc prolongation was the most frequent adverse event.[7,10,15-17] As would be predicted for TDZ effects from previous clinical studies,[18] more frequent QTc prolongation events were noted with TDZ dose escalation. Although limited to a small number of patients, the most dramatic episodes of QTc elongation were observed in patients with the highest QTc interval at baseline. Specifically, patient 3 had a grade 3 QTc elongation and patient 9 experienced the highest frequency of grade 2 QTc prolongations. Both patients exhibited baseline QTc levels that exceeded 440 ms,[19] greater than all other trial patients (Table 3). From a baseline QTc of 445 ms, patient 3 developed severe (grade 3) QTc prolongation to 553 ms on day 7 of TDZ treatment, coinciding with the second day of cytarabine treatment. This was considered a DLT, leading to permanent discontinuation of TDZ for this patient. However, it is noteworthy that following TDZ discontinuation, patient 3 continued to show significant QTc prolongation (supplemental Figure 1). Moreover, after plasma concentrations of TDZ and TDZ 5-sulfoxide (the metabolite associated with cardiotoxicity[19-22]) were reduced to undetectable levels, frequent QTc prolongation events continued to be noted on serial ECGs (supplemental Figure 1). These QTc changes in patient 3 were not associated with fluctuations in magnesium or potassium levels. Other instances of QTc prolongation included grade 2 QTc prolongations (between 481 and 500 ms, NCI-CTCAE version 4.03) in patients 7 and 9 in dose level II, as well as patient 11 in dose level III. No other cardiac-related DLTs were reported.

In addition to cardiac adverse events, neurological DLTs were noted in 2 patients at dose level III (100 mg every 6 hours). Patient 12 experienced grade 3 depressed level of consciousness and patient 13 experienced grade 2 dizziness and gait disturbance (Table 2). Other nonhematologic grade ≥2 adverse events that were not dose-limiting included fatigue (n = 1) and urinary incontinence (n = 1) (Table 2). Frequent grade 3 or greater adverse events predominantly included cytarabine-related myelosuppression and associated complications. These adverse events were observed across all 3 cohorts, independent of the dose of TDZ, and included anemia, reduced platelet, neutrophil, and leukocyte counts. Patients were managed with standardized transfusions and administration of antimicrobial agents according to the institutional standard of care. There were 4 episodes of grade ≥3 sepsis in patients 8 and 11 to 13. All 4 cases of sepsis were considered unrelated to TDZ. There was 1 on-study death (patient 12), which was associated with disease progression. Based on the dose-related neurological DLTs observed in dose level III, we concluded that oral TDZ at 50 mg every 6 hours in combination with intermediate-dose cytarabine was safe and feasible in older patients with relapsed or refractory AML.

Pharmacokinetics and pharmacogenetics

Pharmacokinetic analyses were performed to determine whether a safe dose of TDZ would achieve the target plasma concentrations of TDZ (10 μM) that we previously reported to display antileukemic activity in vitro.[6] Blood samples were collected to measure plasma trough concentration of TDZ and its 4 metabolites on days 1, 5, 8, 10, 17, 22, 29, and 36. As previously reported, the concentration of TDZ and metabolites accumulated upon repeated dosing[23] and steady-state plasma TDZ was achieved

Table 2. Adverse events grade ≥2 possibly related to TDZ

Adverse event	Grade	Dose level I (n = 6)		Dose level II (n = 4)		Dose level III (n = 3)		Total frequency of occurrence
		Frequency per cohort, n (%)	Frequency of occurrence	Frequency per cohort, n (%)	Frequency of occurrence	Frequency per cohort, n (%)	Frequency of occurrence	
General								
Fatigue	2	1 (16)	1	0 (0)	0	0 (0)	0	1
Urinary incontinence	2	0 (0)	0	1 (25)	1	0 (0)	0	1
Neurological								
Gait disturbance	2	0 (0)	0	0 (0)	0	1 (33)	1	1
Dizziness	2	0 (0)	0	0 (0)	0	1 (33)	1	1
Depressed level of consciousness	3	0 (0)	0	0 (0)	0	1 (33)	1	1
Cardiovascular								
QTc interval prolongation (481-500 ms)	2	0 (0)	0	2 (50)	7	1 (33)	2	9
QTc Interval prolongation (≥501 ms on at least 2 separate ECGs)	3	1 (16)	1	0 (0)	0	0 (0)	0	1

approximately by day 5, prior to the administration of cytarabine (Figure 2A; supplemental Figure 2). At dose level III, only patient 11 achieved steady-state plasma TDZ levels. However, for this patient, TDZ was deescalated to 50 mg every 6 hours on day 10 after 2 grade 2 QTc prolongation events. On day 16, TDZ was completely discontinued due to the development of sepsis, which was considered unrelated to TDZ. Steady-state plasma TDZ level was not achieved for patients 12 and 13 because TDZ was discontinued early on days 4 and 2, respectively.

