Antagonism of SET Using OP449 Enhances the Efficacy of Tyrosine Kinase Inhibitors and Overcomes Drug Resistance in Myeloid Leukemia

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Abstract

Purpose: The SET oncoprotein, a potent inhibitor of the protein phosphatase 2A (PP2A), is overexpressed in leukemia. We evaluated the efficacy of SET antagonism in chronic myeloid leukemia (CML) and acute myeloid leukemia (AML) cell lines, a murine leukemia model, and primary patient samples using OP449, a specific, cell-penetrating peptide that antagonizes SET’s inhibition of PP2A.

Experimental Design: In vitro cytotoxicity and specificity of OP449 in CML and AML cell lines and primary samples were measured using proliferation, apoptosis, and clonogenic assays. Efficacy of target inhibition by OP449 was evaluated by immunoblotting and PP2A assay. In vivo antitumor efficacy of OP449 was measured in human HL-60 xenografted murine model.

Results: We observed that OP449 inhibited growth of CML cells including those from patients with blastic phase disease and patients harboring highly drug-resistant BCR-ABL1 mutations. Combined treatment with OP449 and ABL1 tyrosine kinase inhibitors was significantly more cytotoxic to K562 cells and primary CD34+ CML cells. SET protein levels remained unchanged with OP449 treatment, but BCR-ABL1-mediated downstream signaling was significantly inhibited with the degradation of key signaling molecules such as BCR-ABL1, STAT5, and AKT. Similarly, AML cell lines and primary patient samples with various genetic lesions showed inhibition of cell growth after treatment with OP449 alone or in combination with respective kinase inhibitors. Finally, OP449 reduced the tumor burden of mice xenografted with human leukemia cells.

Conclusions: We demonstrate a novel therapeutic paradigm of SET antagonism using OP449 in combination with tyrosine kinase inhibitors for the treatment of CML and AML. Clin Cancer Res; 20(8); 2092–103. ©2014 AACR.

Introduction

Tyrosine kinases play critical biologic roles in the pathogenesis of chronic and acute leukemia. A groundbreaking advance came with the identification of the constitutively active fusion tyrosine kinase, BCR-ABL1, which causes chronic myeloid leukemia (CML; reviewed in ref. 1). Similarly, most acute myeloid leukemia (AML) cells exhibit constitutive phosphorylation of STAT5, a marker for tyrosine kinase activity (2). The mechanism of STAT5 activation is explained by genetic abnormalities in FLT3, KIT, PDGFR, JAK1, and JAK2 kinases in only 35% of AML cases, which suggests that unidentified mechanisms of kinase dysregulation are active in the remainder of these patients.

Clinically, the most successful example of targeted therapy for any cancer has been imatinib (Gleevec; STI571), a small molecule ABL1 tyrosine kinase inhibitor that has been frontline treatment for CML for over a decade. More than 80% of newly diagnosed patients with chronic phase CML achieve durable complete cytogenetic response on imatinib therapy (3). However, 20% to 25% of patients with chronic phase exhibit primary resistance to imatinib or relapse after an initial response. Furthermore, among patients who progress to accelerated or blastic phase disease, responses to imatinib are significantly less frequent and almost always transient. Various mechanisms have been found to account for the resistance to imatinib including BCR-ABL1 kinase-dependent mechanisms (4–6) or BCR-ABL1 kinase-independent mechanisms (7–9). The additional ABL1 kinase inhibitors dasatinib (10, 11) and nilotinib (12–14) have...
Translational Relevance

Molecularly targeted therapy has achieved remarkable success in patients with leukemia with defined oncogenic lesions. However, drug resistance and relapse is common in a large number of cases. The SET oncoprotein is known to be overexpressed in myeloid leukemias including chronic myeloid leukemia (CML) and acute myeloid leukemia (AML). Here, we show that combined targeting of SET and tyrosine kinases provides more efficient and selective inhibition of CML and AML leukemia cell growth from specimens that collectively harbor a broad range of oncogenic lesions. This approach of combined targeting of SET and relevant oncogenic kinase pathways may offer new treatment strategies for treatment-refractory malignancies. To our knowledge, this is the first report showing that SET antagonism in combination with standard targeted therapies may provide an improved treatment option for these patients.

