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Phase II pilot study of oral dasatinib in patients with higher-risk myelodysplastic syndrome (MDS) who failed conventional therapy☆

Vu H. Duong[a], Michael V. Jaglal[b], Ling Zhang[b], Vishakha Kale[c], Jeffrey E. Lancet[b], Rami S. Komrokji[b,*], Alan F. List[b]

[a] University of Maryland Greenebaum Cancer Center, Baltimore, MD, United States
[b] H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, United States
[c] South Texas Veterans Health Care System, San Antonio, TX, United States

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ABSTRACT

Given evidence for the role of Src family kinases, especially Lyn kinase, in myeloblast proliferation and the *in vitro* inhibitory activity of dasatinib on Src and Lyn, we conducted a phase II study to assess overall response to 100 mg/day dasatinib in patients with higher-risk myelodysplastic syndrome (MDS), chronic myelomonocytic leukemia, or acute myeloid leukemia arising from MDS and who had failed prior treatment with azanucleoside analogs. Among 18 patients treated, 3 responded, 4 had stable disease, and 10 experienced disease progression. Toxicities were limited and consistent with previous reports. Dasatinib appears to be safe but with limited efficacy.

1. Introduction

Higher-risk myelodysplastic syndrome (MDS) has a poor prognosis with a high rate of progression to acute myeloid leukemia (AML). Allogeneic hematopoietic stem cell transplant (ASCT) is the only potentially curative option for this disease; however, it is associated with considerable treatment-related morbidity and mortality. The azanucleosides have potent disease-altering effects in patients; however, survival is poor upon treatment failure with no effective salvage alternatives [1–3]. Therefore, novel therapeutics that impact cellular events inherent to the pathobiology of MDS may offer benefit.

Src family kinases (SFKs) consist of 9 members (c-Src, Lyn, Hck, Fyn, Yes, Blk, Lck, Yrk, and Fgr) that regulate multiple cellular functions ranging from proliferation and differentiation to cell migration [4,5]. They are actively involved in signaling pathways important in the initiation and progression of various human malignancies. In AML, SFKs are highly phosphorylated compared to normal hematopoietic progenitors, including the leukemia stem cell-rich compartment (CD34+, CD38−, CD123+) [6]. Lyn kinase in particular plays a critical role in the proliferative response of myeloid leukemia cells to cytokine signals and is constitutively active in AML myeloblasts and MDS megakaryocyte progenitors [7,8].

Inhibition of Lyn kinase activity using different strategies, such as antisense oligonucleotides, small interfering RNA, and Src kinase inhibitors suppresses leukemia cell growth [6,7,9]. We previously reported that Lyn kinase inhibition in leukemic cells suppresses STAT5 activation accompanied by induction of apoptosis in sensitive cells. Furthermore, treatment of primary bone marrow specimens from 13 AML patients and 1 chronic myelomonocytic leukemia (CMML) patient with the dual Src/Abl inhibitor SKI-606 inhibited proliferation of leukemic blasts in a concentration-dependent manner in 5 out of 14 samples with a 50% inhibitory concentration of less than 500 nM [9].

Dasatinib is an orally available multi-kinase inhibitor that is FDA approved for the treatment of chronic myelogenous leukemia [10,11]. It has activity against a broad spectrum of tyrosine kinases, including SFKs, breakpoint cluster region-Abelson (BCR-ABL), c-KIT, platelet-derived growth factor receptor-β, and EphA. It is less potent against 16 other unrelated protein tyrosine kinases and serine/threonine kinases [12,13]. Given evidence for the role of SFKs, especially Lyn kinase, in myeloblast proliferation and the *in vitro* inhibitory activity of dasatinib on Src and Lyn, we conducted

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* Corresponding author at: H. Lee Moffitt Cancer Center and Research Institute, 12902 Magnolia Drive, FOB-3, Room 3117, Tampa, FL 33612, United States. Tel.: +1 813 745 4291; fax: +1 813 745 5617.
E-mail address: rami.komrokji@moffitt.org (R.S. Komrokji).

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a phase II pilot study to assess response to dasatinib treatment in patients with International Prognostic Scoring System (IPSS)-defined higher-risk MDS [14].