Steady-state plasma TDZ concentrations were dose dependent. However, the target concentration of 10 μM TDZ was not achieved in any of the cohorts (Figure 2B). In cohorts II and III, the sum of TDZ and its 2 metabolites exhibiting DRD2 antagonism (2-sulfoxide and 2-sulfone[24-27]) approached 10 μM (Figure 2C). Detectable levels of TDZ and its 2 principal metabolites, including 2-sulfoxide and 5-sulfoxide,[28] were sustained for ~1 week after the last dose of TDZ (day 29) (Figure 2A). By day 36, TDZ and metabolites were no longer detectable in plasma (Figure 2A). TDZ is metabolized by Cytochrome P450 2D6 enzyme (CYP2D6), and *CYP2D6* polymorphism has been associated with variability in drug metabolism.[29-31] *CYP2D6* genotyping of our patients indicated intermediate and extensive metabolizing phenotypes (Figure 2D), which may contribute to interpatient heterogeneity in plasma drug concentrations.[18,23,28] As previously reported, patients with predicted extensive metabolizer phenotypes exhibited lower plasma concentrations for TDZ and metabolites compared with intermediate metabolizers within the same TDZ dose level[31,32] (Figure 2D). We did not observe any cases of poor or ultrarapid metabolizers in this cohort, consistent with their rare occurrence in the wider population.[14,33]

Responses to treatment

To evaluate the effect of TDZ against human AML disease, the trial incorporated a lead-in phase with TDZ administration alone (days 1-5) prior to the combination with cytarabine (days 6-10). Bone marrow and peripheral blood samples were collected to evaluate effects on day 5 in response to TDZ alone, on day 22 after termination of TDZ (12 days after the end of cytarabine), and on day 36 for end point response assessment (Figure 1A). Day 36 coincided with day 14 after the last dose of TDZ and 26 days after the last dose of cytarabine. Patient responses on day 36 are summarized in Table 3. Of the 13 patients, patients 3, 8, 12, and 13 received less than one-third of the scheduled dose of TDZ, and patients 8 and 12 missed the cytarabine treatment and were therefore excluded from this analysis (Table 3). Of the remaining 9 evaluable patients, 1 patient (patient 2) achieved partial remission as defined by a >50% reduction of bone marrow blasts relative to pretreatment levels.[3] Patient 7 showed no response in the bone marrow but displayed a 66% reduction of peripheral blast levels. The 7 remaining patients showed resistant disease patterns on day 36.

Assessment of patient outcomes after the 5-day lead-in period with TDZ administration alone revealed a reduction of peripheral leukemic burden ranging from 19% to 55%, in 8 of the 11 patients (72%) who completed the 5-day portion of the study (Figure 3A). These outcomes appear modest based on established clinical thresholds,[3] yet noteworthy given the brief exposure period to TDZ. Moreover, the effects of TDZ as a monotherapy was further highlighted when the 5-day blast fluctuations were compared with the patients' respective blast trajectories prior to the start of the trial and in the absence of TDZ (Figure 3A). It is important to note that hydroxyurea was concurrently administered in 6 of the 11 patients during the 5-day lead-in with TDZ (Figure 3A), as previously reported in clinical trials where patients present with advanced disease states.[34-36] These included 4 of the 8 patients with partial responses to TDZ (patients 4, 7, 10, and 11), as well as 2 of the 3 patients with no response to TDZ, which continued to show progressive disease trends despite the use of hydroxyurea (patients 1 and 3). Although it cannot be ruled out that the concurrent use of hydroxyurea may have contributed to the cytoreduction during the 5-day lead-in with TDZ and may therefore confound the definitive assessment of DRD antagonism in 4 patients, blast level suppression observed after TDZ monotherapy cannot be attributed to hydroxyurea in at least 4 other patients that did not receive hydroxyurea treatment. Moreover, the overall patterns of blast

Table 3. Patient details and tumor response on day 36

Patient ID	Termination/reason	CG/molecular	Disease stage	Prognosis/classification	Baseline QTc	Day 1 PB blast count, × 10^9/L	Day 36 PB blast count, × 10^9/L	Day 1 BM blast, %	Day 36 BM blast, %
1	Normal	46,XY,del(7)(q11.2)	Refractory	High/refractory score	436.6	11.6	14.6	54	69
2	Normal	46,XY/normal	Relapse	Intermediate/EPI score	407	0	0.4	46	7
3	Early/DLT	46,XX,t(8;14)(p11.2;q32.1)	Relapse-2	Intermediate/refractory score	445	15.1	NE	89	NE
4	Early/disease progression	46,XY, del(9)(q13q22)/FLT3-ITD+	AML-MRC diagnosis	Intermediate/ ELN	409.3	24.8	135.3	87	78*
5	Normal	46,XX/normal	Relapse	High/EPI score	419	1	1.8	61	70
6	Normal	46,XY/FLT3-ITD$^+$	AML-MRC diagnosis	Intermediate/ELN	438	2.9	8.3	25	80
7	Normal	46,XX, del(5)(q13q33)/FLT3-D835+	Relapse	High/ EPI score	407.3	109.7	37.1	94	71*
8	Early/patient request	46,XX, t(4;6)(q23;p25), del(7)(q22q34), del(20)(q11.2q13.1)	Relapse	Intermediate/EPI score	411	5.4	NE	81	NE
9	Normal	46,XY/normal	AML-MRC diagnosis	Intermediate/ELN	455	0	0	45	34
10	Normal	46,XY/normal	Relapse	High/EPI score	419	2.5	1.2	64	74
11	Normal	46,XY, der(22) t(1;22)(q11;p11.2)	Relapse	Intermediate/EPI score	424	12	16.6	73	69†
12	Early/DLT	Complex karyotype	AML-MRC diagnosis	Poor/ELN	432	0	NE	22	NE
13	Early/DLT	46,XY/normal	Relapse	Intermediate/EPI score	NA	0.2	NE	56	NE