Materials and Methods

Cell culture

Certified K562 and LAMA cells were obtained from the American Type Culture Collection. CMK, HL-60, GDM-1, and UT-7 cells were obtained from the German National Resource Center for Biological Material. MOLM-14 cells were generously provided by Dr. Y. Matsuo (Fujisaki Cell Center, Hayashibara Biochemical Labs, Okayama, Japan; ref. 34). Parental Ba/F3 cells were maintained in RPMI 1640 medium supplemented with 2 mmol/L L-glutamine, 10% FCS, 100 U/mL penicillin, 100 μg/mL streptomycin, and 10% conditioned media derived from WEHI-3B cell line for the source of IL-3. Ba/F3 transfecants expressing wild-type BCR-ABL1, BCR-ABL1 T315I, or BCR-ABL1 E255V/T315I were generated and maintained as described previously (16, 19, 35). None of the cell lines used in this study was cultured for longer than 6 months from initial purchase or characterization. All cell lines were cultured in the recommended culture medium at 37°C in 5% CO2. OP449 (molecular weight 9223 g/mol) was reconstituted in PBS as a 10 mmol/L stock. OP449 was prepared as previously described (29). For convenience, we have also included peptide sequence and synthesis details in the supplementary data. For proliferation assays, cells were incubated for 72 hours in the presence or absence of the tyrosine kinase inhibitors including imatinib, nilotinib, dasatinib (LC Labs, Woburn, MA), or ponatinib (Selleck), OP449 with or without okadaic acid (LC Labs), FY720 (Cayman Chemical),
JAK Inhibitor I (Millipore), or AC220 (Selleck). The number of viable cells was determined by Cell Titer 96 Aqueous One solution cell-proliferation assay (Promega). For apoptosis assays, cells were incubated for 48 hours in the presence of the indicated inhibitor(s), and the number of Annexin V+ cells was determined using the Guava Nexin Kit (Millipore).

**PP2A assay**

PP2A activity was determined as previously described (22, 23, 29, 36) using a commercially available assay (Upstate Biotechnology, 17-313). Briefly, protein lysates were prepared in 20 mmol/L imidazole-HCl, 2 mmol/L EDTA, 2 mmol/L EGTA, pH 7.0 with 10 μg/mL each of aprotinin, leupeptin, and pepstatin, 1 mmol/L benzamidine, 1 mmol/L PMSF, and phosphatase inhibitors tablets (Roche). Fifty micrograms of protein was immunoprecipitated with 4 μg of anti-PP2A antibody (1D6; Upstate Biotechnology) and 50 μL of protein-A-agarose beads for 2 hours at 4°C. Beads were washed extensively with lysis buffer first and then added to the phosphatase reaction for measuring dephosphorylation of the phosphopeptide (K-R-pT-I-R-R) according to the manufacturer’s protocol using malachite green phosphatase detection solution. The level of free phosphate is normalized to total amount of PP2Ac immunoprecipitated as measured by densitometry analysis of immunoblots.

**Patient samples**

All CML and AML samples were obtained after patients provided written and oral informed consents for their participation in the study (Supplementary Tables S1 and S2). The study was reviewed and approved by the institutional review boards at Oregon Health & Science University; University of Texas Southwestern Medical Center, Dallas, TX; and M.D. Anderson Cancer Center, Houston, TX. Bone marrow from normal donors was purchased commercially (Lonza). Mononuclear cells (MNC) were isolated by centrifugation through a Ficoll solution of 0.15 M NH₄Cl, 10 mmol/L KHCO₃, and 0.1 M lysis buffer. The number of mononuclear cells (MNC) were isolated by centrifugation through a Ficoll solution of 0.15 M NH₄Cl, 10 mmol/L KHCO₃, and 0.1 M lysis buffer. The number of mononuclear cells (MNC) were isolated by centrifugation through a Ficoll solution of 0.15 M NH₄Cl, 10 mmol/L KHCO₃, and 0.1 M lysis buffer. The number of mononuclear cells (MNC) were isolated by centrifugation through a Ficoll solution of 0.15 M NH₄Cl, 10 mmol/L KHCO₃, and 0.1 M lysis buffer. The number of mononuclear cells (MNC) were isolated by centrifugation through a Ficoll solution of 0.15 M NH₄Cl, 10 mmol/L KHCO₃, and 0.1 M lysis buffer. The number of mononuclear cells (MNC) were isolated by centrifugation through a Ficoll solution of 0.15 M NH₄Cl, 10 mmol/L KHCO₃, and 0.1 M lysis buffer. The number of mononuclear cells (MNC) were isolated by centrifugation through a Ficoll

