Published in final edited form as:

Leuk Lymphoma. 2018 November ; 59(11): 2595–2601. doi:10.1080/10428194.2018.1443330.

A Phase 1 Study of Dasatinib plus All-Trans Retinoic Acid in Acute Myeloid Leukemia

Robert L. Redner^{1,3}, Jan H. Beumer^{1,2,3}, Patricia Kropf^{3,4}, Mounzer Agha^{1,3}, Michael Boyiadzis^{1,3}, Kathleen Dorritie^{1,3}, Rafic Farah^{1,3}, Jing-Zhao Hou^{1,3}, Annie Im^{1,3}, Seah H. Lim^{3,5}, Anastasios Raptis^{1,3}, Alison Sehgal^{1,3}, Susan M. Christner¹, Daniel Normolle⁶, and Daniel E. Johnson^{3,7}

¹Cancer Therapeutics Program, UPMC Hillman Cancer Center, Pittsburgh, PA 15213, USA

²Department of Pharmaceutical Sciences, University of Pittsburgh School of Pharmacy, Pittsburgh, PA 15213, USA

³Division of Hematology-Oncology, Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213, USA

⁶Department of Biostatistics, University of Pittsburgh Graduate School of Public Health

Abstract

Src family kinases (SFKs) are hyperactivated in acute myeloid leukemia (AML). SFKs impede the retinoic acid receptor, and SFK inhibitors enhance all-trans retinoic acid (ATRA)-mediated cellular differentiation in AML cell lines and primary blasts. To translate these findings into the clinic, we undertook a phase I dose-escalation study of the combination of the SFK inhibitor dasatinib and ATRA in patients with high-risk myeloid neoplasms. Nine subjects were enrolled: six received 70 mg dasatinib plus 45 mg/m² ATRA daily, and three received 100 mg dasatinib plus 45 mg/m² ATRA daily for 28 days. Headache and QTc prolongations were the only two grade 3 adverse events observed. No significant clinical responses were observed. We conclude that the combination of 70 mg dasatinib and 45 mg/m² ATRA daily is safe with acceptable toxicity. Our results provide the safety profile for further investigations into the clinical efficacy of this combination therapy in myeloid malignancies.

Keywords

ATRA; dasatinib; phase I; pharmacokinetics; pharmacodynamics; AML

* Address all correspondence to: Robert L. Redner, MD, 2.18A Hillman Research Pavilion, 5117 Centre Ave, Pittsburgh PA 15213, redner@pitt.edu, phone 412-623-3257, FAX 412-623-7768.

⁴Current Address: Fox Chase Cancer Center, Philadelphia PA 19111

⁵Current Address: Brown University Warren Alpert Medical School, Providence, RI 02903

⁷Current Address: Department of Otolaryngology, University of California San Francisco, San Francisco CA 94143

DISCLOSURE STATEMENT

The authors report no conflicts of interest.

INTRODUCTION

Overexpression and hyperactivation of Src-family kinases (SFKs) is a common finding in acute myeloid leukemia (AML) [1, 2, 3]. The SFK family consists of nine different non-receptor tyrosine kinases [4], of which four are known to be expressed in myeloid lineage cells: c-Src, Lyn, Fgr, and Hck [2, 3, 5, 6]. Gene knockout mice that are deficient in Lyn exhibit increased levels of myeloid progenitors [7, 8], and progenitors derived from Hck-deficient mice demonstrate enhanced responses to granulocytic colony-stimulating factor [7, 9]. These genetic models suggest that SFKs may play an inhibitory role in myeloid progenitor pool expansion and myelopoiesis *in vivo*.

We and others have found that SFKs inhibit the activity of the retinoic acid receptor alpha (RAR α) [1, 10, 11]. Conversely, pharmacologic inhibitors of SFKs synergize with all-trans retinoic acid (ATRA) to enhance retinoic acid receptor-dependent transcription. RAR α controls expression of many genes involved in differentiation and apoptosis [12]. We hypothesized that SFK activation contributes to the AML phenotype by impeding the activity of RAR α and the differentiation pathways that RAR α controls. In support of this hypothesis, we and others have found that the combination of SFK-inhibitors plus ATRA enhances expression of RAR α -target genes, induces differentiation of AML cell lines, and induces differentiation of primary AML blasts in culture [10, 13, 14]. The combination of SFK inhibitor plus ATRA should, therefore, enhance the maturation of leukemic cells, and, analogous to the success of ATRA in acute promyelocytic leukemia (APL), improve AML patient outcomes.