ELN, European LeukemiaNet; EPI, European Prognostic Index; ID, identification; NE, nonevaluable.
*Day 22 values indicated because day 36 value was unavailable.
†Value reported in a follow-up later than day 36.

reduction during the 5-day monotherapy was found to be statistically independent of hydroxyurea ($P = 1.00$ by Fisher's exact test; supplemental Figure 3A), the coadministration of which has not always been reported in previously published clinical studies. To dissociate between the effects of TDZ from hydroxyurea on blast level reduction in patients given TDZ and hydroxyurea vs TDZ alone, we opted for controlled in vitro experimental settings where the antileukemic effects of TDZ could be safely interrogated as a single agent in the absence of hydroxyurea, using patient leukemic cells obtained at baseline prior to initiation of the study. Because the potential contributing effect of hydroxyurea was in question, we focused on hydroxyurea-treated patients and selected 3 cases that represented the greatest response to TDZ (patient 7, −55% circulating blasts), medium-level response (patient 4, −21% circulating blasts), or no response (patient 1, +368% circulating blasts) during the 5-day monotherapy (Figure 3A). When these 3 patient samples were treated with TDZ in vitro, their relative sensitivity to TDZ as measured by leukemia cell viability resembled their respective patterns of clinical response to TDZ monotherapy, suggesting that the antileukemic effect of TDZ can be reproduced in the absence of hydroxyurea and is cell-intrinsically regulated (supplemental Figure 3B).

After this 5-day interval, peripheral blast levels continued to decline with the addition of cytarabine (days 6-10), and up to day 22 when TDZ was stopped (supplemental Figure 3C). Within the 5-day monotherapy with TDZ, residual neutrophil counts were not dramatically reduced compared with baseline levels (Figure 3B). Platelet counts were evaluated among patients who did not receive transfusions prior to or during the 5-day monotherapy with TDZ (Figure 3C), and circulating platelet levels were safely managed according to the institutional standard of care. These observations suggest that TDZ can be practically coupled with cytarabine.

TDZ has been historically administered as an antipsychotic drug whose properties are mediated through DRD2 antagonism.[37] Previous in vitro assays using patient AML cells suggested that DRDs also mediate TDZ's selective suppression of human AML, due to a preferential expression pattern in leukemic vs healthy hematopoietic progenitor cells.[6] Specifically, DRD2 protein expression was detected at a maximum of 1% within primitive cell fractions obtained from multiple independent healthy donor samples (supplemental Figure 3D). This provided a threshold to identify preferential DRD2 expression between leukemic and healthy hematopoietic cells. Based on this benchmark, 9 patients harbored DRD2 expression (>1%) at baseline, and 8 of these patients displayed blast reduction after exposure to TDZ. In contrast, <1% DRD2 expression at baseline was associated with lack of a biological response to TDZ (Figure 3D; supplemental Figure 3E).

Despite not achieving target steady-state plasma TDZ concentrations of 10 μM TDZ, our results provide signals of clinical efficacy with the DRD2 antagonist TDZ. Moreover, the association between response to TDZ and patient-specific DRD2 expression supports the relevance of DRD-based therapies for a subset of patients with notable DRD2 levels. To determine the extent of patients that may benefit from a potential DRD-targeted approach, we expanded our analysis to 20 additional AML samples, which revealed that 80% of patients express robust levels of DRD2 (Figure 3E). These observations form the