**Immunoblotting**

CML and AML cell lines were cultured in the presence or absence of OP449. Following the indicated drug exposure time, cells were washed in PBS and lysed in 50 μL lysis buffer (Cell Signaling) supplemented with complete protease inhibitor and phosphatase inhibitor cocktail-2 (Sigma-Aldrich). Equal amounts of protein were fractionated on 4% to 15% Tris-glycine polyacrylamide gels (Bio-Rad), transferred to polyvinylidene difluoride membranes, and probed with the indicated antibodies: BCR, pABL, STAT5, pSTAT5, AKT, pAKT (S473), pAKT (T308), ERK1/2, pERK1/2, P38/MAPK, pP38/MAPK, S6, pS6 ribosomal (S235/236; Cell Signaling), SET, PP2Ac (Millipore), and α-tubulin (Sigma-Aldrich).

**Animal experiments**

RAG2−/−γc−/− mice (20 g) were bred in-house. Animals received a standard diet and water in the Animal Core Facilities of the Center for Applied Medical Research (University of Navarra, Pamplona, Spain). The protocol for animal experiments was approved by the University of Navarra Animal Experimentation Ethics Committee. Xenografts were established after injecting HL-60 cells (4 × 10⁵ cells/animal) in nonirradiated mice. Treatment was initiated 7 days post-implantation of HL-60 cells with 5 mg/kg OP449 or lactated ringer solution control by intraperitoneal injection every 3 days.

**Statistical methods**

Continuous variables were compared by pairwise Student t test for 2 independent samples using Excel software. IC₅₀ values were generated using GraphPad Prism software. Combination indices (CI) were calculated using CalcuSyn software. A CI value less than 1.0 is represents a synergistic drug combination. A P value less than 0.05 was considered statistically significant. * denotes P < 0.05, ** denotes P < 0.01, and *** denotes P < 0.001.

**Results**

OP449, a peptide antagonist of SET, inhibits growth of CML cell lines including cells harboring drug-resistant BCR-ABL1 kinase domain mutations

The SET oncoprotein contributes to various cancers by inhibiting the tumor suppressor PP2A (reviewed in ref. 26). Recently, we reported that OP449 and its precursors selectively bind to SET and increase PP2A activity in leukemia cells (29, 31, 32). Therefore, we first evaluated the efficacy of OP449 against BCR-ABL1–positive CML cell lines. OP449 treatment resulted in selective, dose-dependent inhibition of murine Ba/F3 cells expressing wild-type BCR-ABL1 (IC₅₀: 0.89 μmol/L), whereas parental Ba/F3 cells exhibited no measurable cytotoxicity (Fig. 1A). Importantly, OP449 also demonstrated activity against both the imatinib-insensitive BCR-ABL1E255V/T315I point mutant and the BCR-ABL1E255V/T315I compound mutant (IC₅₀: 1.62 and 1.97 μmol/L, respectively), the latter of which confers high-level resistance to all currently available ABL1 kinase inhibitors including ponatinib.
The efficacy of OP449 for BCR-ABL1–expressing cells extended to the human CML lines K562 and LAMA (IC50: 0.60 and 1.11 μmol/L, respectively). Furthermore, we found that OP449 was approximately 3- to 8-fold more potent than FTY720 (a previously tested pharmacologic activator of PP2A; refs. 22, 24, and 28) at inhibiting the growth of K562 cells (IC50: 0.60 μmol/L vs. 4.68 μmol/L, respectively) and LAMA cells (IC50: 1.11 μmol/L vs. 3.25 μmol/L, respectively; Fig. 1A and B, right). Reduction in viability of BCR-ABL1–positive cells after treatment with OP449 also correlated with induction of apoptosis in both murine and human CML cell lines (Fig. 1B). Importantly, we found that the treatment of K562 cells with OP449 increased PP2A activity in a dose-
dependent manner. For example, a 67% increase in PP2A activity was observed upon treatment with 2.5 μmol/L OP449 (P = 0.01; Fig. 1C, left). In addition, we confirmed that OP449 activity was mediated through activation of PP2A as the effect of OP449 on the viability of K562 cells was significantly rescued by co-treatment with okadaic acid, a known PP2A inhibitor (ref. 37; Fig. 1C, right).

To determine if OP449-mediated activation of PP2A (24, 28). Furthermore, elevated SET levels are significantly increased by OP449 in combination with AML tyrosine kinase inhibitors for the treatment of CML. Furthermore, OP449 alone may be therapeutically beneficial for patients harboring tyrosine kinase inhibitor–resistant BCR-ABL1 mutations.

