Efficacy of the Farnesyl Transferase Inhibitor, Zarnestra™ (R115777), in Chronic Myeloid Leukemia and Other Hematologic Malignancies


From the Department of Leukemia
The University of Texas M.D. Anderson Cancer Center
Houston, Texas,
and *Johnson & Johnson Pharmaceutical Research & Development, L.L.C.

Jorge Cortes, M.D. is a Clinical Research Scholar for the Leukemia & Lymphoma Society

Short title: R115777 in hematologic malignancies

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Address correspondence to:
Jorge Cortes, M.D.
Associate Professor of Medicine
Department of leukemia
M.D. Anderson Cancer Center
1515 Holcombe Blvd. Box 428
Houston, TX 77030
Phone: 713-794-5783
Fax: 713-794-4297
e-mail: jcortes@mdanderson.org

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SUMMARY

We investigated the clinical activity of the farnesyl transferase inhibitor (FTI) ZARNESTRA (R115777) in patients with chronic myelogenous leukemia (CML, n=22) in chronic (CP, n=10), accelerated (AP, n=6), or blastic phase (n=6), and patients with myelofibrosis (MF), or multiple myeloma (MM). ZARNESTRA was administered at 600 mg orally twice daily for 4 weeks every 6 weeks. Seven patients with CML (CP n=6, AP n=1) achieved a complete (CHR n=5) or partial (PHR n=2) hematologic response. Four patients had a minor cytogenetic response. Responses were transient with a median duration of 9 weeks (range, 3 to 23 weeks). Two patients discontinued therapy because of toxicity while in CHR. In MF, 2 had a significant decrease in splenomegaly, one had normalization of WBC and differential, and one became transfusion independent. One patient with MM had a reduction in monoclonal protein of 34%. Adverse events included nausea in 22 patients (55%; all Grade 2 or lower) and fatigue in 19 (48%) (Grade ≥3 in 1). Other Grade 3 or 4 toxicities included skin rash (4 patients, 10%), peripheral neuropathy (2 patients, 5%), and liver toxicity (2 patients, 5%). Patients who responded to therapy had significantly higher plasma VEGF concentrations prior to treatment compared with those who did not respond. Plasma concentrations decreased significantly during therapy among responders. ZARNESTRA, as administered in this study, showed clinical activity in patients with CML and MF, and possibly MM. The effect on VEGF needs to be further investigated to determine whether this might be a possible mechanism of action of ZARNESTRA. Further investigation with ZARNESTRA with alternative schedules and combinations is warranted.
INTRODUCTION

The ras family of proto-oncogenes comprises a group of G-proteins that have the ability to bind guanine nucleotides.\textsuperscript{1,2} Ras is synthesized in the cytoplasm as a precursor protein that requires additional post-translational modifications in order to attach to the inner surface of the plasma membrane, a prerequisite for Ras-mediated signal transduction. These modifications are accomplished by a prenylation reaction involving the attachment of a 15-carbon farnesyl group to the C-terminal cysteine residue. This reaction is mediated by an enzyme called farnesyl protein transferase (FPT).\textsuperscript{3,4} Alternatively, prenylation may be accomplished by addition of a 20-carbon geranylgeranyl isoprenoid mediated by geranylgeranyl-protein transferase (GGPT).

Ras mutations and Ras protein activation are frequent features of malignant transformation. Approximately 30\% of human cancers have been associated with ras mutations.\textsuperscript{5,6} The frequency of ras mutations varies in hematologic malignancies, from 5\% to 15\% in acute lymphoblastic leukemia and up to 65\% in chronic myelomonocytic leukemia\textsuperscript{2,5,6}. Ras activation may occur by mechanisms other than mutations. A prominent example is activation of Ras by the bcr/abl chimeric gene.\textsuperscript{7,8} Therefore, inhibition of Ras activation has been investigated as an antineoplastic therapy.\textsuperscript{9} One approach to Ras inhibition is inhibition of FPT.\textsuperscript{10-12} ZARNESTRA (R115777) is a potent nonpeptidomimetic FPT inhibitor (FTI) with significant antitumor effects in preclinical studies.\textsuperscript{13}