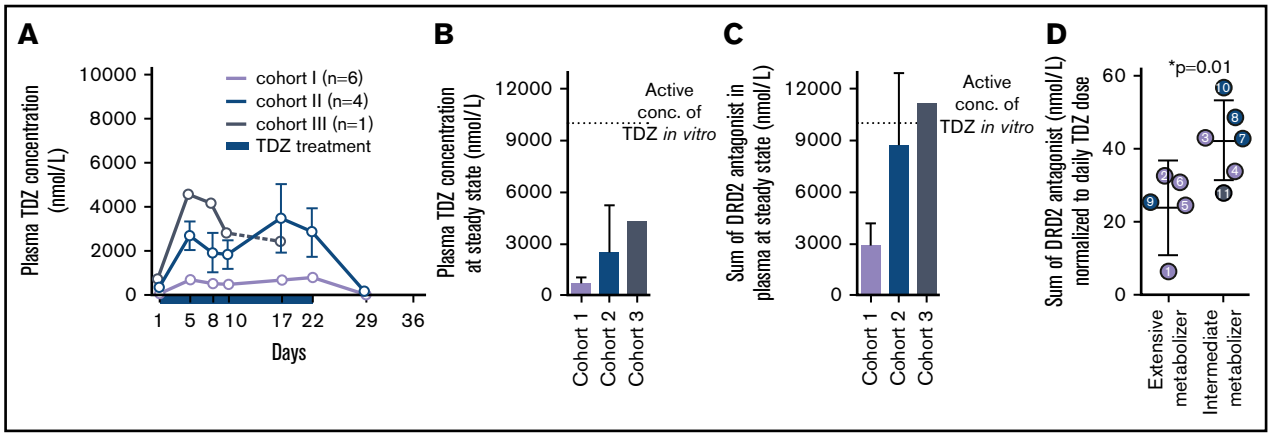

Figure 2. Pharmacokinetic analysis. (A) TDZ trough concentrations were measured on days 1, 5, 8, 10, 17, 22, 29, and 36 for each study cohort. Plasma TDZ concentrations reached a steady state by day 5, and TDZ remained detectable up to day 29. In cohort III, TDZ was discontinued early for patients 12 and 13, and only patient 11 received doses to achieve steady-state plasma TDZ concentrations. This patient received TDZ at dose level III (100 mg every 6 hours) for the first 10 days (solid line), after which TDZ was deescalated to 50 mg every 6 hours (broken line) up to day 16 and discontinued thereafter. Data for cohorts I and II are displayed as mean ± standard error at each assessment point. (B) Plasma TDZ levels at steady state in relation to the target 10 μM concentration (conc.), previously shown to be effective against human AML in vitro.[6] Data are expressed as cohort means and 95% CI. (C) Sum of plasma TDZ and its 2 active metabolites (2-sulfoxide and 2-sulfone) with DRD2 antagonistic effects in relation to the target concentration of 10 μM. Data are expressed as cohort means and 95% CI. (D) Sum of circulating DRD2 antagonist levels for individual patients normalized to daily dose of TDZ. Patient variation in plasma DRD2 antagonist level was associated with *CYP2D6* genotype and its predicted drug metabolism phenotypes (difference between group means: 18.22, 95% CI: 4.1 to 32.3; $P = .01$).

foundation for the application of DRD-based targeted therapies for a substantial proportion of adult AML patients.

Discussion

Current therapeutic options for older AML patients with relapsed or refractory disease are associated with poor therapeutic responses and poor survival outcomes.[3,38] In patients at second or third relapse, median overall survival is further reduced to as low as 3 months,[3] indicating an urgent need for novel therapies and targets. In pursuit of novel drug targets against AML disease, we previously carried out a high throughput chemical screen that led to the identification of DRD2 antagonist TDZ, a former antipsychotic drug with newly described selectivity for cancer cells over normal counterparts.[6] In vitro treatment testing of leukemic vs healthy hematopoietic progenitors with TDZ led to a suppression of clonogenic growth and leukemic disease initiating capacity in primitive leukemia cells.[6] The readout of these functional assays has been associated with leukemic blast production in patients,[39-43] suggesting a potential therapeutic benefit. In addition to AML, anticancer effects of TDZ have been reported in a wide range of cancers, including lymphomas,[44] brain tumors,[45-47] breast,[48] and ovarian.[49,50] This body of clinical evidence related to anticancer effects of TDZ motivated this phase 1 study, facilitating an initial step toward evaluating the translational potential of DRD2 antagonism in AML disease.

The primary objective of our clinical trial was to evaluate the safety and feasibility of oral TDZ at routine therapeutic doses,[51] in combination with intermediate-dose cytarabine in older AML patients with relapsed or refractory disease. The trial included a 5-day lead-in with TDZ alone, as well as a combination phase with cytarabine, providing a unique opportunity to interrogate the effect of TDZ as a monotherapy and together with cytarabine within a single trial. We found that despite the presence of poor cytogenetic features that are associated with adverse therapy responses,[3] leukemic burden was reduced in 8 of 11 patients following the 5-day exposure to TDZ monotherapy (Figure 3A), and the effects were associated with the level of DRD2 expression at baseline (Figure 3D). After this 5-day monotherapy with TDZ, the peripheral leukemic burden continued to decline with the addition of cytarabine (day 6-10) up to day 22 when TDZ was stopped (supplemental Figure 3C). After day 22, we noted an increase in blast levels as patients approached the end point on day 36 (Table 3). These outcomes suggest that DRD antagonism may be required chronically to sustain durable effects on AML blast suppression. However, the neuroleptic symptoms and cardiotoxicity observed here and in previous reports[9] precludes a safe dosing with TDZ for extended periods. Possible modifications include gradual intrapatient dose escalation to improve on tolerability,[46,52] and/or prioritization of younger AML patients to reduce the risk of cardiotoxicity. This is particularly important, because the median age of patients in all 3 cohorts was >65, which is an independent predictor of QTc prolongation.[10] More importantly, chronic regimens may be achievable with alternative formulations of TDZ that are more readily tolerated.

