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Src Family Kinase Inhibition as a Novel Strategy to Augment Melphalan-Based Regional Chemotherapy of Advanced Extremity Melanoma

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Abstract

Background—Src kinase inhibition has been shown to augment the efficacy of chemotherapy. Dasatinib, a dual Src/Abl kinase inhibitor approved for the treatment of CML, is under investigation as monotherapy for tumors with abnormal Src signaling, such as melanoma. The goal of this study was to determine if Src kinase inhibition using dasatinib could enhance the efficacy of regionally administered melphalan in advanced extremity melanoma.

Methods—The mutational status of c-kit and patterns of gene expression predictive of dysregulated Src kinase signaling were evaluated in a panel of 26 human melanoma cell lines. The effectiveness of dasatinib was measured by quantifying protein expression and activation of Src kinase, focal adhesion kinase, and Crk-associated substrate (p130^{CAS}), in conjunction with in vitro cell viability assays using seven melanoma cell lines. Utilizing a rat model of regional chemotherapy, we evaluated the effectiveness of systemic dasatinib in conjunction with regional melphalan against the human melanoma cell line, DM443, grown as a xenograft.

Results—Only the WM3211 cell line harbored a c-kit mutation. Significant correlation was observed between Src-predicted dysregulation by gene expression and sensitivity to dasatinib in vitro. Tumor doubling time for DM443 xenografts treated with systemic dasatinib in combination with regional melphalan (44.8 days) was significantly longer ($p = 0.007$) than either dasatinib (21.3 days) or melphalan alone (24.7 days).

Conclusions—Systemic dasatinib prior to melphalan-based regional chemotherapy markedly improves the efficacy of this alkylating agent in this melanoma xenograft model. Validation of this concept should be considered in the context of a regional therapy clinical trial.

The proto-oncogene c-Src encodes a nonreceptor tyrosine kinase, the expression and activity of which have been found to contribute to proliferation, angiogenesis, adhesion, invasion,

and metastasis.¹⁻⁴ Src, therefore, offers a promising molecular target for anticancer therapy, with its expression frequently elevated in a variety of cancers including colon, pancreatic, lung, and breast cancer, as well as melanoma.⁵⁻¹⁰ Dasatinib is approved by the FDA for the treatment of chronic myelogenous leukemia or Philadelphia chromosome-positive acute lymphoblastic leukemia. It is the most potent Src kinase inhibitor currently in clinical development with an IC₅₀ of 0.5 nM for Src kinase.¹¹ Src kinase inhibitors have also been under investigation as a potential therapeutic option in melanoma. However, a phase 2 trial of dasatinib for advanced melanoma failed to demonstrate a clinical benefit.¹² In this trial, however, patients were not pre-selected based on genetic confirmation of c-kit or altered Src signaling.

Dasatinib not only inhibits the Src-family kinases, but also functions as an inhibitor of a variety of other kinases, including Bcr-Abl, c-kit, EphA2, and PDGF- β . Pertinent to melanoma, c-kit mutations have been observed in 39 % of the mucosal, 36 % of the acral, and 28 % of chronic sun-damaged cutaneous melanomas.¹³ c-kit is therefore an important oncogene in melanoma, and tyrosine kinase inhibitors targeting c-kit, including dasatinib, may hold promise for c-kit positive melanoma. Three phase 2 studies of imatinib have been completed in unselected metastatic melanoma patients without demonstration of clinical benefit.¹⁴⁻¹⁶ However, two other phase 2 trials of imatinib in metastatic melanoma patients harboring a c-kit mutation or amplification of c-kit resulted in significant clinical responses.^{17,18}

The management of advanced melanoma has historically involved the alkylating agents dacarbazine and temozolomide, for systemic disease, and melphalan for in-transit disease.¹⁹⁻²¹ A phase 2 trial of dasatinib combined with dacarbazine for advanced melanoma is currently under investigation. However, no preclinical studies have examined whether dasatinib can augment the response to regional melphalan. We therefore wanted to examine the effects of dasatinib on cell growth and signal transduction in human melanoma cell lines, one of which harbors a c-kit mutation. More specifically, the purpose of this study was to determine if there would be in vitro and/or in vivo evidence of augmented efficacy between the Src-targeted agent, dasatinib, and the alkylating agent, melphalan.