In this study, we investigated the activity of ZARNESTRA in patients with Philadelphia chromosome (Ph)-positive chronic myeloid leukemia (CML), myelofibrosis (MF), or multiple myeloma (MM). In addition, plasma concentrations of vascular
endothelial growth factor (VEGF) have been found to be elevated in CML,\textsuperscript{14,15} and increased expression of VEGF correlates with poor prognosis.\textsuperscript{15} In addition, one of the proposed effects of FPT inhibition is suppression of angiogenesis with decreased expression of VEGF.\textsuperscript{16} Because of the clinical significance of VEGF in CML, we investigated whether ZARNESTRA has any in vivo effect on VEGF and other angiogenic factors to determine whether any clinical effect may be mediated through this mechanism.

**PATIENTS AND METHODS**

Adult patients 18 years or older with CML, MF or MM were eligible for this study. Eligibility criteria included the following: 1) performance status (ECOG) $\leq 2$; 2) serum creatinine $<2$ mg/dL and total bilirubin $<2$ mg/dL; 3) no evidence of severe heart disease (NYHA class III or IV); and 4) signed informed consent. Patients were eligible regardless of whether or not they had ras mutations. Patients with CML were eligible whether they were in the chronic (CP), accelerated (AP), or blastic phase (BP). Patients in CP should have received prior therapy with interferon alpha (IFN-A) and failed to achieve or sustain a hematologic or cytogenetic response, or be intolerant to IFN-A. Patients in AP or BP were eligible irrespective of their prior therapy. At the time the study was conducted, imatinib mesylate (Gleevec\textsuperscript{TM}) was not widely available; therefore prior therapy with imatinib was not required. Patients with MM were required to be refractory or have relapsed after therapy with a regimen such as VAD (vincristine, doxorubicin and dexamethasone). Patients who had not received a stem cell transplant were either ineligible or had refused transplant. Patients with MM were required to have
a quantifiable serum or urine paraprotein, bone marrow plasmacytosis of 5% or more, and an absolute neutrophil count of $\geq 10^9$/L and platelet count of $\geq 75 \times 10^9$/L.

Prior to the start of therapy, all patients had a complete history and physical exam, a complete blood count, SMA-12, a bone marrow (BM) aspiration (and biopsy when indicated) and cytogenetics. The M-protein spike was measured in patients with multiple myeloma. All of these studies were repeated periodically while the patient was receiving treatment.

**Treatment schedule**

Patients received ZARNESTRA at a starting dose of 600 mg orally twice daily (BID) for 4 weeks every 6 weeks (i.e., 4 weeks on, 2 weeks off). Patients who developed Grade $\geq 3$ toxicity (graded according to the NCI Common Toxicity Criteria v2.0) had their treatment interrupted. For patients who had evidence of antitumor activity, therapy was re-instituted upon improvement of the toxicity to Grade $\leq 1$ with a dose reduction to 300 mg orally BID. Therapy was interrupted for patients with CML in CP or MM if their absolute neutrophil count was $< 10^9$/L or their platelet count $< 50 \times 10^9$/L. Therapy was resumed at the same dose if recovery above these levels occurred within 2 weeks, or at 300 mg orally BID if recovery was longer than 2 weeks. Patients in AP or BP of CML or with MF could continue therapy uninterrupted with counts below these levels when it was considered that the cytopenias were related to the disease. Patients with CML could have hydroxyurea for the first month of therapy for white cell counts (WBC) $> 30 \times 10^9$/L or anagrelide for platelets $> 700 \times 10^9$/L. Hydroxyurea was not allowed after the first month of therapy, but anagrelide could be continued beyond this time if needed. Patients
who achieved a response could receive continued treatment for up to 12 months. The
dose of ZARNESTRA could be adjusted to achieve an absolute neutrophil count of
\( \geq 10^9/L \) and a platelet count of \( \geq 50 \times 10^9/L \), and the schedule could be modified as needed
to achieve this target, (i.e., 14-day treatment every 28 days).