Our safety study provides preliminary signs of potential clinical efficacy, despite the inability to achieve plasma TDZ levels that match concentrations demonstrated to be effective in vitro.[6] This suggests that the in vivo potency of TDZ may not directly correspond to the half maximal effective concentrations defined using in vitro assays.[53] The biological effects in patients achieved at these modest plasma concentrations reinforce the therapeutic relevance of DRD2 targeting in human AML disease. In line with our observations here, previous studies with larger patient cohorts have reported reduced rates of solid tumor incidence in schizophrenic patients, which has been proposed to be associated with DRD antagonist treatment.[54,55] In the current study, the antileukemic effect of TDZ was observed predominantly in the peripheral blood and did not meet the established clinical remission criteria for AML. However, these measurements and outcomes using TDZ alone resemble that of

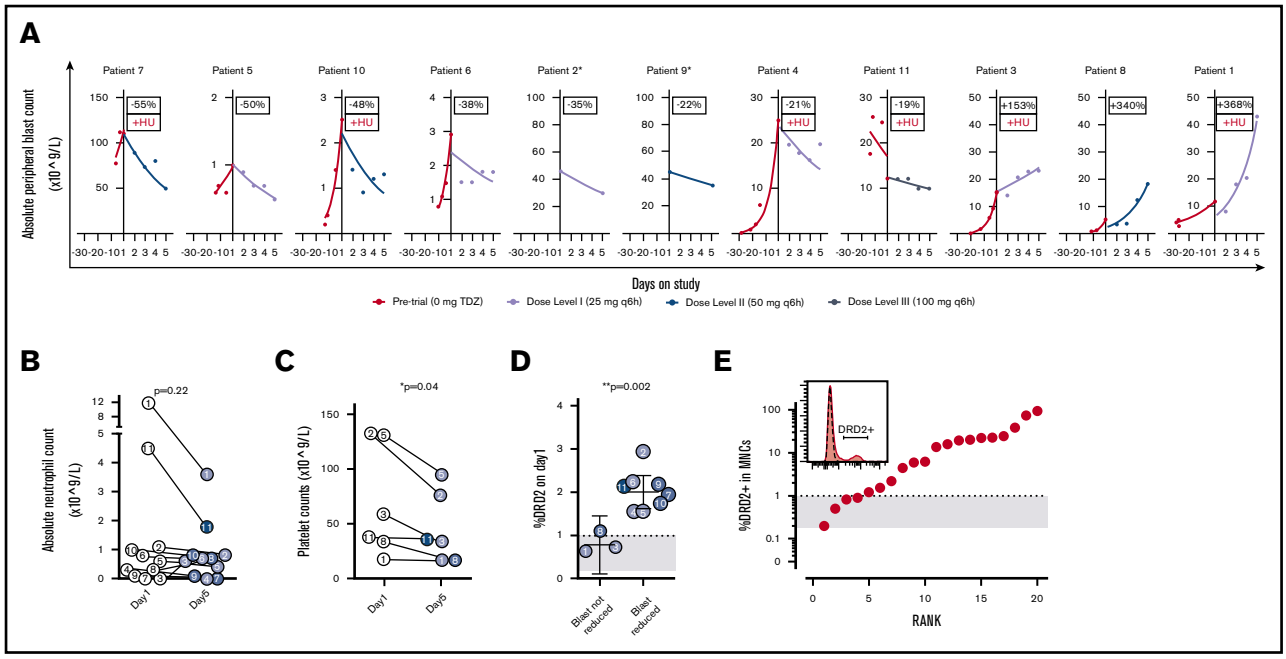

Figure 3. Response. (A) Response to a 5-day treatment with TDZ as a monotherapy was monitored at the level of peripheral blood ABC. Response for patients 2 and 9 was determined by percent bone marrow blast due to a lack of circulating blasts as indicated by the asterisk. Patients 12 and 13 were excluded from this analysis because they did not complete the 5-day treatment with TDZ. Red curves indicate disease progression based on ABC prior to the start of TDZ treatment. Blue color coding indicates TDZ dose level. Insets depict percent change in blast counts at day 5 compared with day 1. "+HU" indicates concurrent use of hydroxyurea during the 5-day treatment with TDZ. (B) Absolute neutrophil counts after exposure to up to 100 mg of TDZ every 6 hours based on data from 11 patients that completed the 5-day monotherapy with TDZ. Data are expressed as cohort means and 95% CI (mean change from day 1 to day 5: −1.00, 95% CI: −2.7 to 0.7, $P = .22$). (C) Platelet counts after a 5-day treatment with up to 100 mg of TDZ every 6 hours. Data points include patients that did not receive platelet transfusions within the first 5 days of TDZ, or up to 4 days prior to the start of the trial. Data are expressed as cohort means and 95% CI (mean change from day 1 to day 5: −23.00, 95% CI: −45.4 to −0.54, $P = .04$). (D) Baseline levels of DRD2 protein expression in MNCs of patients that showed no blast response vs patients that showed blast reduction after the 5-day treatment with TDZ (difference between group means: 1.20, 95% CI: 0.56 to 1.84; $P = .002$). The shaded area indicates the upper and lower 99% CI for DRD2 levels in healthy primitive cells as defined in supplemental Figure 3D. (E) DRD2 protein expression in MNCs of 20 AML patient samples (16 diagnosis and 4 relapse cases) not including on-study patients. The shaded area indicates the upper and lower 99% CI for DRD2 levels in healthy primitive cells ($CD34^+$) as defined in supplemental Figure 3D. Inset depicts a representative flow cytometry plot for DRD2 expression in AML (red histogram), overlayed on the staining control (dotted histogram).

early generation FLT3 inhibitors in clinical testing that also reduced leukemia blast levels with greater potency in the peripheral blood vs the bone marrow.[56] Similar to our findings with TDZ reported here, the preliminary results using FLT3 inhibitors formed the foundation for more informed future clinical trials and the development of refined versions of the drug. Subsequent generations of FLT3 inhibitors have evolved into some of the most promising targeted therapies when combined with induction chemotherapy,[57] with improved outcomes in patients that express the drug target in a minority of blast cells,[57] similar to minor fractions reported for DRD expression in this cohort.