MATERIALS AND METHODS

Cell Lines

Twenty-six human melanoma cell lines were selected for in vitro studies. Expanded in vitro studies were performed on seven cell lines and two were eventually utilized as xenografts using our rat ILI model. The melanoma cell line WM3211 was provided by Dr. Meenhard Herlyn (Molecular and Cellular Oncogenesis Program, The Wistar Institute, Philadelphia, PA), PR-Mel was provided by Dr. Stefania D'Atri (Istituto Demopatico Dell'Immaacolata-Istituto di Ricovero e Cura a Carattere Scientifico, Rome, Italy) and Duke Melanoma (DM) cell lines were obtained courtesy of Dr. Hilliard Seigler (Duke University, Durham, NC). Cells were maintained at 37 °C, 5 % CO₂ in Iscove's modified Dulbecco's medium supplemented with 10 % fetal bovine serum and 2 mM l-glutamine.

c-kit Mutation Assay

DNA was isolated from melanoma cells lines using the DNeasy kit (Qiagen). PCR amplification was performed using HotStart *Taq* DNA polymerase (Qiagen) in a 50 µl reaction volume. Primer sequences and thermocycle settings are reported in the supplemental methods. The PCR products were purified (Qiaquick PCR purification kit; Qiagen) and sequenced by the Duke University DNA Analysis Facility using the Applied Biosystems Dye Terminator Cycle Sequencing system with AmpliTaq DNA Polymerase and ABI 377 PRISM DNA sequencing instruments and analysis software.

Analysis of Src-Predicted Dysregulation

Using cDNA, binary regression in combination with a series of gene expression training sets derived from in vitro studies of a family of human primary mammary epithelial cell lines overexpressing Src was used to define a prediction of dysregulated Src-signaling across the melanoma cell lines using affymetrix gene expression data obtained as previously reported.²² c-kit expression was taken directly from the gene expression data. The probe ID is 205051_s_at, which corresponds to the KIT gene.

Cell Survival Assay

Cell viability assays were performed as we described previously, and briefly within the supplemental methods.²³ Using a colorimetric assay, cell viability was defined as the absorbance measured in the treatment group divided by the absorbance measured in the control group. The fraction of viable cells was plotted as a function of the concentration of melphalan. Area under the dose–response curve (AUC) was calculated between 0 to 125 µM melphalan.

Western Blot Analysis

Subconfluent melanoma cell lines were treated with DMSO or dasatinib (10, 100, and 1,000 µM in DMSO) in culture for 24 h. Western blot analysis was performed as previously described.²³ Antibodies against β-actin, phosphorylated focal adhesion kinase (FAK) (Ty576/577), and phosphorylated p130^{CAS} were purchased from Cell Signaling Technology (Danvers, MA). Antibodies against Src, FAK, and p130^{CAS} were purchased from Millipore (Billerica, MA). The antibody against phosphorylated Src was purchased from Invitrogen (Carlsbad, CA).

Animals and Tumor Inoculation

Female athymic nude rats (Duke University, Durham, NC) were housed in a temperature-controlled room with a 12-h light–dark cycle. To facilitate tumor growth, rats were irradiated with 500 cGy 2 days before subcutaneous injection of either 5 million DM443 or 9 million WM3211 cultured cells suspended in 0.15 ml of a 2:1 solution of PBS:Matrigel (Matrigel, BD Bioscience, Bedford, MA) into the right hind limb. Xenografts were measured every other day with vernier calipers. Tumor volume was calculated as $(\text{length} \times \text{width}^2)/2$.