To translate our *in vitro* findings to the clinic, we undertook a phase I trial of the combination of dasatinib - an FDA approved SFK inhibitor – and ATRA, in relapsed, refractory or elderly, non-induction candidate AML patients. Dasatinib is a potent, broad spectrum inhibitor of 5 tyrosine kinases/kinase families: BCR-ABL, SFKs, c-KIT, PDGF receptor β , and ephrin receptor kinases [15]. It has received FDA approval for use in chronic, accelerated, and blast phase CML. The recommended starting dosage for subjects with chronic phase CML is 100 mg administered orally once daily (QD). ATRA is a Vitamin A analog that serves as the natural ligand for RAR α [12]. Retinoic acid signaling plays a key role in normal myeloid development [16], best exemplified by the observation that the defective RAR α signaling pathway that characterizes APL causes myeloid differentiation arrest [12]. ATRA has received FDA approval for use in APL at a dose of 45 mg/m², administered in 2 divided doses daily.

METHODS

PATIENT SELECTION

Eligibility required patients to have histologically confirmed diagnosis of non-APL AML, based on WHO criteria [17]. Patients above age 18 were eligible if they provided informed consent, had ECOG performance status of 0–3, and had a life expectancy of at least 2 months. Patients aged less than 65 were considered eligible if they had refractory or relapsed AML. Subjects above age 65 were also considered eligible if they were *de novo* not considered candidates for induction chemotherapy. In 2013, the protocol was amended to

also include patients with IPSS-high-risk MDS [18] who were either not a candidate for, or who failed DNA methyltransferase inhibitor therapy. Adequate organ function was defined as total bilirubin less than twice the upper limit of normal (ULN), hepatic enzymes $<2.5 \times$ ULN, creatinine $<2 \times$ ULN, and QTc prolonged beyond 470 msec. Patients were excluded if they had received growth factors within 14 days prior to screening labs, hydroxyurea within 7 days, or were currently receiving other cancer therapy. Patients receiving potent inhibitors of CYP3A4 were also excluded.

TREATMENT PLAN

The study was a single center trial performed at the UPMC Hillman Cancer Center, Pittsburgh, PA. The primary objective was to determine the safety and tolerability of the combination of dasatinib and ATRA in AML patients and to identify the maximally tolerated dose (MTD) and dose-limiting toxicities (DLT). Subjects were considered evaluable for toxicity if they completed 22 of 28 days of treatment. DLT was defined as any grade 3 or higher non-hematological toxicity or a delay of therapy greater than 2 weeks due to investigational agent-associated toxicity. Our study design was to evaluate three cohorts using a fixed dose of ATRA in combination with an escalating dose of dasatinib, using a standard 3+3 dose-escalation design, with 3 patients at each dose level. If 1/3 patients experiences DLT, 3 more patients are added at the same level, and if 0/3 or 1/6 experience DLT, the dose for the next cohort is escalated by one level. If $>1/6$ patients experience DLT, de-escalation continues until $1/6$ patients at a dose level experience DLT; this level was to be defined as the MTD. ATRA was to be administered at 22.5 mg/m^2 po bid (rounding to the nearest 10 mg). The three dose levels of dasatinib were dose level (DL) 1 at 70 mg po qd; DL2 at 100 mg po qd; and DL3 at 140 mg po qd. Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria (NCI CTCAE Version 4.0). Study drugs were administered daily for 28 days, with clinical observation for two additional weeks to determine late adverse events.

Supportive therapy was administered at the discretion of the attending physician(s). Growth factors were not permitted. Antacids, if needed, were administered at least 2 hours prior to or 2 hours after the dose of dasatinib.

Patients were considered evaluable for toxicity if they completed 22 days of administration (75%) of study drugs. Any AEs that occurred in subjects who withdrew from the study before completing 22 days of therapy were not considered DLTs, but are presented in this manuscript (see Table 2 and Supplementary Table 1). All subjects who consented to undergo screening are listed in Supplementary Table 1. Dasatinib was supplied by Bristol Myers Squibb (BMS); ATRA (Tretinoin) was purchased from commercial sources. The protocol was approved by the University of Pittsburgh IRB. All patients provided written informed consent. The full protocol is listed at [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00892190) identifier NCT00892190.