Future studies are required to corroborate these preliminary findings in the absence of concurrent hydroxyurea or within 2-armed trials, to more discretely determine the antileukemic potential of DRD antagonism as a monotherapy. Also, larger patient cohorts and modified formulations of TDZ that display reduced neurological and cardiac liability should be considered for future trials. Such analogs are currently under investigation by our group. Our study offers a conceptual advancement toward understanding the biology and vulnerabilities of AML through DRDs and holds promise for a novel therapeutic target to combat AML disease.

Acknowledgments

The authors would like to thank the patients for participation in the study and providing clinical specimens. They would also like to acknowledge Cathy Ross, Aimee Hill, Pamela O'Hoski, and Tammy Degelder at the Juravinski Hospital and Cancer Centre, Lisa Rudd-Scott, Donna McCarthy, and Beverly Pond at the Ontario Clinical Oncology Group, and Monika Lenkiewicz, Monica Graham, and Kennedy Makondo at the McMaster Stem Cell and Cancer Research Institute.

This work was supported by the Owen and Marta Boris Foundation, Hamilton Health Sciences as well as the Canadian Cancer Society (grant 703204) and Canadian Institutes of Health Research (CIHR-FRN #153158). M.B. is supported by the Canadian Chair Program and holds the Canada Research Chair in human stem cell biology.

Authorship

Contribution: L.A. wrote the manuscript and analyzed and interpreted the data; A.L.B. analyzed and interpreted the data and provided input on the manuscript; M.A. designed the study protocol and provided input on the manuscript; T.J.C. interpreted the data; D.P.L.

designed the study protocol and provided input on the manuscript; R.G.T. and R.B.K. designed and analyzed the PK studies and provided input on the manuscript; J.A.J. designed the study protocol, oversaw data management and AE data analysis, and provided input on the manuscript; A.X. designed the study protocol and provided input on the manuscript; M.N.L. designed the study protocol, directed the study, and provided input on the manuscript; B.L. designed the study protocol, conducted the study, and provided input on the manuscript; R.F. designed the study protocol, conducted the study, and wrote the manuscript; and M.B. designed the study protocol and wrote the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

ORCID profile: M.B., 0000-0002-9304-5130.

Correspondence: Mickie Bhatia, Stem Cell and Cancer Research Institute, McMaster University, Faculty of Health Sciences, 1280 Main St West, MDCL 5029, Hamilton, ON L8S 4K1, Canada; e-mail: mbhatia@mcmaster.ca; and Ronan Foley, Juravinski Hospital and Cancer Centre, 711 Concession St, Second floor laboratory, Room 201, Hamilton, ON L8V 5C2, Canada; e-mail: foleyr@hhsc.ca.