**Definitions of response and disease stages**

AP was defined as the presence of any one of the following: 1) peripheral blood
(PB) or BM blasts \( \geq 15\% \); 2) PB or BM blasts + promyelocytes \( \geq 30\% \); 3) PB or BM
basophils \( \geq 20\% \); 4) platelets \( <100 \times 10^9/L \) unrelated to therapy; 5) clonal evolution; or 6)
hemoglobin \( <7\,\text{g/dL} \) unrelated to therapy or bleeding. Blast phase was defined as the
presence of \( \geq 30\% \) blasts in the PB or BM, or extramedullary disease outside the liver or
spleen.

Responses in CML were defined as previously reported.\(^{17}\) Briefly, a complete
hematologic response (CHR) was defined as WBC \( <10 \times 10^9/L \), platelet count \( <450
\times 10^9/L \), no immature PB cells (blasts, promyelocytes, myelocytes), and disappearance of
all signs and symptoms of leukemia. This was further categorized by the best cytogenetic
response: 1) complete: Ph-positive 0\%, 2) partial: Ph-positive: 1\% to 34\%, and 3) minor:
Ph-positive 35\% to 90\%. A major cytogenetic response included complete plus partial
cytogenetic responses (Ph-positive less than 35\%). Partial hematologic response (PHR)
was defined as CHR except for the persistence of immature cells in PB and/or persistent
splenomegaly or thrombocytosis (\( >450 \times 10^9/L \)) but at least 50\% less than pretreatment.

In MM, a CR was defined as disappearance of serum and/or urine M-protein on 2
determinations at least 4 weeks apart, with \(<5\% \) plasma cells in the BM and PB,
resolution of any soft-tissue plasmacytomas present at the start of therapy, and resolution of all signs and symptoms of disease. A PR was defined as a reduction of the monoclonal protein by $\geq 50\%$ on two determinations at least 4 weeks apart, reduction in soft-tissue plasmacytoma by $\geq 50\%$, and decrease in bone pain from Grade 2 to $\leq 1$.

In MF, CR was defined as normalization of the PB for at least four weeks, with an absolute neutrophil count $>10^9$/L with no immature cells, and platelet count $>100 \times 10^9$/L. Partial response was considered by the presence of at least 2 of the following: 1) hemoglobin increase by $\geq 2\text{g/dL}$ and to $>9\text{g/dL}$, plus independence from transfusions, 2) a platelet count increase by 100% and to $>50 \times 10^9$/L, plus transfusion independence, 3) a neutrophil count increase by 100% and to $>10^9$/L, and 4) reduction of splenomegaly by 50%.

**Laboratory correlative studies**

Assessment of ras mutations was performed by sequencing. Enzyme-linked immunosorbent assay (ELISA) for VEGF, basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF) and tumor necrosis growth factor-$\alpha$ (TNF-$\alpha$) was performed using commercially available kits from R&D Systems (Minneapolis, MN). We followed the protocols recommended by the manufacturer. Briefly, plasma was collected using EDTA and stored at -82°C. Patients’ plasma samples were added to separate microplates, each containing a specific monoclonal antibody. The mixtures were incubated at room temperature for 2 hours. The plates were washed 3 times to remove any unbound substances. Enzyme-linked polyclonal antibodies specific for each protein were added to the wells, and mixtures were incubated at room temperature for 2 hours.
followed by another washing to remove any unbound antibody or enzyme reagent. A substrate solution was added to the wells, and a blue color developed. The intensity of the blue color was proportionate to the amount of cytokine bound in the initial step. The color development was stopped, and the intensity of the color was measured and compared with a standard curve. Reading was done at 450-nm wavelength per the manufacturer’s recommendations.

**Statistical Analysis**

The Kruskall-Wallis test was used to compare values between groups.