Chemotherapeutic Drugs In Vivo

Dasatinib was provided by LC Laboratories® (Woburn, MA) and was prepared as a 3 mg/ml stock in polyethyleneglycol 400 (Fluka, Sigma-Aldrich), diluted in 0.9 % saline and administered by oral gavage at a volume of 10 ml/kg for each individual rat (~2 ml per rat). Melphalan was purchased from Sigma (St. Louis, MO) and was prepared at 1 mg/ml in 0.9 % sodium chloride solution (0.9 % saline). Before infusion, the stock drug solutions were further diluted (0.9 % saline for melphalan) to achieve a final infusate concentration of 90 mg/kg (melphalan) in a volume of 22.5 ml. Dasatinib or vehicle was given at a dose of 30 mg/kg daily for 6 days.

In Vivo Treatment Protocol and ILI Technique

Once the tumor volume had reached ~1.2–1.6 cm³, rats were randomly assigned to each treatment group. Daily treatment with dasatinib or vehicle (30 mg/kg by oral gavage) for 6 days was initiated. ILI was performed 8 h after the third dose of dasatinib, as previously described and outlined briefly in the supplemental methods and shown in Fig. 3a.^{24–26}

Tumor Measurement and End Point

Tumors were measured, as described above. The volume of any ulcer or eschar was subtracted from the overall tumor volume. Large ulcers and eschars were considered study endpoints by our IACUC and therefore were euthanized and excluded from analysis. The response to treatment was followed until either a 400 % change in tumor volume was achieved or 60 days had elapsed. Complete response was defined as destruction of the underlying subcutaneous xenograft, rather than ulcer or eschar formation.

Statistical Analysis

Mann–Whitney *U* test (two-tailed) was performed for tumor regressions. *p* values < 0.05 were considered significant. Correlation coefficients were obtained using simple regression analysis.

RESULTS

Identification of Cell Lines

Mutational status of c-kit and patterns of gene expression predictive of dysregulated Src kinase signaling and c-kit expression were evaluated in a panel of 26 melanoma cell lines (Supplemental Table 1). Twenty-five cell lines were wild-type (WT) for c-kit, with only WM3211 harboring the L576 c-kit mutation as previously reported (Supplemental Fig. 1).²⁷ A panel of seven cell lines, representing a spectrum of dysregulated Src signaling, was selected for further analysis of baseline sensitivity to dasatinib, including the single L576C c-kit mutant line, WM3211.

Effect of Dasatinib on Src Kinase, FAK, and p130^{CAS} In Vitro

The effectiveness of dasatinib on cell signaling was measured by activation of Src kinase, FAK, and Src-associated substrate (p130^{CAS}) in conjunction with in vitro cell viability. FAK and p130^{CAS} are the downstream substrates of Src family kinases and play important role in

cell adhesion and migration.²⁸ Phosphorylation of Src was decreased in DM443, PRMel, DM440, and WM3211 cell lines treated with dasatinib, but the level of Src phosphorylation was not changed in DM6, DM366, or DM738. Phosphorylation of FAK and p130^{CAS} was decreased in the DM443 cell line, and completely blocked in the WM3211 cell line (Fig. 1 and Supplemental Fig. 2).

In Vitro Response to Dasatinib Mono- and Combination Therapy

In vitro response to dasatinib monotherapy was observed in 4 of 7 cell lines (Fig. 2a). No correlation was observed between c-kit expression and sensitivity to dasatinib at each concentration; however, we did observe a significant correlation between Src predicted dysregulation and the inhibition rate of proliferation with dasatinib alone at each concentration ($r^2 = 0.502$), illustrated in Supplemental Fig. 3.