OP449 inhibits the growth of AML cells harboring various genetic lesions

PP2A activity has been previously shown to be significantly reduced in AML cells, and pharmacologic restoration of PP2A activity causes growth inhibition of leukemic cells (24, 28). Furthermore, elevated SET levels are significantly correlated with poor disease outcome in AML (33). To evaluate the efficacy of OP449 in AML cells, we first tested a panel of 5 AML cell lines harboring different genetic lesions (Fig. 3A). MOLM-14 cells (which harbor a FLT3-ITD mutation; IC50: 0.59 μmol/L), GDM-1 (overexpressing CSF1R; IC50: 0.82 μmol/L), and HL-60 (NRASQ61K; IC50: 0.94 μmol/L) were the most sensitive to OP449. Other cell lines demonstrated comparable intermediate sensitivity.
including CMK cells (JAK3A572V; IC50 = 1.73 μmol/L) and GM-CSF–dependent UT7 cells (IC50: 1.78 μmol/L; Fig. 3A).

Each of these cell lines exhibited similar levels of SET expression (Fig. 3B). Similar results were obtained when SET levels were normalized with total protein levels of PP2Ac, a subunit of PP2A (Supplementary Fig. S2A).

Although the ratio of the SETα and SETβ (lower band) proteins were demonstrated to be prognostic for more aggressive disease in chronic lymphocytic leukemia (CLL; ref. 29), no significant differences in band intensities were noted in the AML cell lines. We also confirmed that treatment with OP449 resulted in a dose-dependent increase in PP2A activity in HL-60 and MOLM-14 cell lines (normalized to total PP2Ac protein levels; Fig. 3C). Furthermore, we demonstrated time-dependent reduction in levels of phosphorylated S6 ribosomal protein and ERK in HL-60 cells and phosphorylated STAT5, AKT, and S6 ribosomal protein in MOLM-14 cells, suggesting efficacy of OP449 at inhibiting downstream signaling in AML cells (Fig. 3D). Of note, the basal phosphorylation of STAT5 and AKT was very low in HL-60 cells, precluding identification of any significant differences over time (data not shown). These results show that OP449 is cytotoxic to AML cells driven by diverse oncogenic genetic lesions.
AML patient samples show overexpression of SET and are sensitive to SET inhibition by OP449

A recent study reported that SET is overexpressed in 28% of AML samples and its expression is associated with poor prognosis (33). To determine whether the efficacy of OP449 extends to primary AML samples, we screened a small cohort of 9 AML patient samples for expression of SET as compared with normal CD34<sup>+</sup> cells. We observed that 7 of 9 patient samples show significantly increased SET expression as compared with normal CD34<sup>+</sup> cells (Fig. 4A). Similar results were obtained when SET levels were normalized with total PP2Ac levels (Supplementary Fig. S2B). In addition, consistent with previous studies (33), we found SET protein levels were significantly increased in a subset of AML specimens (3/9; 30%) when normalized within the disease. No discernible disease subtype seemed overrepresented among those samples with SET overexpression (Supplementary Table S2). However, examination of a larger patient cohort would be required for more conclusive analysis of disease subtype and to establish correlation between SET expression...
levels and OP449 sensitivity. Next, we tested the efficacy of OP449 in primary AML samples as compared with normal CD34$^+$ cells and observed that OP449 significantly reduced the viability of AML cells (with IC$_{50}$ ranges from 0.6 to 0.9 $\mu$mol/L for most of the samples except one sample (AML-05), which was not very responsive to OP449 treatment; Fig. 4B). Overall, similar to our findings in CML cells, OP449 inhibited growth of primary AML patient cells with variety of genetic lesions, including cells from a patient positive for the FLT3-ITD mutation.

**OP449 inhibits AML tumor growth in a murine leukemia model**

Previous studies have reported pharmacologic activation of PP2A reduces leukemia burden in CML cells (21–23). We have also shown that OP449 is effective in reducing tumor growth in a Burkitt lymphoma model (29). To evaluate this compound’s potential antitumor efficacy against AML cells in vivo, we tested efficacy of OP449 in RAG2$^{-/-}$ γc$^{-/-}$ xenograft mice bearing human leukemia HL-60 cell-derived tumors. Animals were treated with 5 mg/kg OP449 or vehicle by intraperitoneal injection every 3 days. We observed that treatment with OP449 significantly inhibited tumor growth measured over time ($P = 0.0067$; Fig. 5A) and resulted in a more than 2-fold reduction in tumor burden measured at the end of the experiment as compared with vehicle-treated controls (Day 18: 1.14 ± 0.06 g vs. 0.45 ± 0.08 g, respectively; $P < 0.001$; Fig. 5B and C). These results demonstrate the in vivo efficacy of OP449 in a murine leukemia model.