**RESULTS**

Between February 2001 and October 2001, 40 patients were treated. These include 22 patients with CML, 10 with MM and 8 with MF. The clinical characteristics of patients by diagnosis are presented in Table 1. Thirteen patients were investigated for N- or K-ras mutations; only 1 patient (8%; CML-BP) had a mutation (K-ras). The results will be described by disease group.

**Chronic Myeloid Leukemia**

Twenty-two patients with CML in CP (n=10), AP (n=6) or BP (n=6) were treated. The median time from diagnosis to therapy was 23 months (range, 5 to 82 months). All patients had received at least one prior therapy for CML besides hydroxyurea, and the median number of prior regimens was 2 (range, 1-4). Twenty patients (91%) had received prior IFN-A; the 2 patients who had not received IFN-A were in BP and had failed other
therapies (imatinib mesylate and troxacitabine, respectively). Two patients received pegylated IFN-A after failing conventional IFN-A. Seventeen patients (77%) had received imatinib and were resistant, refractory or intolerant to it. Six patients had received other investigational agents including troxacitabine (n=2), homoharringtonine + ara-C (n=2), decitabine (n=1), or oral idarubicin (n=1); 1 patient had relapsed in AP after an allogeneic bone marrow transplant.

Patients received ZARNESTRA therapy for a median of 8 weeks (range, 1 to 25 weeks). Seven patients (0.32; 95%CI 0.14 to 0.55) achieved a complete (n=5) or partial (n=2) hematologic response. All but one of the responses occurred among patients in CP. Thus, 6 of 10 patients in CP (0.6; 95%CI 0.26 to 0.88) responded. All responses occurred in the absence of hydroxyurea and only one patient (PHR) required anagrelide. Among responders, 4 (1 AP, 3 CP) had reductions in the percentage of Ph-positive metaphases. Three of these patients achieved a minor cytogenetic response; the fourth started with 90% metaphases Ph-positive and decreased on therapy to 65%. The responses were transient, with a median duration of response of 9 weeks (range, 3 to 23 weeks). Five patients discontinued therapy because of loss of response and 2 because of toxicity. Two patients had an ongoing CHR (one of them also a minor cytogenetic response) at the time treatment was discontinued because of toxicity (fatigue and skin toxicity, respectively). These patients received therapy for 7 and 12 weeks, respectively. All other patients had lost their response at the time they stopped therapy. Eleven patients (50%; CP n=8, AP n=3) were alive after a median follow-up of 11 months. The median survival was 42 weeks. Among patients in CP, the median survival has not been reached. Four patients
have transformed to accelerated (n=2) or blastic (n=2) phase, and 2 of them died after transformation.

**Myelofibrosis**

Eight patients with MF were treated. Two patients had received therapy with transfusion support only, 3 had received recombinant human erythropoietin (rhEPO) and transfusion support, and 3 had received multiple therapies including hydroxyurea (n=2), interferon (n=2), rhEPO, 9-nitrocamptothecin, thalidomide, and anabolic steroids (n=1 each). Six patients had splenomegaly with a median of 12.5 cm below the costal margin (BCM) (range, 3 to 18 cm), and 3 had hepatomegaly of 6, 8, and 15 cm BCM.

Four patients received only 1 course of therapy: 2 discontinued therapy because of toxicity (Grade 3 skin rash), 1 was lost to follow-up, and 1 had progression to AML. Two patients were treated for 22 and 28 weeks, respectively, and had a reduction in spleen size from 18 cm BCM (in both cases) to 9 and 10 cm BCM, respectively. Both remained PRBC-transfusion dependent. Two patients are still receiving therapy 24 and 33 weeks after being included on this trial. One patient had received thalidomide and had become RBC-transfusion independent with that therapy. However, the patient developed progressive splenomegaly, with an increase in WBC and a differential left shift. Thalidomide was discontinued and ZARNESTRA was started. The bone marrow at the time showed extensive myelofibrosis. The spleen decreased in size from 6 cm BCM to undetectable, the WBC count normalized and the immature forms disappeared from the peripheral blood. The patient continues to be RBC-transfusion independent. The second patient (Figure 1) had received rhEPO and thalidomide without a response, and was
requiring transfusions of RBC at least once every week. Two months after the start of therapy, he became transfusion independent and his platelet count improved from 50 x10⁹/L to 150 x10⁹/L. The response has been sustained after 33 weeks of therapy.