The effect of dasatinib monotherapy and in combination with melphalan was assessed. Dasatinib in combination with melphalan inhibited cell proliferation to a greater extent in six cell lines compared with melphalan treatment alone (Fig. 2b). No correlation was observed between sensitivity to dasatinib in combination with melphalan and Src predicted dysregulation or c-kit expression (data not shown).

Effect of Dasatinib on Src Kinase, FAK, and p130^{CAS} In Vivo

The ability of dasatinib to enhance the effects of high dose regionally infused chemotherapy was examined in our rat xenograft model of extremity melanoma.^{23–25,29}

Thirty milligram per kilogram of dasatinib was determined to be the highest dose not associated with drug-related mortality. We therefore used a modified dosing schema of 30 mg/kg as shown in Supplemental Fig. 4A for the xenograft treatment experiments. Additional studies looking at the effect of the 30 mg/kg dosing on Src signaling in tumors demonstrated that maximal inhibition of phosphor-P130 and phosphor-FAK occurred after 3 dosages of dasatinib (Supplemental Fig. 4B).

In Vivo Response to Dasatinib in Combination with Melphalan Isolated Limb Infusion

For preclinical proof of concept we utilized our regional rat model of isolated limb infusion to examine the effects of systemic dasatinib and regional melphalan on tumor growth. We attempted to use both the DM443 and WM3211 xenografts representing Src dysregulated signaling and c-kit mutated tumors, respectively. Unfortunately, tumorigenesis was not seen in WM3211 xenografts (attempted in 12 rats), which prevented analysis of this combination treatment strategy in a c-kit mutated xenograft. DM443 xenografts grew slower with combined dasatinib and melphalan therapy with a tumor doubling time of 44.8 days, which was significantly longer ($p = 0.007$) than either dasatinib alone (21.3 days) or melphalan alone (24.7 days; Fig. 3b).

DISCUSSION

Src, a membrane-associated nonreceptor tyrosine kinase, belongs to the Src family kinase group (SFK).^{4,30} SFKs play a critical role in cellular signal transduction pathways,

regulating diverse processes including cell division, motility, adhesion, angiogenesis, and survival. The prosurvival and proliferative Src pathway can be inhibited by dasatinib. The benefit of dasatinib as monotherapy, however, was not shown in a phase 2 trial in advanced melanoma.¹² On the other hand, a phase 1 trial of dasatinib combined with dacarbazine in metastatic melanoma demonstrated the objective response rate to be 13.8 %, the clinical benefit rate to be 72.4 %, the 6-month progression-free survival to be 20.7 %, and the 12-month overall survival to be 34.5 %.³¹ The benefit of dasatinib for patients harboring the c-kit mutation also has been reported.^{12,32} In vitro, dasatinib combined with temozolomide significantly improved response in melanoma cell lines compared to either drug alone,³³ and dasatinib in combination with cisplatin was associated with reduced cell viability.³⁴ Therefore, we aimed to determine if systemically administered dasatinib could improve the efficacy of regionally administered chemotherapy in tumors sensitive to Src inhibition, including those harboring a c-kit mutation, using our rat ILI model. Unfortunately, there were only two reported cell lines harboring the c-kit mutation, neither of which grow as a tumor in immunodeficient animals.

In our previous study, bevacizumab, the monoclonal antibody against vascular endothelial growth factor (VEGF), significantly enhanced tumor response to ILI with melphalan in two melanoma xenografts, DM443 and DM738, increasing quadrupling time 37 and 113 %, respectively ($p = 0.03$).³⁵ It was determined that bevacizumab significantly decreases tumor vascular permeability, leading to a marked increase in melphalan delivery and DNA adduct formation.³⁵ Src activation is associated with increased expression of proangiogenic cytokines such as VEGF and interleukin-8 (IL-8).³⁶ IL-8 is a chemokine that increases endothelial permeability during early stages of angiogenesis. Inhibiting Src blocks IL-8-mediated VEGFR2 activation and decreases vascular permeability.³⁷ In this context, dasatinib may have the ability to increase the efficacy of melphalan by altering vascular permeability, thereby leading to an increase in melphalan delivery to the tumor.