**OP449 synergistically reduces growth of AML cells in combination with relevant kinase inhibitors and chemotherapy**

To determine if combined inhibition of relevant tyrosine kinases and SET antagonism is more effective for inhibiting the growth of AML cells, we assessed the effects of combinations of OP449 with either FLT3 or JAK kinase inhibitors in MOLM-14 and CMK cells, respectively. Treatment of MOLM-14 cells with 2.5 $\mu$mol/L OP449 or 1 nmol/L AC220 alone resulted in reduced cell viability by 58% and 75%, respectively; combined treatment significantly and synergistically reduced cell growth by close to 96% (CI value: 0.723; Fig. 6A, Table S3). Similarly, for CMK cells we observed ~40% reduction in viability with 2.5 $\mu$mol/L OP449 and ~22% with 500 nmol/L JAK inhibitor 1 (a pan-JAK family kinase inhibitor) alone, whereas the combination reduced viability by approximately 65% ($P < 0.001$ as compared with each drug alone) and was highly synergistic (CI value: 0.158; Fig. 6B and Supplementary Table S3). In addition, HL-60 cells showed ~40% reduced growth with 1 $\mu$mol/L OP449 and ~60% reduced growth with 250 nmol/L cytarabine (AraC), whereas combination treatment led to a 94% reduction in viability (CI value: 0.630; $P <
0.001 as compared with each drug alone; Fig. 6C and Supplementary Table S3). These results suggest that select combinations of OP449 with relevant tyrosine kinase inhibitors or chemotherapy are capable of improved, synergistic suppression of growth of AML cells harboring various mutationally activated kinase pathways.

Discussion

Although great strides have been made in the treatment of the patients with CML and AML harboring well-characterized genetic lesions, intrinsic and acquired drug resistance is a persistent clinical problem. Therefore, the development of novel therapeutic approaches is urgently required to treat these patients. Recent studies have proposed the SET oncoprotein as a novel therapeutic target for the treatment of leukemia (26, 29). Perrotti and colleagues reported that SET is overexpressed in CML cells, resulting in decreased PP2A activity (22, 23, 36). Similarly, SET overexpression in patients with CLL (29) and AML is associated with poor prognosis (33). Furthermore, the pharmacologic activation of PP2A by FTY720 reduced viability of CML cells by inhibiting BCR-ABL1 kinase signaling (22, 23). However, given potential toxicity concerns encountered for FTY720 in clinical evaluation in multiple sclerosis (38, 39), evaluation of this compound in patients with CML blastic phase has not been pursued. Therefore, investigation of alternative SET-targeted agents for reactivation of PP2A is warranted.

Recently, we reported OP449, a novel compound that antagonizes SET’s inhibition of PP2A in CLL and non-Hodgkin lymphoma (29). In this report, we evaluated the efficacy of OP449 for inhibiting the growth of CML and AML cells. We showed that SET antagonism leads to growth suppression, enhanced apoptosis, and impaired clonogenicity of tyrosine kinase inhibitor-sensitive and –resistant CML and AML cell lines and primary patient cells. We also demonstrated the in vivo efficacy of OP449 in an acute myeloid leukemia xenograft model. Because a fine balance between kinase and phosphatase activity is critical for normal cell growth, we tested the efficacy and specificity of the combination of SET antagonism using OP449 with specific tyrosine kinase inhibitors or chemotherapy in various CML and AML cell lines and primary patient samples. We showed that combined targeting of specific tyrosine kinases or chemotherapy and SET is both synergistic and selective for inhibiting leukemia cell growth both in patients with CML and AML as compared with monotherapy. Notably, these findings extended to difficult to treat myeloid disease, including patients with blastic phase CML harboring highly drug-resistant BCR-ABL1 mutations and patients with AML with FLT3-ITD, JAK3 mutant, or RAS mutant-positive cells.