References

1. Stone RM, Berg DT, George SL, et al. Postremission therapy in older patients with de novo acute myeloid leukemia: a randomized trial comparing mitoxantrone and intermediate-dose cytarabine with standard-dose cytarabine. *Blood*. 2001;98(3):548-553.
2. Döhner H, Estey EH, Amadori S, et al; European LeukemiaNet. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. *Blood*. 2010;115(3):453-474.
3. Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood*. 2017;129(4):424-447.
4. Ivanoff S, Gruson B, Chantepie SP, et al. 5-Azacytidine treatment for relapsed or refractory acute myeloid leukemia after intensive chemotherapy. *Am J Hematol*. 2013;88(7):601-605.
5. Ritchie EK, Feldman EJ, Christos PJ, et al. Decitabine in patients with newly diagnosed and relapsed acute myeloid leukemia. *Leuk Lymphoma*. 2013; 54(9):2003-2007.
6. Sachlos E, Risueño RM, Laronde S, et al. Identification of drugs including a dopamine receptor antagonist that selectively target cancer stem cells. *Cell*. 2012;149(6):1284-1297.
7. Kemper AJ, Dunlap R, Pietro DA. Thioridazine-induced torsade de pointes. Successful therapy with isoproterenol. *JAMA*. 1983;249(21): 2931-2934.
8. Harrigan EP, Miceli JJ, Anziano R, et al. A randomized evaluation of the effects of six antipsychotic agents on QTc, in the absence and presence of metabolic inhibition. *J Clin Psychopharmacol*. 2004;24(1):62-69.
9. Menkes DB, Knight JC. Cardiotoxicity and prescription of thioridazine in New Zealand. *Aust N Z J Psychiatry*. 2002;36(4):492-498.
10. Reilly JG, Ayis SA, Ferrier IN, Jones SJ, Thomas SH. QTc-interval abnormalities and psychotropic drug therapy in psychiatric patients. *Lancet*. 2000; 355(9209):1048-1052.
11. Faderl S, Wetzler M, Rizzieri D, et al. Clofarabine plus cytarabine compared with cytarabine alone in older patients with relapsed or refractory acute myelogenous leukemia: results from the CLASSIC I Trial. *J Clin Oncol*. 2012;30(20):2492-2499.
12. Storer BE. Design and analysis of phase I clinical trials. *Biometrics*. 1989;45(3):925-937.
13. Wójcikowski J, Maurel P, Daniel WA. Characterization of human cytochrome p450 enzymes involved in the metabolism of the piperidine-type phenothiazine neuroleptic thioridazine. *Drug Metab Dispos*. 2006;34(3):471-476.
14. Gaedigk A, Simon SD, Pearce RE, Bradford LD, Kennedy MJ, Leeder JS. The CYP2D6 activity score: translating genotype information into a qualitative measure of phenotype. *Clin Pharmacol Ther*. 2008;83(2):234-242.
15. Ewer MS, Ewer SM. Cardiotoxicity of anticancer treatments. *Nat Rev Cardiol*. 2015;12(9):547-558.
16. Buckley NA, Whyte IM, Dawson AH. Cardiotoxicity more common in thioridazine overdose than with other neuroleptics. *J Toxicol Clin Toxicol*. 1995; 33(3):199-204.
17. Buckley NA, Sanders P. Cardiovascular adverse effects of antipsychotic drugs. *Drug Saf*. 2000;23(3):215-228.
18. LLerena A, Berecz R, de la Rubia A, Dorado P. QTc interval lengthening is related to CYP2D6 hydroxylation capacity and plasma concentration of thioridazine in patients. *J Psychopharmacol*. 2002;16(4):361-364.
19. Hartigan-Go K, Bateman DN, Nyberg G, Mårtensson E, Thomas SH. Concentration-related pharmacodynamic effects of thioridazine and its metabolites in humans. *Clin Pharmacol Ther*. 1996;60(5):543-553.
20. Hale PW Jr, Poklis A. Thioridazine-5-sulfoxide cardiotoxicity in the isolated, perfused rat heart. *Toxicol Lett*. 1984;21(1):1-8.
21. Hale PW Jr, Poklis A. Cardiotoxicity of thioridazine and two stereoisomeric forms of thioridazine 5-sulfoxide in the isolated perfused rat heart. *Toxicol Appl Pharmacol*. 1986;86(1):44-55.
22. Gottschalk LA, Dinovo E, Biener R, Nandi BR. Plasma concentrations of thioridazine metabolites and ECG abnormalities. *J Pharm Sci*. 1978;67(2): 155-157.
23. Ng CH, Crammer JL. Measurement of thioridazine in blood and urine. *Br J Clin Pharmacol*. 1977;4(2):173-183.

24. Kilts CD, Knight DL, Mailman RB, Widerlöv E, Breese GR. Effects of thioridazine and its metabolites on dopaminergic function: drug metabolism as a determinant of the antidopaminergic actions of thioridazine. *J Pharmacol Exp Ther*. 1984;231(2):334-342.

25. Bylund DB. Interactions of neuroleptic metabolites with dopaminergic, alpha adrenergic and muscarinic cholinergic receptors. *J Pharmacol Exp Ther*. 1981;217(1):81-86.

26. Daniel WA, Syrek M, Haduch A, Wójcikowski J. Pharmacokinetics and metabolism of thioridazine during co-administration of tricyclic antidepressants. *Br J Pharmacol*. 2000;131(2):287-295.

27. Vanderheeren FA, Muusze RG. Plasma levels and half lives of thioridazine and some of its metabolites. I. High doses in young acute schizophrenics. *Eur J Clin Pharmacol*. 1977;11(2):135-140.

28. Berecz R, de la Rubia A, Dorado P, Fernández-Salguero P, Dahl ML, LLerena A. Thioridazine steady-state plasma concentrations are influenced by tobacco smoking and CYP2D6, but not by the CYP2C9 genotype. *Eur J Clin Pharmacol*. 2003;59(1):45-50.

29. Eap CB, Finkbeiner T, Gastpar M, Scherbaum N, Powell K, Baumann P. Replacement of (R)-methadone by a double dose of (R,S)-methadone in addicts: interindividual variability of the (R)/(S) ratios and evidence of adaptive changes in methadone pharmacokinetics. *Eur J Clin Pharmacol*. 1996;50(5):385-389.

30. Dorado P, Peñas-LLedó EM, de la Rubia A, LLerena A. Relevance of CYP2D6 -1584C>G polymorphism for thioridazine:mesoridazine plasma concentration ratio in psychiatric patients. *Pharmacogenomics*. 2009;10(7):1083-1089.

31. Bertilsson L, Dahl ML, Dalén P, Al-Shurbaji A. Molecular genetics of CYP2D6: clinical relevance with focus on psychotropic drugs. *Br J Clin Pharmacol*. 2002;53(2):111-122.

32. Linnet K, Wiborg O. Influence of Cyp2D6 genetic polymorphism on ratios of steady-state serum concentration to dose of the neuroleptic zuclopenthixol. *Ther Drug Monit*. 1996;18(6):629-634.