2. Materials and methods

2.1. Study design

This was a single-center, open-label, 2-stage phase II study of dasatinib in patients with intermediate-2 or high-risk MDS by IPSS, CMML, and MDS/AML with ≤30% blasts (ClinicalTrials.gov Identifier NCT00624585). The primary objective was to estimate the overall response rate after 16 weeks of study treatment according to International MDS Working Group (IWG) 2006 Criteria for Response Assessment [15]. Secondary objectives were to assess the rate of hematologic improvement, time to AML progression, median duration of response, and overall survival, as well as to assess the relationship between response to study treatment and inhibition of Src-Tyr^{416} phosphorylation in bone marrow myeloblasts. The study was approved by the Moffitt Cancer Center Scientific Review Committee and University of South Florida Institutional Review Board. Informed written consent was obtained from all patients.

2.2. Patient eligibility and selection

Patients ≥18 years of age with a documented bone marrow diagnosis of MDS or MDS/myeloproliferative neoplasm (MPN) with intermediate-2 or high-risk IPSS scores were eligible for study treatment. Patients with AML with multilineage dysplasia with <30% blasts who declined induction chemotherapy or were deemed unfit for induction chemotherapy were also eligible. Patients previously treated with azanucleosides were eligible, provided that their last dose was administered no less than 2 months before the first dose of dasatinib. Prior investigational therapy was permitted if discontinued at least 4 weeks before dasatinib treatment. Patients were also required to have an ECOG performance status of 0–2, adequate liver function (total bilirubin <2 times the upper limit of normal (ULN) and AST/ALT ≤ 2.5 times the institutional ULN) and renal function (serum creatinine <1.5 times the ULN), and a prothrombin time and partial thromboplastin time of less than 1.5 times the ULN. Patients with a leukocyte count >50,000 off hydroxyurea for greater than 72 h, with another malignancy that required radiotherapy or systemic treatment within the past 3 years, or who were receiving concurrent therapy for MDS or AML were excluded. Patients with medical conditions that were judged potentially to increase the risk of toxicity on dasatinib were excluded, including those with pleural or pericardial effusions, active coronary arterial disease or congestive heart failure, prolonged QT syndrome, significant arrhythmias, and significant bleeding disorders. Bisphosphonate use was prohibited. Pregnant or breastfeeding women were excluded, and females of child-bearing age were required to use adequate contraception.

2.3. Treatment

All patients received a continuous daily oral dose of dasatinib 100 mg. In the absence of at least a partial remission after 8 weeks of treatment or drug intolerance, the dose was increased to 150 mg/day. Final response to study treatment was assessed after 16 weeks of dasatinib treatment. Responding patients continued dasatinib for up to 48 weeks in the absence of disease progression, limiting toxicity, or secondary treatment failure. Treatment was held for any ≥ grade 3 non-hematological adverse event with suspected drug association until resolution to < grade 2. Upon resolution of the toxicity, dasatinib was dose-reduced to 70 mg if the toxicity occurred at the 100-mg dose and to 50 mg if the toxicity occurred at the 70-mg dose. Patients who did not experience resolution of non-hematological adverse effects to < grade 2 within 4 weeks were removed from the study. Similarly, dasatinib treatment was interrupted and dose-reduction permitted for a decline of 50% in the absolute neutrophil count (ANC) if the baseline ANC was ≤500/μL and for a decrease in ANC to <500/μL for patients with a baseline ANC >500/μL. Dose modification for thrombocytopenia was only done at the completion of a 28-day cycle, and dasatinib was held when the platelet count fell below 30,000/μL and resumed at the next lower dose upon recovery to ≥30,000/μL. Patients who were intolerant of 50 mg of daily dasatinib were removed from the study. Study treatment was discontinued for progressive disease, withdrawal of consent, pregnancy, or any clinical adverse event, laboratory abnormality, or intercurrent illness, which, in the opinion of the investigators, indicated that continued treatment with dasatinib was not in the best interest of the patient.