Mechanistically, we demonstrated that the treatment of CML and AML cells with OP449 increased PP2A activity in a dose-dependent manner and OP449-mediated cytotoxicity could be partially neutralized by inhibition of PP2A with okadaic acid. Together these results validate that OP449 inhibits leukemia cell growth through a PP2A-dependent mechanism as would be expected by a SET-targeting agent. Furthermore, we showed that treatment of CML and AML cells with OP449 decreased phosphorylation of STAT5, AKT, and ERK. Importantly, in CML cells OP449 not only decreased tyrosine phosphorylation but also reduced protein levels of BCR-ABL1. It has been suggested that increased PP2A activity is associated with increased tyrosine phosphatase activity of SHP-1 in CML cells, which in turn dephosphorylates BCR-ABL1 and leads to proteosome-dependent
degradation of BCR-ABL1 (22, 23). Previous studies have shown that overexpression or amplification of BCR-ABL1 is associated with decreased imatinib sensitivity in patients with CML (40). Our results suggest that SET antagonism using OP449 in combination with standard therapy may offer additional therapeutic advantage to those patients having disease persistence and tyrosine kinase inhibitor resistance by reducing oncoprotein levels.

With the recent addition of ponatinib to the pharmacopoeia of clinically approved ABL1 inhibitors for CML, control of resistance because of point mutations in the kinase domain of BCR-ABL1, including the T315I mutant, seems to be largely tenable. However, BCR-ABL1 compound mutations confer high-level resistance to multiple drugs including ponatinib (16, 18, 19). Our data suggest that even cells that express highly recalcitrant BCR-ABL1 mutations such T315I and the E255V/T315I compound mutant remain sensitive to OP449, warranting further exploration of SET antagonism as a therapeutic strategy in these patients.

The treatment of AML remains challenging because the molecular abnormalities in AML cells are more complex and more heterogeneous than those found in CML cells. Recently, selective inhibitors have been developed for many genes and pathways that are altered in AML. However, a successful implementation of treatment with these agents has been impeded by an incomplete understanding of the genetic changes that drive the disease process. Given the efficacy we demonstrated with OP449 for a variety of mutationally and functionally activated targets in AML cell lines and primary patient cells, we believe SET antagonism represents a promising paradigm for the treatment of genetically heterogeneous AML cells.

The therapeutic strategy of combined targeting of SET and kinase pathways has broader application to various cancers.
For instance, Piazza and colleagues recently reported that mutations in SET binding protein 1 (SETBP1) in atypical CML lead to elevated SET protein levels and reduced PP2A activity (41). SETBP1 deregulation has also been reported in patients with AML (42, 43), myelodysplastic syndromes, myelofibrosis, myeloproliferative neoplasms (44–49), and T-cell precursor ALL (50). Although the precise mechanistic signaling consequences of such variants have not yet been fully characterized, our findings warrant investigation of the efficacy of SET antagonism in cells harboring such mutations, as well as in tandem with inhibitors of potentially simultaneously dysregulated kinase signaling pathways.

Taken together, our findings demonstrate the potential for development of new therapeutics such as OP449 for chronic and acute leukemias that act by antagonizing SET and increasing the activity of PP2A. Importantly, we show for the first time that combined targeting of SET and relevant oncogenic kinase pathways not only increases efficacy but also maintains selective toxicity for leukemia progenitor cells, which suggests that this approach may also offer a new treatment strategy for targeting residual disease in patients in remission on kinase inhibitor therapy. Therefore, our data advocate for the further investigation of such agents in combination with approved kinase inhibitors. Moreover, further preclinical testing of OP449 for efficacy and toxicity may advance it into clinical trials. Overall, this and future studies will help guide the establishment of a novel paradigm for combined targeting of phosphatase and tyrosine kinase signaling pathways to offer improved therapeutic options in patients with treatment-refractory malignancies.

Disclosure of Potential Conflicts of Interest
M.P. Vitek is CEO for Oncotide Pharmaceuticals, Inc. M.P. Vitek has ownership interest (including patents) in Oncotide Pharmaceuticals, Inc. D.J. Christensen is President and Chief Scientific Officer for Oncotide Pharmaceuticals, Inc. D.J. Christensen has ownership interest (including patents) for Oncotide Pharmaceuticals. B.J. Druker has a commercial research grant from Oncotide Pharmaceuticals (NIH STTR subcontract), Novartis (clinical trial funding), Bristol-Myers Squibb (clinical trial funding), and ARIAD pharmaceuticals. B.J. Druker has ownership interest (including patents) in MolecularMD, BluePrint Medicines, MilliPore via Dana-Farber Cancer Institute, and Technologies developed at Oregon Health and Science University. B.J. Druker is a consultant/advisory board member for MolecularMD, BluePrint Medicines, Gilead Sciences, Cell Therapeutics Inc., AstraZeneca, Cyphere, Lorus Therapeutics, and Agios. No potential conflicts of interest were disclosed by the other authors.

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