Three patients have died at 6, 12 and 39 weeks, respectively, from the start of therapy with ZARNESTRA. Five patients are alive with a median follow-up of 32 weeks (range, 22 to 49 weeks).

**Multiple Myeloma**

Ten patients with refractory or relapsed MM were treated. Patients had received a median of 4 prior treatment regimens (range, 1 to 6), including VAD in 7 patients, thalidomide in 7, and autologous stem cell transplant in 3. The median time on therapy was 7 weeks (range, 2 to 14). The median M-paraprotein spike at the start of therapy was 2.7 g/dL (range, 1.3 to 6.2 g/dL) compared with 2.9 g/dL (range, 2.1 to 7.3 g/dL) (p=0.10) at the end of therapy. One patient had a reduction of the monoclonal protein of 34% and one of 6%. All others had no change or an increase in the monoclonal protein.

Six patients died 18.5 weeks (range, 4 to 48 weeks) from the start of therapy. Four patients are alive with a median follow-up of 41 weeks (range, 12 to 48 weeks).

**Toxicity**

The median duration of therapy was 7 weeks (range, 1 to 28 weeks). Toxicity is presented in Table 2. Nausea and vomiting were the most common side effects, occurring in 22 patients (55%), but were always Grade ≤2 and responded to symptomatic treatment. Fatigue was noted in 19 patients (48%) and resulted in discontinuation of therapy in 1
(3%) patient with CML after 12 weeks on therapy while still in CHR with a minor cytogenetic response. The symptoms persisted after a dose reduction to 200 mg PO BID and finally resolved 4 weeks after treatment was discontinued. Grade 3 or 4 skin toxicity was noted in 4 patients (10%), including one patient with CML who had to discontinue therapy while still in CHR. This was in the form of a diffuse, pruritic rash, which recurred upon re-challenge at a lower dose. Peripheral neuropathy Grade 3 or 4 was seen in 3 patients (8%) in the form of painful dysesthesias in the lower extremities. Symptoms resolved after discontinuation of the therapy but one patient developed progressive peripheral neuropathy that had not resolved at the time of her death from progressive disease 3 months after discontinuation of ZARNESTRA. Hematologic toxicity can more uniformly be assessed among patients who started with normal or high platelets (n=22) and neutrophils (n=26), or hemoglobin (n=11) at the start of therapy. Grade ≥3 neutropenia was seen in 15 patients (58%), thrombocytopenia in 6 (27%), and anemia in 2 (18%). Twenty-three patients (58%) required dose interruptions and/or reductions because of myelosuppression or extramedullary toxicity.

**Effects on angiogenic factors**

Plasma concentrations of angiogenic factors, including VEGF, bFGF, HGF, and TNF-α were measured at baseline and after 7 and 14 days of therapy. Sequential evaluation was available for 25 patients (63%): 15 with CML, 6 with MM, and 4 with MF. As previously reported, plasma concentrations of VEGF, bFGF, and HGF were elevated at baseline among patients with CML.¹⁴ Similar increments were seen among patients with MM and MF and were not statistically significantly different compared with
patients with CML. Sequential measures did not show any significant change during the first 2 weeks of therapy (Table 3). However, when the change in VEGF concentrations during the first 14 days of therapy was evaluated for responders (i.e., CML patients with at least a PHR, or myelofibrosis with hematologic improvement) versus nonresponders, a statistically significant decline was observed in patients who responded to therapy compared with those who did not respond (Figure 2). As shown, this difference is mostly attributable to very high baseline concentrations among patients who responded (median 668.8 pg/mL, range 28.8 to 1166.3 pg/mL) compared with nonresponders (median 78.88 pg/mL, range 21.2 to 469.8 pg/mL) (p=0.009). The only value among responders within the range for normal individuals as identified in our laboratory was for a patient with myelofibrosis; the median concentration at the start of treatment for patients with CML who responded was 674.9 pg/mL (range, 144.9 to 1166.3 pg/mL) compared to 98.6 pg/ml (range, 19.6 to 1085 pg/ml; p=.001) for CML non-responders. There was no significant difference in the values after 7 and 14 days of therapy between responders and nonresponders. No significant difference was observed in any of the other angiogenic factors analyzed.