TUMOR RESPONSE ASSESSMENT

All patients had complete blood counts performed twice weekly. Bone marrow aspirates and biopsies were performed at entry to study and at days 14 and 28.

STATISTICAL ANALYSIS

The statistics presented are descriptive. Patients who enrolled but did not take any treatment were excluded from all analyses. Subjects were evaluable for toxicities if they received at least 22 days of study drug. Adverse events that were possibly, probably, or definitely related to treatment were considered toxicities. Because of the patients' underlying diagnoses, only non-hematologic toxicities are reported.

PHARMACOKINETIC ANALYSIS

Blood samples were collected in heparin-anticoagulated vacutainers covered in aluminum foil to prevent degradation of ATRA. Samples were immediately put on ice and spun down for 10 min at 4 °C at 1000 x g. With direct lab lights off, 1 mL of plasma was stored in an amber microcentrifuge tube for determination of ATRA, while the remaining plasma was stored for determination of dasatinib. Samples were stored at -70 °C or colder until analysis. Samples were taken on days 1 and 28 before and 0.5, 1, 1.5, 2, 3, 4, 6, and 8 h after simultaneous administration of dasatinib and ATRA. Concentrations of dasatinib were quantitated with a validated LC-MS/MS assay as previously described [19]. Concentrations of ATRA were quantitated by HPLC-UV based on an earlier method [20]. ATRA was analyzed in 0.5 mL of plasma, utilizing retinoic acid p-hydroxyanilide as internal standard, resulting in a calibration range of 10–1000 ng/mL. Plasma pharmacokinetic parameters were derived from the data by non-compartmental methods with PK Solutions 2.0™ (Summit Research Services, Montrose, CO, USA).

RESULTS

ADVERSE EVENTS

All AEs were reviewed by an internal DSMC. Table 1 lists the characteristics of the study subjects. We entered six subjects at Dose Level 1 (70 mg dasatinib qd; 22.5 mg/m² ATRA bid). Three completed at least 24 days of treatment, and were considered evaluable for toxicity. Of those who began study drugs but were not evaluable: Subject 1 experienced a mild headache within hours of taking the second dose of ATRA. He went to an emergency room, and was monitored overnight. The headache resolved with mild narcotic analgesia. He exhibited no focal neurologic deficits; no imaging or spinal fluid sampling was performed. He did not receive further study medication, and elected to withdraw consent. He died six month later, without recurrence of headache nor other neurological events. Subject 7 received 13 days of study drugs, but expired secondary to progressive fungal pneumonia (unrelated to study treatment). Subject 9 received 19 days of study drugs but expired secondary to resistant enterococcus sepsis (unrelated to study treatment). While on study, he experienced grade 3 prolongation of QTc: he had a history of coronary artery disease and coronary artery bypass grafting, and recent occurrence of pulmonary embolism. Subjects 8 and 11 completed all 28 days of treatment; patient 10 received 24 days of study drugs, and was followed for adverse events through day 42, but refused the day 28 bone marrow and PK sampling. Of the three subjects who were evaluable, none experienced a DLT. In Table 2 we list the non-hematological adverse events that were deemed to be possibly, probably or definitely related to treatment for all subjects who began study medications. Of three subjects entered onto Dose Level 2 (100 mg dasatinib qd; 22.5 mg/m² ATRA bid), none

received the requisite 22 days of study drugs to qualify them as evaluable for toxicity. Subject 18 elected to withdraw from the study after developing neutropenic enterocolitis (unrelated to study treatment); Subject 13 experienced grade 2 rash (possibly related to study treatment) of his scrotum and penis, with erythema and pain, after 15 days of study drugs; the rash was not biopsied, and resolved after discontinuing the study drugs. It recurred after re-challenge with the study drug. The patient was taken off study after he failed to complete 22 days of treatment. Subject 19, who had a history of migraine headaches, developed grade 2 headache (possibly related to study treatment), without other neurologic signs or symptoms, after 12 days on study. He did not undergo imaging nor spinal fluid analysis. His symptoms resolved with mild analgesia, but he elected to withdraw consent.

PHARMACOKINETICS

Pharmacokinetic data were collected and analyzed for ATRA in seven patients and dasatinib in nine patients. Pharmacokinetic parameters are listed in Table 3. Day 28 dasatinib PK was available in only 2 patients, and showed >1.4 fold the half-life, C_{max} , and AUC values relative to day 1 (data not shown).

