Chemotherapy and Targeted Therapy Combinations in Advanced Melanoma

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Abstract For three decades, clinical trials with chemotherapy in melanoma have failed to show superiority of any one regimen over another. Dacarbazine remains the only "standard" agent. With response rates of <10% and median progression-free survival of 2 months or less in contemporary trials, there is a need to improve systemic therapy. Combination chemotherapy is associated with higher response rates than single-agent therapy but this has not translated into improved survival. An increasing number of potential therapeutic targets have been identified. For some, pharmacologic inhibitors are available, including sorafenib for BRAF, farnesyltransferase inhibitors for NRAS, PD-0325901 for mitogen-activated protein kinase/extracellular signal-regulated kinase kinase, rapamycin analogues for mammalian target of rapamycin, and agents that inhibit either vascular endothelial growth factor or its receptors. Several multitargeted kinase inhibitors have potency against the fibroblast growth factor receptor, c-kit, and platelet-derived growth factor receptor. Small-molecule inhibitors of c-met and Akt are in preclinical development. Another class of agents indirectly affect aberrant signaling, including inhibitors of chaperones and proteasomes. Several targeted agents seem to enhance the cytotoxicity of chemotherapy in preclinical models. The mechanism by which signaling inhibition might synergize with chemotherapy requires more study so that rational combinations move forward. Very few targeted agents have been studied rigorously in this fashion.

The effect of systemic therapy on survival for patients with advanced melanoma remains unproven. Combination regimens, containing multiple chemotherapeutic agents, multiple biological agents, or both, have not improved survival compared with single-agent therapy (1). The best-studied single-agent chemotherapies for melanoma have objective response rates of <20%, with complete responses observed in <5% of cases (2).

For the purposes of this article, the term chemotherapy is intended to include drugs that induce cell death by interfering with a vital cellular structure or process. Admittedly, the line between targeted therapy and chemotherapy is increasingly blurred as we dissect the mechanisms of chemotherapy and some of the newer agents. The most logically consistent definition of targeted therapies might be those agents for which the molecular target was known as the compound was selected from drug screens. Even this discriminator is flawed but it serves as a point of departure for the following commentary.

Although one can speculate about rational combinations of targeted therapy with chemotherapy, a thorough preclinical evaluation is rarely done to justify a particular drug development program. In fact, most current clinical trials in melanoma, combining a novel targeted agent with a chemotherapeutic agent, use dacarbazine. This is neither the most rational nor the most efficacious approach to build new and more useful combinations. It would be preferable to have a combination of agents for which the selection is scientifically grounded. Not all chemotherapies cause the same intracellular consequences, allowing for the possibility that the signaling effects of nontargeted chemotherapy could be used to advantage in combination with certain targeted signaling inhibitors.

Targeting Antiapoptotic Mediators

BCL-2, BCL-xL, "X-linked inhibitor of apoptosis," and nearly all members of the BH3-only class of antiapoptotic proteins have been reported as overabundant in melanoma and may confer resistance to chemotherapy (3). It is not clear that any one antiapoptotic protein functions as a master suppressor of apoptosis in melanoma. Therefore, the therapeutic value of inhibiting a single member of this large protein family in melanoma is a matter of speculation.

Oblimersen, an antisense oligonucleotide against BCL-2 mRNA, has been tested extensively in clinical trials. This agent had been shown preclinically to down-regulate BCL-2 mRNA and to enhance the cytotoxicity of chemotherapy. In a phase 1/2 trial among patients with advanced melanoma, the down-regulation of BCL-2 in tumor biopsy specimens was shown for most patients (4). Data from this small trial were the basis for a phase 3 trial among 771 patients with metastatic melanoma (5). The failure of this trial to show a survival advantage by the addition of BCL-2 antisense to dacarbazine compared with

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dacarbazine alone leaves more questions than answers. Was the amount of BCL-2 down-regulation shown in the phase 1/2 trial sufficient to provide the anticipated sensitization to chemotherapy? Is there a subpopulation of melanoma patients for whom this strategy would be of particular value? Lacking the ability to answer these questions with clinical data or correlative study of the patients' tumors, one is left to conclude that the drug is ineffective and that the target may be invalid. The latter would require the demonstration that more complete inhibition of BCL-2 lacks chemosensitizing activity. As inhibitors of other antiapoptotic proteins, including BCL-xL, are developed clinically, some of the questions raised by the BCL-2 antisense trials will reappear.