2.4. Evaluation and follow-up

Patients were evaluated by history and physical examination every 4 weeks, and complete blood counts (CBC) were obtained every 2 weeks. Bone marrow aspiration and biopsy (including cytogenetics by karyotyping and phospho-Src-Tyr^{416} by immunohistochemical staining) were performed at screening, at 8 and 16 weeks of treatment, at discontinuation of study therapy, and every 16 weeks in responding patients. If a patient continued on study drug beyond 16 weeks but did not experience a hematologic response, CBC was obtained every 2 weeks until response was documented. After hematologic response was achieved, CBCs were monitored every 4 weeks.

2.5. Assessment of toxicity and response

All patients who received study drug were assessed for response and toxicity. Adverse events were assessed at every visit according to NCI Common Terminology Criteria for Adverse Events version 3.0 and deemed to be likely related, possibly related, not likely related, or not related to the study drug. All responses were assessed according to the 2006 IWG Response Criteria.

2.6. Immunohistochemical staining for phospho-Src-Tyr^{416}

Immunohistochemical staining for phospho-Src-Tyr^{416} was performed using a Ventana Discovery XT automated system (Ventana Medical Systems, Tucson, AZ) according to the manufacturer's recommended protocol with proprietary reagents. Slides were deparaffinized on the automated system with EZ Prep solution (Ventana). Enzymatic retrieval method was used in Protease 1 (Ventana). The rabbit primary antibody that recognizes the human activation loop phospho-Src-Tyr^{416} (number 05–677, Millipore, Darmstadt, Germany) was used at a 1:200 concentration in PSS diluent (Ventana) and incubated for 60 min. The Ventana OmniMap anti-mouse secondary antibody was applied for 16 min. The detection system used was the Ventana ChromoMap kit, and slides were then counterstained with hematoxylin. Slides were then dehydrated and coverslipped as per normal laboratory protocol.

2.7. Statistics

Sample size was calculated using Simon's minimax 2-stage design. Eighteen patients were to be accrued in the first stage of the study. If 2 or fewer responses were observed, then the trial would be terminated for futility. Otherwise, if at least 3 responses were observed in the first stage, then an additional 8 evaluable patients would be enrolled during stage 2. If 6 or fewer responses were observed by the end of stage 2, then the protocol treatment would be deemed ineffective. However, if 7 or more responses were observed, the response rate to the protocol treatment would be at least 30%. The overall significance level of the design was 0.14 with a power of 0.70.

Overall survival was defined as the time from enrollment to the date of death, and patients who were lost to follow-up were censored at the time they were last known to be alive. Time to AML progression was defined as the time from enrollment to the date of initial diagnosis of AML with ≥20% blasts. Those who died without disease progression as defined by IWG 2006 criteria were censored at the time of death. Both outcomes were assessed by the Kaplan–Meier method.

3. Results

3.1. Patients

Eighteen patients were enrolled in the study at the Moffitt Cancer Center. Baseline patient characteristics are summarized in Table 1. Patients were generally older (median age 73.5) with a male predominance (55%). Most patients had refractory anemia with excess blasts (RAEB)-2 MDS (55%), and all patients failed treatment with at least one azanucleoside analog. Three patients who had lower-risk disease were enrolled but were still evaluated for response and toxicity. Upon central review, 3 patients were found to have AML with bone marrow blast percentage >30%.

3.2. IWG response and disposition

Three of the 18 patients (17%) had a response to study treatment, two of whom achieved marrow complete remission (mCR) without hematologic improvement. One responder was a 73-year-old male with RAEB-2 (13% blasts) at baseline and an IPSS score of 2.5. He achieved mCR and subsequently received an ASCT 4 months after starting study therapy, but died 9 months after his transplant without evidence of relapse or disease progression. The second patient achieving mCR was a 68-year-old female with RAEB-2 (13% blasts) and an IPSS score of 1.5. She discontinued therapy after 23 weeks due to lack of improvement in peripheral blood counts and died 6 months later without disease progression. The third patient with clinical benefit was a 75-year-old female with acute myelomonocytic leukemia with 14% blasts and 18% promonocytes at baseline, who experienced a >50% reduction in bone marrow blasts to 1.5%