RESPONSE TO STUDY DRUGS

Though our cohort is small, we have analyzed the response in an exploratory manner to determine if the subjects show alteration in blood counts or marrow progenitors. The absolute neutrophil counts and peripheral blast counts for subjects who received more than 14 days of study drug are detailed in Figure 1. Subject 10 developed an increase in neutrophils, though blasts also increased; Subjects 11 and 8 remained severely neutropenic with few circulating blasts throughout the study. Subject 7 and 9, both of whom were taken off study because of progressive infections, showed no increase in neutrophils and a rise in blast counts.

Flow cytometric analysis of marrow specimens from the subjects who received day 14 or day 28 marrow aspirates are listed in Table 4. Though it is difficult to draw conclusions based on such a small sample size, it is interesting to note that subject 11 had fewer total blasts in the day 14 specimen (decreasing by 50%). Subject 8 showed stable blast counts, and had a decrease in the CD34/CD33 and CD117/CD33 population on day 14.

DISCUSSION

ATRA has been shown to induce *in vitro* differentiation in many non-APL AML cell lines, including HL-60, THP-1, MOLM-14, HF-6, and U937 cells [16]. In non-APL AML blast cells, Lehmann *et al.* found that blasts from more than half of 23 non-APL AML patients were moderately sensitive to ATRA *in vitro* [21]. Though there have been no published trials of ATRA as a single agent for AML, several case reports suggest that non-APL AML patients may derive benefit from treatment with ATRA. Qian *et al.* reported positive effects of single-agent ATRA in four t(8;21) AML patients who were originally misdiagnosed as APL [22]. Several large European studies have sought to establish a role for ATRA therapy as a supplement to standard induction therapy for non-APL AML [23, 24, 25], with

discrepant results, though recent data suggests that patients with mutated NPMc may show improvement when treated with ATRA-based regimens [26].

Our study was designed with the intent of enhancing the activity of ATRA and RAR α signaling in patients with non-APL myeloid malignancies through addition of an SFK inhibitor (dasatinib). We found that the combination of dasatinib and ATRA has minimal toxicity when administered at doses of 70 mg qd and 22.5 mg/m² bid, respectively. We observed only two grade 3 toxicities. One subject, with a history of coronary artery disease and coronary artery bypass grafting, had grade 3 prolongation of QTc, a known potential side effect of dasatinib. One subject had grade 3 headache, a recognized side effect of ATRA. Because of slow accrual, the trial was closed before the MTD was reached.

We encountered two grade 3 Adverse Events. The first, a headache in Subject 1, occurred on day 1 of DL1. It resolved with analgesia; there was no evidence of other neurologic toxicity; there was little suspicion for leptomeningeal disease. Headache is a common AE with ATRA, possibly related to increased intracranial pressure; our patient did not undergo CNS sampling nor CT scanning, so we are unable to report further on the etiology of his headache. This was not considered to be a DLT because, according to the pre-specified criteria of the study protocol, AEs occurring in patients who did not complete at least 22 days of study drug were not considered evaluable, and would not be considered DLTs. Subject 9, with a history of coronary artery disease and coronary artery bypass grafting, and recent occurrence of pulmonary embolism, on the 8th day of receiving study drugs developed grade 3 prolongation of QTc, a known potential side effect of dasatinib.

ATRA has recognized effects on metabolic capacity, induces its own metabolism and can cause drug-drug interactions [27]. CYP3A4 appears to play a major role in dasatinib metabolism [28]. Dasatinib treatment inhibits the activities of liver microsome CYP2C8 (K_i of 3.6 μ M) and CYP3A4 (K_i = 1.9 μ M) [28]. ATRA is metabolized by the CYP450 system, but specific isoenzymes have not been defined [20]. There is, therefore, potential for drug interaction or interference. For these reasons, the study design included pharmacokinetic (PK) studies on both study drugs at day 1 and again on day 28. The dasatinib exposures in the current study were slightly higher than those previously reported, with a C_{max} of 56 (SD 66) ng/mL at 1.5 h after a 100 mg dose [29]. ATRA is reported to increase the activity of CYP2E1 (83%) and NAT (29%), but not CYP1A2, 2C19, 2D6, or 3A4 [27]. Unexpectedly, we observed an almost 50% increase in dasatinib exposure in 2 patients after a month of combination therapy, however, the small numbers did not allow statistical evaluation. In addition, dasatinib exposure has large between- and within patient variability, with coefficients of variation of up to 100% for both AUC and C_{max} [29, 30]. ATRA PK in the current study was in line with previous reports [20].