Targeting Oncogenes and Downstream Signaling Pathways

In the shadow of the success with imatinib in chronic myelogenous leukemia and gastrointestinal stromal tumors, melanoma awaits effective molecularly targeted therapy. The question that remains unanswered is whether melanoma is more like chronic myelogenous leukemia and gastrointestinal stromal tumor or more like the prevalent cancers that lack a defining somatic genetic event. BRAF is the most frequently mutated oncogene identified to date in melanoma, present in 60% to 70% of cases (Table 1; ref. 6). This serine-threonine kinase mediates signaling through the mitogen-activated protein kinase (MAPK) pathway. Immediately upstream of BRAF is NRAS, which is mutated and constitutively activated in another 15% of melanomas (7). Given that the two events are mutually exclusive, a striking 75% to 85% of melanomas harbor an activating mutation in this pathway. The less frequent activating mutations in fibroblast growth factor receptor (15%) and amplification of microphthalmia-associated transcription factor (20%) leave the MAPK as a deserving focus of targeted therapy strategies (8, 9). Soon after BRAF mutations were described in metastatic melanoma, a similarly high frequency of BRAF mutations was reported in benign nevi (10). The therapeutic value of agents that target the MAPK pathway is still uncertain. NRAS inhibition remains a therapeutic challenge. The only clinical agents used to date that affect RAS activity are the farnesyltransferase inhibitors. These agents impair the posttranslational modification of the RAS proteins and prevent their membrane localization, which is required for signaling activity (11). Clearly, farnesylation is not a process that is limited to the RAS proteins; thus, this class of agents is surely limited by lack of specificity. Nonetheless, in preclinical models and in limited correlative studies in clinical trials, there is evidence that RAS activity is impaired by farnesyltransferase inhibitors (12). In pancreatic cancer, where KRAS mutations are present in 95% of cases, farnesyltransferase inhibitors have little, if any, activity (13). Yet to be determined is whether more effective RAS inhibition would produce more significant antitumor effects. The therapeutic value of farnesyltransferase inhibitors is currently being evaluated in a phase 2 trial. Combination studies with farnesyltransferase inhibitors and chemotherapy in an NRAS mutant population seem justified by preclinical data (14).

Table 1. Oncogenes and tumor suppressor genes in melanoma

Oncogenes	Tumor suppressor genes
BRAF, 70% mutated	*p16*, 50-70% loss
MITF, 20% amplified	*PTEN*, 45% loss
NRAS, 15% mutated	*APAF-1*, 40% loss
FGFR, 15% mutated	*p53*, 15% loss

Because activating mutations in BRAF allow the kinase to remain constitutively active irrespective of upstream NRAS activity, NRAS is an unattractive target in BRAF mutant melanoma. RAF inhibition is a focus of several ongoing clinical trials, but significant gaps in our knowledge exist. The only agent with BRAF inhibitory activity that has reached clinical trials is sorafenib. This agent was selected for clinical development before BRAF was identified as an oncogene relevant to melanoma and is most potent against CRAF (15). Nonetheless, the homology in the ATP binding sites of the RAF kinases provides for potent cross-reactivity against BRAF. Even later in the clinical development of sorafenib, it was found to be a potent inhibitor of vascular endothelial growth factor receptors. Its activity against BRAF remains the focus of interest in the context of melanoma.

In preclinical models, depletion of mutant BRAF with RNA interference in melanoma cells that harbor the mutation inhibits growth and induces apoptosis in cell culture (16). However, this same method of depleting BRAF fails to entirely abrogate the growth capacity of these same cells when xenografted into an immunodeficient mouse (17). These data suggest that in a tumor environment more representative of the human disease, melanomas that harbor BRAF mutations have accessory signaling pathways that can sustain growth. Nonetheless, in these experiments, the melanoma cells deprived of mutant BRAF grew less rapidly than unaltered melanoma cells. Although small interfering RNA experiments are a precise method of interrogating a therapeutic target, they poorly represent the effects of pharmacologic inhibition.

In melanoma cell lines, sorafenib induces cell cycle arrest and apoptosis (18). The activity of the MAPK is clearly inhibited. Mirroring the small interfering RNA data, however, sorafenib administration to an immunodeficient mouse bearing a mutant BRAF melanoma xenograft only slows tumor growth compared with controls. Fewer animal studies have addressed the question of whether sorafenib is effectively blocking the MAPK pathway while failing to regress the xenografts.