Dasatinib also blocks with high potency the activity of receptor tyrosine kinases, such as c-kit. Malignancies with an activating mutation in c-kit, such as gastrointestinal stromal tumors, demonstrate significant clinical benefit when treated with small molecule inhibitors of c-kit, including imatinib. Our data showed that dasatinib is effective against the WM3211 cell line harboring an L576P c-kit mutation in exon 11, which is the most common mutation in melanoma and also is found in GIST.³⁸ Two phase 2 trials of imatinib in metastatic melanoma harboring c-kit mutations or amplification resulted in significant clinical responses.^{17,18} Due to our inability to grow a c-kit mutated cell line in vivo, we were unable to determine the effectiveness of this strategy preclinically. However, given the frequency of this mutation in studies of acral lentiginous melanoma, it may be worth testing this strategy in patients with this mutation. The identification of predictive biomarkers for the sensitivity of dasatinib is important. Consistent with previous reports, we found a significant correlation between Src predicted dysregulation and the sensitivity to dasatinib, but no correlation was observed between c-kit expression and sensitivity to dasatinib.^{12,27,39} Caveolin-1 is a Src substrate required for anchorage-dependent cell growth that participates in the modulation of c-Src kinase activity and mediates the activation of Fyn by integrins.⁴⁰ Jilaveanu et al.⁴¹ suggests that caveolin-1 may be a candidate for use as predictive biomarker. Biomarkers predictive of response to dasatinib are under investigation in some clinical trials.

Regional chemotherapy with melphalan is a widely accepted therapeutic approach for patients with in-transit melanoma.⁴² We have recently demonstrated that ADH-1, a novel pentapeptide that targets and disrupts *N*-cadherin adhesion complexes, in combination with melphalan or temozolomide, dramatically improved tumor response by increasing drug delivery in a rat model.⁴³ Other targeted agents such as a poly(ADP-ribose) polymerase (PARP) inhibitor or sorafenib appear to make tumors more susceptible to chemotherapy by lowering the tumor's apoptotic threshold.^{23,44,45} With the concept in mind that targeted therapy can augment the response of clinically relevant cytotoxic agents, this study evaluated the role of Src inhibition with dasatinib combined with the alkylating agent, melphalan, and demonstrated improved efficacy with this combination therapy in a rat ILI model. Although we were not able to investigate the benefit of this treatment regimen in c-kit mutant xenografts, we suspect that dasatinib, which not only inhibits Src but also c-kit, would prove to be efficacious in a clinical trial when combined with an alkylating agent. While this study provides preclinical evidence that systemic dasatinib in combination with melphalan markedly improves the efficacy of this regionally administered cytotoxic agent, validation of this concept should be considered in the context of a regional therapy clinical trial.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Montero JC, Seoane S, Ocana A, Pandiella A. Inhibition of SRC family kinases and receptor tyrosine kinases by dasatinib: possible combinations in solid tumors. *Clin Cancer Res.* 2011; 17(17):5546–52. [PubMed: 21670084]
2. Kim LC, Song L, Haura EB. Src kinases as therapeutic targets for cancer. *Nat Rev Clin Oncol.* 2009; 6(10):587–95. [PubMed: 19787002]
3. Yeatman TJ. A renaissance for SRC. *Nat Rev Cancer.* 2004; 4(6):470–80. [PubMed: 15170449]
4. Summy JM, Gallick GE. Src family kinases in tumor progression and metastasis. *Cancer Metastasis Rev.* 2003; 22(4):337–58. [PubMed: 12884910]
5. Jallal H, Valentino ML, Chen G, Boschelli F, Ali S, Rabbani SA. A Src/Abl kinase inhibitor, SKI-606, blocks breast cancer invasion, growth, and metastasis in vitro and in vivo. *Cancer Res.* 2007; 67(4):1580–8. [PubMed: 17308097]
6. Homsy J, Cubitt C, Daud A. The Src signaling pathway: a potential target in melanoma and other malignancies. *Expert Opin Ther Targets.* 2007; 11(1):91–100. [PubMed: 17150037]
7. Duxbury MS, Ito H, Zinner MJ, Ashley SW, Whang EE. Inhibition of SRC tyrosine kinase impairs inherent and acquired gemcitabine resistance in human pancreatic adenocarcinoma cells. *Clin Cancer Res.* 2004; 10(7):2307–18. [PubMed: 15073106]
8. Masaki T, Igarashi K, Tokuda M, et al. pp60c-src activation in lung adenocarcinoma. *Eur J Cancer.* 2003; 39(10):1447–55. [PubMed: 12826049]
9. Frame MC. Src in cancer: deregulation and consequences for cell behaviour. *Biochim Biophys Acta.* 2002; 1602(2):114–30. [PubMed: 12020799]
10. Aligayer H, Boyd DD, Heiss MM, Abdalla EK, Curley SA, Gallick GE. Activation of Src kinase in primary colorectal carcinoma: an indicator of poor clinical prognosis. *Cancer.* 2002; 94(2):344–51. [PubMed: 11900220]