**Discussion**

R115777 is a nonpeptidomimetic inhibitor of FPT that has demonstrated activity in patients with acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS). Our study demonstrated its modest activity in patients with CML and MF. No activity was noted in patients with MM.
Preclinical studies have demonstrated significant anti-CML activity of FTI\textsuperscript{21,22}. Peters et al. demonstrated inhibition of proliferation of \textit{bcr/abl}-transformed BaF3 cells and primary human CML cells after incubation with SCH66336, another nonpeptidomimetic FTI\textsuperscript{21}. Mice treated with SCH66336 after being injected with \textit{bcr/abl}-BaF3 cells survived, whereas those who received no treatment after the injection of these cells died within 4 weeks\textsuperscript{21}. Similar results were obtained in a \textit{bcr/abl}-positive acute lymphoblastic leukemia murine model using SCH66336\textsuperscript{22}. In addition, SCH66336 has been reported to overcome resistance to imatinib. Hoover et al. reported that SCH66336 inhibited proliferation of imatinib-resistant cell lines and hematopoietic colony formation from patients with CML unresponsive to imatinib\textsuperscript{23}, and Nakajima et al. demonstrated apoptosis induced by SCH66336 in imatinib-resistant cells\textsuperscript{24}. In this clinical trial, we report demonstrable albeit modest activity in 33\% of patients with CML.

Most of the responses were transient but it is possible that therapy may have been discontinued prematurely in some patients. The study allowed for the use of hydroxyurea only for the first 4 weeks, and responding patients were withdrawn (or discontinued) from therapy at the first sign of rising WBC. This frequently occurred at the end of the periods when ZARNESTRA was not taken. Since FTIs are mostly cytostatic, it is reasonable to hypothesize that a more prolonged exposure may be required to demonstrate a sustained response. Thus, alternative schedules, including using lower-dose long-exposure schedules might be preferable and are currently being investigated. Interestingly, we did not observe responses among patients in BP, whereas Karp et al.\textsuperscript{18} reported a partial response in 2/2 patients with Ph-positive CML in BP. The clinical
results reported here with ZARNESTRA, and the reported synergy with imatinib\textsuperscript{23,24} provide the basis for ongoing studies combining FTI and imatinib.

The responses observed in patients with MF are intriguing. Although 2 patients showed only modest (but clinically significant) decreases in splenomegaly, 2 patients had a more notable response with normalization of the peripheral blood abnormalities, 1 of them (Figure 1) becoming transfusion independent. R115777 has been reported to have significant in vitro activity in MF with myeloid metaplasia and other myeloproliferative disorders. Concentrations of 5 to 24 nM selectively inhibited the in vitro growth of myeloid and megakaryocytic progenitor colonies obtained from patients with MF, essential thrombocytemia and polycythemia vera.\textsuperscript{25} Further exploration of this agent in myeloproliferative disorders is ongoing.