Our study was not designed to assess efficacy, and we did not see a significant clinical effect on the AML by administration of the study drugs for one 28-day period. However, it is intriguing to note that one subject showed fewer blasts in the day 14 marrow compared to day 0, and one subject showed a decrease in the CD34/CD33 and CD117/CD33 populations.

The molecular mechanism whereby dasatinib enhances ATRA activity in AML is as yet unclear. Congelton et. al have suggested a mechanism dependent upon c-RAF and ERK [11]. Sarry's group [31] has found that ATRA induces LYN activation, which in turn reduces RARA activity, and suggests that dasatinib inhibitory effects on LYN activation may be responsible for the synergistic effects. This effect is pronounced in AML patients with IDH1 R123H mutation, which Sarry's group has found to activate C/EBP ϵ . Our subjects were not tested for IDH mutations.

We conclude that administration of the combination of dasatinib and ATRA is safe and well tolerated at doses of 70 mg dasatinib qd and ATRA 22.5 mg/m² bid. The study closed before accrual could be completed at a higher dosing cohort. The MTD was not reached. It should be noted that in our study design the AE experienced by Subject 1 was not considered a DLT; in other phase I designs a Grade 3 headache might have been considered a DLT. Our results provide the safety profile for further investigations into the clinical efficacy of this combination therapy in patients with high grade myeloid malignancies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

The authors would like to thank Rita Johnson for valuable discussions. The authors would also like to thank the patients and nursing staff of the UPMC Hillman Cancer Center.

FUNDING

This project was supported by grants from the Leukemia and Lymphoma Society TRP6028–10 and BMS CA180–333. This project used the UPMC Hillman Cancer Center Cancer Pharmacokinetics and Pharmacodynamics Facility (CPPF), Clinical Protocol and Data Management (CPDM) Facility, and Biostatistics Facility (BF) and was supported in part by an award from the National Institutes of Health, USA P30-CA47904 and National Cancer Institute. The funding agencies had no role in the design or analysis of the trial.

This project was supported by grants from the Leukemia and Lymphoma Society TRP6028–10 and BMS CA180–333. This project used facilities of the UPMC Hillman Cancer Center and was supported in part by award P30-CA47904.

REFERENCES

1. Johnson DE. Src family kinases and the MEK/ERK pathway in the regulation of myeloid differentiation and myeloid leukemogenesis. *Adv Enzyme Regul.* 2008;48:98–112. [PubMed: 18155170]
2. Willman CL, Stewart CC, Griffith JK, et al. Differential expression and regulation of the c-src and c-fgr protooncogenes in myelomonocytic cells. *Proc Natl Acad Sci U S A.* 1987 7;84(13):4480–4. [PubMed: 2440024]
3. Willman CL, Stewart CC, Longacre TL, et al. Expression of the c-fgr and hck protein-tyrosine kinases in acute myeloid leukemic blasts is associated with early commitment and differentiation events in the monocytic and granulocytic lineages. *Blood.* 1991 2 15;77(4):726–34. [PubMed: 1825181]
4. Thomas SM, Brugge JS. Cellular functions regulated by Src family kinases. *Annu Rev Cell Dev Biol.* 1997;13:513–609. [PubMed: 9442882]
5. Corey SJ, Anderson SM. Src-related protein tyrosine kinases in hematopoiesis. *Blood.* 1999 1 1;93(1):1–14. [PubMed: 9864140]