11. Lombardo LJ, Lee FY, Chen P, et al. Discovery of N-(2-chloro-6-methyl- phenyl)-2-(6-(4-(2-hydroxyethyl)- piperazin-1-yl)-2-methylpyrimidin-4-ylamino)thiazole-5-carboxamide (BMS-354825), a dual Src/Abl kinase inhibitor with potent antitumor activity in preclinical assays. *J Med Chem.* 2004; 47(27):6658–61. [PubMed: 15615512]
12. Kluger HM, Dudek AZ, McCann C, et al. A phase 2 trial of dasatinib in advanced melanoma. *Cancer.* 2011; 117(10):2202–8. [PubMed: 21523734]
13. Curtin JA, Busam K, Pinkel D, Bastian BC. Somatic activation ofKIT in distinct subtypes of melanoma. *J Clin Oncol.* 2006; 24(26):4340–6. [PubMed: 16908931]
14. Kim KB, Eton O, Davis DW, et al. Phase II trial of imatinib mesylate in patients with metastatic melanoma. *Br J Cancer.* 2008; 99(5):734–40. [PubMed: 18728664]
15. Wyman K, Atkins MB, Prieto V, et al. Multicenter Phase II trial of high-dose imatinib mesylate in metastatic melanoma: significant toxicity with no clinical efficacy. *Cancer.* 2006; 106(9):2005–11. [PubMed: 16565971]
16. Ugurel S, Hildenbrand R, Zimpfer A, et al. Lack of clinical efficacy of imatinib in metastatic melanoma. *Br J Cancer.* 2005; 92(8):1398–405. [PubMed: 15846297]
17. Guo J, Si L, Kong Y, et al. Phase II, open-label, single-arm trial of imatinib mesylate in patients with metastatic melanoma harboring ckit mutation or amplification. *J Clin Oncol.* 2011; 29(21):2904–9. [PubMed: 21690468]
18. Carvajal RD, Antonescu CR, Wolchok JD, et al. KIT as a therapeutic target in metastatic melanoma. *JAMA.* 2011; 305(22):2327–34. [PubMed: 21642685]
19. Brady MS, Brown K, Patel A, Fisher C, Marx W. A phase II trial of isolated limb infusion with melphalan and dactinomycin for regional melanoma and soft tissue sarcoma of the extremity. *Ann Surg Oncol.* 2006; 13(8):1123–9. [PubMed: 16791448]
20. Eggermont AM, Kirkwood JM. Re-evaluating the role of dacarbazine in metastatic melanoma: what have we learned in 30 years? *Eur J Cancer.* 2004; 40(12):1825–36. [PubMed: 15288283]
21. Lindner P, Doubrovsky A, Kam PC, Thompson JF. Prognostic factors after isolated limb infusion with cytotoxic agents for melanoma. *Ann Surg Oncol.* 2002; 9(2):127–36. [PubMed: 11888868]