The minimal activity in MM was disappointing. In vitro studies have suggested that FTIs may have significant activity in this disease. Bolick et al. reported significant activity of FTI-277 in human myeloma cell lines.\textsuperscript{26} A different FTI, perillic acid, showed significant activity against myeloma cell lines and primary cells from patients, while relatively sparing non-myeloma bone marrow elements.\textsuperscript{27} Alsina et al recently reported results on 12 evaluable patients with MM treated with this agent\textsuperscript{28}. Half of the patients had a reduction in the monoclonal protein of <25\% compatible with disease stabilization. The dose and schedule used in our trial may not have been ideal and a more prolonged therapy may be required. Alsina et al used a dose of 300 mg twice daily for 3 weeks, repeated every 4 weeks. Four of the 6 patients with a response were able to stay on therapy for at least 4 cycles (i.e., 16 weeks), whereas the median time on treatment for
our patients was 7 weeks (range, 2 to 14 weeks). Therefore, schedules of ZARNESTRA that allow for a more prolonged administration should be investigated further.

The actual mechanism of action of FTI is not yet clear. Clinical responses in this trial and others\textsuperscript{18-20} have been unrelated to Ras mutations. As mentioned earlier, Ras can be activated by other mechanisms. However, farnesylation is not unique to Ras,\textsuperscript{2,11,29} and Ras-independent mechanisms of cell growth inhibition by FTI such as gain of RhoB that has been prenylated by GGPT\textsuperscript{30} and inhibition of the mitotic kinasins CENP-E and CENP-F\textsuperscript{31} have been reported. Additionally, Ras can be prenylated by GGPT.\textsuperscript{2,11,29} Although combinations of FTI and GGPTase inhibitors may be synergistic,\textsuperscript{26,32} initial animal studies have resulted in significant toxicity,\textsuperscript{32} which may be related to the ubiquitous nature of proteins requiring GGPT. Thus, dual inhibition is unlikely to be beneficial in the clinic.

One recently proposed mechanism of action of FTIs is through inhibition of angiogenesis. Several investigators have reported that different FTIs inhibit angiogenesis in a variety of models.\textsuperscript{16,33-36} Ras activation (e.g., via mutations) upregulates VEGF expression,\textsuperscript{37,38} and inhibition of Ras through FPT inhibition has led to significant decrease in the expression and secretion of VEGF.\textsuperscript{16,33-36} We have previously reported a significant increase in angiogenesis in patients with acute and chronic leukemias.\textsuperscript{14} Patients with CML had the highest increase in VEGF plasma concentrations, and increased cellular concentrations of VEGF are associated with an adverse outcome, independent of other prognostic factors.\textsuperscript{15} These results suggest a possible role of VEGF in the pathogenesis and or progression of CML. In this report, we identified a significant decrease in the plasma concentrations of VEGF among patients who responded to
ZARNESTRA. In addition, patients who responded had a higher baseline VEGF plasma concentration. The sample size studied is small and this observation will require further confirmation in other studies. However, these results showed for the first time in vivo a possible antiangiogenic effect of ZARNESTRA and suggests that patients with high concentrations of VEGF might be more likely to respond to this agent.

As mentioned, toxicity limited continuation of therapy in several patients, including some having an adequate response. The starting dose selected for this trial was within the MTD reported in other trials. However, the 600 mg twice daily dose had not been given on a 4 week schedule. Karp et al.18 using a 21-day schedule, observed dose-limiting toxicities at 1200 mg PO BID, although doses of 900 mg PO BID were associated with frequent dose interruptions. Similarly, Kurzrock et al. reported frequent dose interruptions using doses of 900 mg PO BID20 in patients with MDS. In both studies, the majority of patients requiring dose interruptions were older than 70 years. However, considering that significant FPT inhibition occurs at doses of 300 mg BID and that no clear correlation existed between dose and response, lower doses may be more desirable, particularly for prolonged therapy.