6. Abram CL, Courtneidge SA. Src family tyrosine kinases and growth factor signaling. *Exp Cell Res.* 2000 10;254(1):1–13. [PubMed: 10623460]
7. Mermel CH, McLemore ML, Liu F, et al. Src family kinases are important negative regulators of G-CSF-dependent granulopoiesis. *Blood.* 2006 10 15;108(8):2562–8. [PubMed: 16772601]
8. Harder KW, Parsons LM, Armes J, et al. Gain- and loss-of-function Lyn mutant mice define a critical inhibitory role for Lyn in the myeloid lineage. *Immunity.* 2001 10;15(4):603–15. [PubMed: 11672542]
9. Lowell CA, Fumagalli L, Berton G. Deficiency of Src family kinases p59/61hck and p58c-fgr results in defective adhesion-dependent neutrophil functions. *J Cell Biol.* 1996 5;133(4):895–910. [PubMed: 8666673]
10. Miranda MB, Redner RL, Johnson DE. Inhibition of Src family kinases enhances retinoic acid induced gene expression and myeloid differentiation. *Mol Cancer Ther.* 2007 12;6(12 Pt 1):3081–90. [PubMed: 18065491]
11. Congleton J, MacDonald R, Yen A. Src inhibitors, PP2 and dasatinib, increase retinoic acid-induced association of Lyn and c-Raf (S259) and enhance MAPK-dependent differentiation of myeloid leukemia cells. *Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, UK.* 2012 6;26(6):1180–8.
12. Melnick A, Licht JD. Deconstructing a disease: RARalpha, its fusion partners, and their roles in the pathogenesis of acute promyelocytic leukemia. *Blood.* 1999 5 15;93(10):3167–215. [PubMed: 10233871]
13. Kropf PL, Wang L, Zang Y, et al. Dasatinib promotes ATRA-induced differentiation of AML cells. *Leukemia.* 2010;24(3):663–5. [PubMed: 20033056]
14. Miranda MB, Johnson DE. Signal transduction pathways that contribute to myeloid differentiation. *Leukemia.* 2007 7;21(7):1363–77. [PubMed: 17443228]
15. Schittenhelm MM, Shiraga S, Schroeder A, et al. Dasatinib (BMS-354825), a dual SRC/ABL kinase inhibitor, inhibits the kinase activity of wild-type, juxtamembrane, and activation loop mutant KIT isoforms associated with human malignancies. *Cancer research.* 2006 1 1;66(1):473–81. [PubMed: 16397263]
16. Collins SJ. The role of retinoids and retinoic acid receptors in normal hematopoiesis. *Leukemia.* 2002 10;16(10):1896–905. [PubMed: 12357341]
17. Campo E, Swerdlow SH, Harris NL, et al. The 2008 WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications. *Blood.* 2011 5 12;117(19):5019–32. [PubMed: 21300984]
18. Greenberg P, Cox C, LeBeau MM, et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. *Blood.* 1997 3 15;89(6):2079–88. [PubMed: 9058730]
19. Twardowski PW, Beumer JH, Chen CS, et al. A phase II trial of dasatinib in patients with metastatic castration-resistant prostate cancer treated previously with chemotherapy. *Anticancer Drugs.* 2013 8;24(7):743–53. [PubMed: 23652277]
20. Conley BA, Egorin MJ, Sridhara R, et al. Phase I clinical trial of all-trans-retinoic acid with correlation of its pharmacokinetics and pharmacodynamics. *Cancer Chemother Pharmacol.* 1997;39(4):291–9. [PubMed: 9025769]
21. Lehmann S, Bengtzen S, Broberg U, et al. Effects of retinoids on cell toxicity and apoptosis in leukemic blast cells from patients with non-M3 AML. *Leuk Res.* 2000 1;24(1):19–25. [PubMed: 10634641]
22. Qian SX, Li JY, Hong M, et al. Acute myeloid leukemia in four patients with t(8;21) treated with all-trans retinoic acid as a single agent. *Leuk Lymphoma.* 2008 5;49(5):998–1001. [PubMed: 18464121]
23. Schlenk RF, Frohling S, Hartmann F, et al. Phase III study of all-trans retinoic acid in previously untreated patients 61 years or older with acute myeloid leukemia. *Leukemia.* 2004 11;18(11):1798–803. [PubMed: 15385923]
24. Schlenk RF, Lubbert M, Benner A, et al. All-trans retinoic acid as adjunct to intensive treatment in younger adult patients with acute myeloid leukemia: results of the randomized AMLSG 07–04 study. *Ann Hematol.* 2016 12;95(12):1931–1942. [PubMed: 27696203]