In clinical trials, single-agent sorafenib has been associated with few objective responses and a modest degree of tumor stabilization (19). The rationale for combining sorafenib with chemotherapy derives from preclinical studies conducted *in vitro* and in animal models. Sorafenib has been shown to synergize with the proapoptotic stimulus adaphostin in a broad range of tumor cell lines (20). Likewise, sorafenib enhances the activity of a broad range of chemotherapeutics in xenograft models. Relatively little investigation of this interaction has been conducted in melanoma models but some evidence of chemotherapy sensitization has been reported (18).

Two clinical trials combining sorafenib with chemotherapy are completed or in progress. Rigorous preclinical studies to identify the most appropriate chemotherapy partner for sorafenib have not been conducted. The most extensively evaluated

combination has been that of sorafenib with carboplatin and paclitaxel (21). These two chemotherapy agents have long been known to induce synergistic results in nonmelanoma models and enhancement of the activity of each agent by sorafenib has been observed in nonmelanoma models. In a large, single-arm phase 2 trial, this regimen was associated with an objective response rate and progression-free survival that far exceeds that reported in similarly large trials of other agents in melanoma. Considering that most patients in this trial had progressed during prior systemic therapy for metastatic disease, the results of this trial are encouraging.

How this regimen addresses the therapeutic value of BRAF inhibition remains unclear. The clinical outcome of patients with BRAF mutations was nearly identical to that of patients without BRAF mutations in their tumors. This has led some to speculate that the enhancement of chemotherapy effects by sorafenib might derive from its effect on factors other than BRAF. The likely antiangiogenic effect of vascular endothelial growth factor receptor inhibition is one obvious consideration. We have thus studied the effect of single-agent sorafenib on MAPK pathway activity and tumor perfusion as a surrogate for antiangiogenic effect (22, 23). Among a subset of patients who underwent serial tumor biopsies or dynamic contrast-enhanced magnetic resonance imaging, we found evidence that the MAPK pathway is inhibited and tumor perfusion is significantly decreased. Thus, both mechanisms seem manifest. Further study is required to determine which one predominates and in which subpopulation of patients. With more intensive correlative studies, the next generation of clinical trials with sorafenib should shed more light on this area.

A randomized phase 3 trial comparing this regimen with the chemotherapy agents alone is currently under way. This double-blind trial among 800 patients with metastatic melanoma will definitively address the contribution of sorafenib to this chemotherapy. In parallel, a phase 2/3 trial combining sorafenib with dacarbazine is ongoing. As with the carboplatin and paclitaxel regimen, there is no scientific basis on which this doublet is founded. In a preliminary report of the ongoing trial, the objective response rate seems to exceed that expected for dacarbazine alone (7). A randomized trial comparing sorafenib and dacarbazine to dacarbazine alone is currently accruing patients. These two randomized trials are well powered and should establish or refute this combination chemotherapy strategy.

Considering the preclinical data summarized herein and the clinical experience to date, it is uncertain whether agents that target BRAF more specifically and more potently will be of greater or lesser value. With these open questions remaining from the development of sorafenib, such agents are soon to enter the clinic.

An alternative strategy to targeting BRAF is the inhibition of MEK (MAPK/extracellular signal-regulated kinase kinase), the immediate downstream signaling component in the MAPK pathway. Preclinical data support the induction of apoptosis *in vitro* and xenograft growth inhibition *in vivo* (25). The only clinical evidence in melanoma comes from a recently reported phase 1 trial of PD-0325901 in which melanoma patients were well represented (26). Serial biopsies were attempted on all patients to investigate target inhibition. Nearly all patients had nearly complete inhibition of the MAPK pathway. Yet only 2 of 27 melanoma patients showed objective responses. Whereas the data are very immature, lacking even BRAF mutation status of the patients, one is left with the preliminary conclusion that inhibiting MAPK/extracellular signal-regulated kinase kinase will be insufficient to alter the natural history of the majority of melanoma patients. A single-agent phase 2 trial is under way and includes patients with melanoma. Based on preclinical data, the combination of MEK inhibition with chemotherapy is justified (25) but remains to be clinically investigated.

The clinical experience with inhibitors of mammalian target of rapamycin, c-kit, and platelet-derived growth factor receptor β as single agents have not yielded objective responses in the context of phase 2 trials. CCI-779, a selective inhibitor of mammalian target of rapamycin, was associated with little evidence of clinical activity in melanoma (27). Nonetheless, this agent and other rapamycin analogues have been shown to affect their targets *in vivo*. The lack of single-agent antitumor efficacy does not preclude a chemotherapy-enhancing effect, which is suggested by preclinical studies. Imatinib mesylate is an agent that is potent against both c-kit and platelet-derived growth factor receptor β, targets that are worthy of study based on preclinical evidence (28, 29). Single-agent imatinib was not associated with significant activity in phase 2 trials in melanoma (30). Again, the value of this agent in combination with chemotherapy is unknown. Unlike other agents discussed in this review, imatinib seems to have limited capacity for combination with chemotherapy due to an enhancement of myelosuppression presumably derived from c-kit inhibition.