22. Augustine CK, Jung SH, Sohn I, et al. Gene expression signatures as a guide to treatment strategies for in-transit metastatic melanoma. *Mol Cancer Ther.* 2010; 9(4):779–90. [PubMed: 20371714]
23. Augustine CK, Toshimitsu H, Jung SH, et al. Sorafenib, a multikinase inhibitor, enhances the response of melanoma to regional chemotherapy. *Mol Cancer Ther.* 2010; 9(7):2090–101. [PubMed: 20571072]
24. Toshimitsu H, Yoshimoto Y, Augustine CK, et al. Inhibition of poly(ADP-ribose) polymerase enhances the effect of chemotherapy in an animal model of regional therapy for the treatment of advanced extremity malignant melanoma. *Ann Surg Oncol.* 2010; 17(8):2247–54. [PubMed: 20182810]
25. Yoshimoto Y, Augustine CK, Yoo JS, et al. Defining regional infusion treatment strategies for extremity melanoma: comparative analysis of melphalan and temozolomide as regional chemotherapeutic agents. *Mol Cancer Ther.* 2007; 6(5):1492–500. [PubMed: 17483437]
26. Ueno T, Ko SH, Grubbs E, et al. Modulation of chemotherapy resistance in regional therapy: a novel therapeutic approach to advanced extremity melanoma using intra-arterial temozolomide in combination with systemic O6-benzylguanine. *Mol Cancer Ther.* 2006; 5(3):732–8. [PubMed: 16546988]
27. Woodman SE, Trent JC, Stemke-Hale K, et al. Activity of dasatinib against L576P KIT mutant melanoma: molecular, cellular, and clinical correlates. *Mol Cancer Ther.* 2009; 8(8):2079–85. [PubMed: 19671763]
28. Westhoff MA, Serrels B, Fincham VJ, Frame MC, Carragher NO. SRC-mediated phosphorylation of focal adhesion kinase couples actin and adhesion dynamics to survival signaling. *Mol Cell Biol.* 2004; 24(18):8113–33. [PubMed: 15340073]
29. Ueno T, Ko SH, Grubbs E, Pruitt SK, Friedman HS, Tyler DS. Temozolomide is a novel regional infusion agent for the treatment of advanced extremity melanoma. *Am J Surg.* 2004; 188(5):532–7. [PubMed: 15546565]
30. Parsons SJ, Parsons JT. Src family kinases, key regulators of signal transduction. *Oncogene.* 2004; 23(48):7906–9. [PubMed: 15489908]