25. Burnett AK, Hills RK, Green C, et al. The impact on outcome of the addition of all-trans retinoic acid to intensive chemotherapy in younger patients with nonacute promyelocytic acute myeloid leukemia: overall results and results in genotypic subgroups defined by mutations in NPM1, FLT3, and CEBPA. *Blood*. 2010 2 04;115(5):948–56. [PubMed: 19965647]
26. Martelli MP, Gionfriddo I, Mezzasoma F, et al. Arsenic trioxide and all-trans retinoic acid target NPM1 mutant oncoprotein levels and induce apoptosis in NPM1-mutated AML cells. *Blood*. 5 28;125(22):3455–65. [PubMed: 25795919]
27. Adedoyin A, Stiff DD, Smith DC, et al. All-trans-retinoic acid modulation of drug-metabolizing enzyme activities: investigation with selective metabolic drug probes. *Cancer Chemother Pharmacol*. 1998;41(2):133–9. [PubMed: 9443626]
28. Luo FR, Yang Z, Camuso A, et al. Dasatinib (BMS-354825) pharmacokinetics and pharmacodynamic biomarkers in animal models predict optimal clinical exposure. *Clin Cancer Res*. 2006 12 1;12(23):7180–6. [PubMed: 17145844]
29. Demetri GD, Lo Russo P, MacPherson IR, et al. Phase I dose-escalation and pharmacokinetic study of dasatinib in patients with advanced solid tumors. *Clin Cancer Res*. 2009 10 01;15(19):6232–40. [PubMed: 19789325]
30. Dai G, Pfister M, Blackwood-Chirchir A, et al. Importance of characterizing determinants of variability in exposure: application to dasatinib in subjects with chronic myeloid leukemia. *J Clin Pharmacol*. 2008 11;48(11):1254–69. [PubMed: 18779376]
31. Boutzen H, Saland E, Larrue C, et al. Isocitrate dehydrogenase 1 mutations prime the all-trans retinoic acid myeloid differentiation pathway in acute myeloid leukemia. *J Exp Med*. 2016 4 4;213(4):483–97. [PubMed: 26951332]

FIGURE 1.
Peripheral blood absolute neutrophil and blast counts for subjects receiving more than 14 days of study drug.

Table 1

. Baseline patient characteristics.

	DL 1 [70 mg dasatinib qd, 22.5 mg ATRA bid] <i>Evaluable</i>	DL 1 [70 mg dasatinib qd, 22.5 mg ATRA bid] <i>Nonevaluable</i>	DL 2 [100 mg dasatinib qd, 22.5 mg ATRA bid] <i>Nonevaluable</i>	TOTAL
Number of subjects	3	3	3	9
Age (range)	69.6 (56–80)	69.6(59–85)	64.3(56–79)	68.2 (56–85)
ECOG status	0.7 (0–1)	1 (1)	0.7 (0–1)	0.8
Refractory AML	3	3	2	8
High grade MDS	0	0	1	1

Table 2.

All Adverse Events, graded by NCI CTCAE Version 4.0.

GRADE	1	2	3
Vomiting	1	2	0
Nausea	0	2	0
Myalgia	0	1	0
Headache	2	1	1
Prolonged QTc	2	2	1
Diarrhea	2	0	0
Neck pain	0	1	0
Rash	0	1	0
Fatigue	0	1	0

Table 3.

Mean (SD) pharmacokinetic parameters of ATRA and dasatinib.

	Dose	C _{max} (ng/mL)	T _{max} (h)	t _{1/2} (h)	AUC _{0-t} (ng•h/mL)	AUC _{0-inf} (ng•h/mL)	V _{ss} (L)	CL (L/h)
ATRA						1		
N=7	22.5 mg/m ²	277 (115)	1.6 (0.4)	0.81 (0.36)	578 (230)	594 (232)	101 (47)	44.2 (19.5)
Dasatinib								
N=6	70 mg	77 (96)	1.7 (2.2)	2.9 (1.5)	178 (160)	206 (173)	2309 (1391)	490 (246)
N=3	100 mg	103 (49)	2.5 (1.3)	2.5 (0.4)	373 (92)	434 (78)	1070 (430)	235 (38)
N=9	All	-	1.9 (1.9)	2.8 (1.2)	-	-	1896 (1280)	405 (233)

Subject	11	10	9	8								
Dose Level	DL1			DL1								
	D0	D14	D28	D0	D14	D28	D0	D14	D28			
Total Blast Count	56%	28%	57%	14%	35%		86%	85%		41%	43%	57%
CD14/CD45 (monocytes)	14	8	9	8	8		0	0		2	31	1
CD36/CD64 (monocytes)	99	89	91	6	10		0	1		2	NA	NA
CD13/CD16 (maturing myeloid)	0	NA	NA	2	NA		1	NA		2	NA	NA
CD13/CD11b (maturing myeloid)	1	NA	NA	6	NA		0	NA		2	NA	NA
CD34/CD33 (myeloblast)	0	1	0	24	25		0	0		51	20	43
CD117/CD33 (immature myeloid)	0	17	54	12	9		0	77		87	34	84

Total Blast Count was performed morphologically