In summary, ZARNESTRA used as a single agent had activity of short duration in one third of previously treated CML patients. Considering the preclinical data discussed above, exploration of combination therapy with FTI and imatinib mesylate is warranted in CML. The effect observed on VEGF concentrations is interesting and will need confirmation in a larger population. If these results can be corroborated, the effect on VEGF may suggest a new and important therapeutic target in CML. The data in MF are intriguing, and further investigations in this disease are warranted.
Table 1. Clinical characteristics of patients by disease

<table>
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<tr>
<th>Characteristics</th>
<th>CML</th>
<th>MM</th>
<th>MF</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>22</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Median age in years (range)</td>
<td>53 (26-79)</td>
<td>59 (44-79)</td>
<td>58 (37-76)</td>
</tr>
<tr>
<td>Median performance status (range)</td>
<td>1 (0-2)</td>
<td>1 (0-1)</td>
<td>1 (0-2)</td>
</tr>
<tr>
<td>Median No. prior therapies (range)*</td>
<td>2 (1-4)</td>
<td>4 (1-7)</td>
<td>2 (0-5)</td>
</tr>
<tr>
<td>Median WBC x10^9/L (range)</td>
<td>16 (1.8-66.5)</td>
<td>4.4 (2.8-7.3)</td>
<td>5.3 (2.1-108.4)</td>
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<td>Median platelets x10^9/L (range)</td>
<td>71 (5-3895)</td>
<td>232 (100-411)</td>
<td>37 (7-626)</td>
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<tr>
<td>Median Hgb g/L (range)</td>
<td>9.8 (7.1-15.4)</td>
<td>10.2 (8.5-13.8)</td>
<td>8.7 (6.7-13.5)</td>
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<td>Ras mutations</td>
<td>N-ras</td>
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<td>0/3</td>
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<tr>
<td></td>
<td>K-ras</td>
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<td>0/3</td>
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<td></td>
<td>AP</td>
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<tr>
<td></td>
<td>BP</td>
<td>6</td>
<td>N/A</td>
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<tr>
<td>Median M protein in g/dL (range)</td>
<td>N/A</td>
<td>2.7 (1.3-6.2)</td>
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<td>Median percentage bone marrow plasma cells (range)</td>
<td>N/A</td>
<td>30 (5-50)</td>
<td>N/A</td>
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</table>

* Excluding hydroxyurea or supportive care (e.g., transfusions).
Table 2. Adverse events related to treatment with ZARNESTRA

<table>
<thead>
<tr>
<th>Event</th>
<th>No. of patients with event (%)</th>
<th>Any Grade</th>
<th>Grade 3 or 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nausea/vomiting</td>
<td>22 (55)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td>19 (48)</td>
<td>1 (3)</td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>13 (33)</td>
<td>4 (10)</td>
<td></td>
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<tr>
<td>Diarrhea</td>
<td>13 (33)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Pain</td>
<td>10 (25)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Elevated transaminases / bilirubin</td>
<td>9 (23)</td>
<td>2 (5)</td>
<td></td>
</tr>
<tr>
<td>Elevated creatinine</td>
<td>9 (23)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Peripheral neuropathy</td>
<td>7 (18)</td>
<td>3 (8)</td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Plasma concentrations of angiogenic factors in patients treated with ZARNESTRA

<table>
<thead>
<tr>
<th>Angiogenic factor</th>
<th>Median in pg/mL</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Day 7</td>
</tr>
<tr>
<td>VEGF</td>
<td>97.9</td>
<td>108.2</td>
</tr>
<tr>
<td>bFGF</td>
<td>16.3</td>
<td>11.7</td>
</tr>
<tr>
<td>HGF</td>
<td>926.8</td>
<td>933.9</td>
</tr>
<tr>
<td>TNFα</td>
<td>7.16</td>
<td>7.06</td>
</tr>
<tr>
<td>IFNγ</td>
<td>6.62</td>
<td>6.68</td>
</tr>
<tr>
<td>VEGF Responders</td>
<td>668.8</td>
<td>213.1</td>
</tr>
<tr>
<td>VEGF Non-responders</td>
<td>78.8</td>
<td>96.6</td>
</tr>
</tbody>
</table>

NS = not significant; p-value is based on change in plasma concentrations from baseline.
Figure 1.
Figure 2.

A. Non-Responders

B. Responders
Figure 1. Response to ZARNESTRA in a patient with myelofibrosis.

Figure 2. Change in VEGF plasma concentrations in non-responders (A) versus responders (B). (CML = ______, MM = ________, MF = ________).
REFERENCES


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