31. Algazi AP, Weber JS, Andrews SC, et al. Phase I clinical trial of the Src inhibitor dasatinib with dacarbazine in metastatic melanoma. *Br J Cancer*. 2011; 106:85–91. [PubMed: 22127285]
32. Woodman SE, Davies MA. Targeting KIT in melanoma: a paradigm of molecular medicine and targeted therapeutics. *Biochem Pharmacol*. 2010; 80(5):568–74. [PubMed: 20457136]
33. Eustace AJ, Crown J, Clynes M, O'Donovan N. Preclinical evaluation of dasatinib, a potent Src kinase inhibitor, in melanoma cell lines. *J Transl Med*. 2008; 6:53. [PubMed: 18823558]
34. Homsí J, Cubitt CL, Zhang S, et al. Src activation in melanoma and Src inhibitors as therapeutic agents in melanoma. *Melanoma Res*. 2009; 19(3):167–75. [PubMed: 19434004]
35. Turley RS, Fontanella AN, Padussis JC, et al. Bevacizumab-induced alterations in vascular permeability and drug delivery: a novel approach to augment regional chemotherapy for in-transit melanoma. *Clin Cancer Res*. 2012; 18(12):3328–39. [PubMed: 22496203]
36. Kilarski WW, Jura N, Gerwins P. Inactivation of Src family kinases inhibits angiogenesis in vivo: implications for a mechanism involving organization of the actin cytoskeleton. *Exp Cell Res*. 2003; 291(1):70–82. [PubMed: 14597409]
37. Petreaca ML, Yao M, Liu Y, Defea K, Martins-Green M. Transactivation of vascular endothelial growth factor receptor-2 by interleukin-8 (IL-8/CXCL8) is required for IL-8/CXCL8-induced endothelial permeability. *Mol Biol Cell*. 2007; 18(12):5014–23. [PubMed: 17928406]
38. Grossmann AH, Grossmann KF, Wallander ML. Molecular testing in malignant melanoma. *Diagn Cytopathol*. 2012; 40(6):503–10. [PubMed: 22619125]
39. Hodi FS, Friedlander P, Corless CL, et al. Major response to imatinib mesylate in KIT-mutated melanoma. *J Clin Oncol*. 2008; 26(12):2046–51. [PubMed: 18421059]
40. Wary KK, Mariotti A, Zurzolo C, Giancotti FG. A requirement for caveolin-1 and associated kinase Fyn in integrin signaling and anchorage-dependent cell growth. *Cell*. 1998; 94(5):625–34. [PubMed: 9741627]
41. Jilaveanu LB, Zito CR, Aziz SA, et al. In vitro studies of dasatinib, its targets and predictors of sensitivity. *Pigment Cell Melanoma Res*. 2011; 24(2):386–9. [PubMed: 21320292]
42. Beasley GM, Petersen RP, Yoo J, et al. Isolated limb infusion for in-transit malignant melanoma of the extremity: a well-tolerated but less effective alternative to hyperthermic isolated limb perfusion. *Ann Surg Oncol*. 2008; 15(8):2195–205. [PubMed: 18528730]
43. Augustine CK, Yoshimoto Y, Gupta M, et al. Targeting N-cadherin enhances antitumor activity of cytotoxic therapies in melanoma treatment. *Cancer Res*. 2008; 68(10):3777–84. [PubMed: 18483261]
44. Tentori L, Portarena I, Graziani G. Potential clinical applications of poly(ADP-ribose) polymerase (PARP) inhibitors. *Pharmacol Res*. 2002; 45(2):73–85. [PubMed: 11846617]
45. Virag L, Szabo C. The therapeutic potential of poly(ADP-ribose) polymerase inhibitors. *Pharmacol Rev*. 2002; 54(3):375–429. [PubMed: 12223530]

FIGURE 1.

Dasatinib inhibits autophosphorylation of Src, FAK and p130^{CAS} in vitro. Melanoma cell lines DM443 and PRMe1 were treated with 10, 100, and 1,000 nM dasatinib or DMSO vehicle control for 24 h and the level of phosphorylation of Src, FAK, and p130^{CAS} were measured by Western blotting to assess response to dasatinib.

FIGURE 2.

Dasatinib enhances in vitro response to melphalan chemotherapy. **a** Response of ten human melanoma-derived cell lines to treatment with dasatinib alone is shown. **b** Response of seven human melanoma-derived cell lines to treatment with dasatinib in combination with melphalan. Results are average $\pm$ SEM of at least three.

FIGURE 3.

Dasatinib augments response to regional chemotherapy with melphalan. **a** Schematic illustration of dasatinib treatment protocol in combination with melphalan ILI. **b** Fold change in tumor volume is plotted as a function of time following ILI with melphalan or saline. Error bars are SEM for six animals. Dasatinib in combination with melphalan ILI (*red*), saline with melphalan ILI (*yellow*), dasatinib with saline ILI (*green*), and saline with saline ILI (*blue*) are shown.