33. van Schaik RH. 6. Dose adjustments based on pharmacogenetics of CYP450 enzymes. *EJIFCC*. 2008;19(1):42-47.

34. Smith BD, Levis M, Beran M, et al. Single-agent CEP-701, a novel FLT3 inhibitor, shows biologic and clinical activity in patients with relapsed or refractory acute myeloid leukemia. *Blood*. 2004;103(10):3669-3676.

35. Bug G, Ritter M, Wassmann B, et al. Clinical trial of valproic acid and all-trans retinoic acid in patients with poor-risk acute myeloid leukemia. *Cancer*. 2005;104(12):2717-2725.

36. Gojo I, Beumer JH, Pratz KW, et al. A phase 1 study of the PARP inhibitor veliparib in combination with temozolomide in acute myeloid leukemia. *Clin Cancer Res*. 2017;23(3):697-706.

37. Seeman P, Lee T. Antipsychotic drugs: direct correlation between clinical potency and presynaptic action on dopamine neurons. *Science*. 1975;188(4194):1217-1219.

38. Stein EM, DiNardo CD, Pollyea DA, et al. Enasidenib in mutant *IDH2* relapsed or refractory acute myeloid leukemia. *Blood*. 2017;130(6):722-731.

39. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med*. 1997;3(7):730-737.

40. Tehranchi R, Woll PS, Anderson K, et al. Persistent malignant stem cells in del(5q) myelodysplasia in remission. *N Engl J Med*. 2010;363(11):1025-1037.

41. Griffin JD, Löwenberg B. Clonogenic cells in acute myeloblastic leukemia. *Blood*. 1986;68(6):1185-1195.

42. Browman G, Goldberg J, Gottlieb AJ, et al. The clonogenic assay as a reproducible in vitro system to study predictive parameters of treatment outcome in acute nonlymphoblastic leukemia. *Am J Hematol*. 1983;15(3):227-235.

43. Preisler HD. Prediction of response to chemotherapy in acute myelocytic leukemia. *Blood*. 1980;56(3):361-367.

44. Nagel D, Spranger S, Vincendeau M, et al. Pharmacologic inhibition of MALT1 protease by phenothiazines as a therapeutic approach for the treatment of aggressive ABC-DLBCL. *Cancer Cell*. 2012;22(6):825-837.

45. Dolma S, Selvadurai HJ, Lan X, et al. Inhibition of dopamine receptor D4 impedes autophagic flux, proliferation, and survival of glioblastoma stem cells. *Cancer Cell*. 2016;29(6):859-873.

46. Huang X, He Y, Dubuc AM, et al. EAG2 potassium channel with evolutionarily conserved function as a brain tumor target. *Nat Neurosci*. 2015;18(9):1236-1246.

47. Cheng HW, Liang YH, Kuo YL, et al. Identification of thioridazine, an antipsychotic drug, as an antiglioblastoma and anticancer stem cell agent using public gene expression data. *Cell Death Dis*. 2015;6(5):e1753.

48. Ke XY, Lin Ng VW, Gao SJ, Tong YW, Hedrick JL, Yang YY. Co-delivery of thioridazine and doxorubicin using polymeric micelles for targeting both cancer cells and cancer stem cells. *Biomaterials*. 2014;35(3):1096-1108.

49. Rho SB, Kim BR, Kang S. A gene signature-based approach identifies thioridazine as an inhibitor of phosphatidylinositol-3′-kinase (PI3K)/AKT pathway in ovarian cancer cells. *Gynecol Oncol*. 2011;120(1):121-127.

50. Byun HJ, Lee JH, Kim BR, et al. Anti-angiogenic effects of thioridazine involving the FAK-mTOR pathway. *Microvasc Res*. 2012;84(3):227-234.

51. Kane JM. Schizophrenia. *N Engl J Med*. 1996;334(1):34-41.

52. Abbate E, Vescovo M, Natiello M, et al. Successful alternative treatment of extensively drug-resistant tuberculosis in Argentina with a combination of linezolid, moxifloxacin and thioridazine. *J Antimicrob Chemother*. 2012;67(2):473-477.

53. Tuntland T, Ethell B, Kosaka T, et al. Implementation of pharmacokinetic and pharmacodynamic strategies in early research phases of drug discovery and development at Novartis Institute of Biomedical Research. *Front Pharmacol*. 2014;5:174.

54. Mortensen PB. Neuroleptic medication and reduced risk of prostate cancer in schizophrenic patients. *Acta Psychiatr Scand*. 1992;85(5):390-393.

55. Dalton SO, Mellemkjaer L, Thomassen L, Mortensen PB, Johansen C. Risk for cancer in a cohort of patients hospitalized for schizophrenia in Denmark, 1969-1993. *Schizophr Res*. 2005;75(2-3):315-324.

56. Stone RM, DeAngelo DJ, Klimek V, et al. Patients with acute myeloid leukemia and an activating mutation in FLT3 respond to a small-molecule FLT3 tyrosine kinase inhibitor, PKC412. *Blood*. 2005;105(1):54-60.

57. Stone RM, Mandrekar SJ, Sanford BL, et al. Midostaurin plus chemotherapy for acute myeloid leukemia with a FLT3 mutation. *N Engl J Med*. 2017;377(5):454-464.