Inhibitors of c-met, Akt, and the fibroblast growth factor receptor are well justified for clinical investigation based on their role in melanoma biology (31–33). Inhibitors of these signaling mediators are in preclinical development and, in the case of fibroblast growth factor receptor, early clinical development. No data exist about their clinical utility in melanoma.

Indirect Strategies for Targeting Aberrant Signaling

The inhibition of protein chaperones and proteasomes is a distinct area of clinical investigation. It is more difficult to justify the rationale for these agents as specific melanoma therapies. However, there is evidence that inhibition of heat shock proteins and proteasomes can inhibit the growth of melanoma cells *in vitro* and *in vivo* (34, 35).

The geldanamycin derivatives 17-allylamino-17-demethoxy-geldanamycin and 17-dimethylaminoethylamino-17-demethoxy-geldanamycin are currently in clinical development for numerous types of cancer, but experience in melanoma is lacking. Given the fact that heat shock protein 90, the primary target of these agents, is involved in the posttranslational modification of numerous client proteins, their therapeutic index in melanoma remains difficult to predict. Although it has been speculated that heat shock protein 90 inhibition might selectively target mutant BRAF for degradation, there is evidence both in support of and against this hypothesis (34).

The proteasome inhibitor bortezimib has been studied preclinically in melanoma and seems to enhance the cytotoxicity of temozolomide *in vitro* and *in vivo* (35). In melanoma and in other tumors, evidence suggests that the indirect effects of this agent on NF-κB correlate with the antitumor effects of this agent (36, 37). It is clear that NF-κB inhibition is a target of therapeutic interest in melanoma but it is unclear whether

bortezimib acts via this mechanism. Although temozolomide may not be a rational partner for this agent, data are sufficiently promising to warrant clinical investigation and a phase 1/2 trial of this combination is ongoing.

Conclusions

The list of potential therapeutic targets in melanoma is growing at a faster pace than the clinical development of agents against them. Faced with a backlog of agents and somewhat limited resources for conducting thorough clinical investigations, the selection of agents and regimens with strong preclinical rationale must take precedence over empirical motivations. Confronted with apparent biological relevance of MAPK pathway mutations in melanoma, efforts to target this signaling axis deserve increased focus. The preclinical and early clinical data suggest that this single pathway may not be the Achilles heel of the disease. Perhaps melanoma will never be found to have such a susceptible point of intervention as gastrointestinal stromal tumor and chronic myelogenous leukemia. Chemotherapy serves as the readily available and, at this time, best-justified partner for signaling inhibition in the current generation of trials. The choice of chemotherapy partners for targeted therapy requires a rigorous preclinical justification as the empirical choice of dacarbazine or temozolomide may neglect important mechanistic interactions. Whereas this strategy is being validated or refuted, the hope that signaling inhibitors can be combined with each other toward a greater therapeutic effect remains and will require the attention of the next generation. The exploration of targeted therapy combinations is limited by legal and regulatory impediments. Given the unique ability of the Cancer Therapeutics Evaluation Program to sponsor such trials, it is imperative that emerging targeted agents be included in the Cancer Therapeutics Evaluation Program portfolio.

Open Discussion

Dr. Atkins: Do you want to comment about the correlative science plans for the Intergroup trial with sorafenib that's going forward?

Dr. Flaherty: A total of 800 patients are going to be randomized into this trial: 400 to carboplatin-taxol alone and 400 to sorafenib with the chemotherapy. We get to study what chemotherapy does or doesn't do and in whom it does and doesn't work. Harriet Kluger at Yale is using a tissue microarray-based method, wherein a small piece of a paraffin-embedded tumor will be taken to look at a diverse array of protein and phospo-protein markers. We're going to be looking at proapoptotic and antiapoptotic proteins. We're going to be looking at vascular endothelial growth factor pathway elements and predictors of outcome. What we've also proposed, and we've just got a score that's going to require at least one revision to get funded, is a genetic study looking at the known players: NRAS, BRAF, and the tumor suppressors pTEN, p16, and APAF-1. We will look for mutation, amplification, and then loss of the tumor suppressors, in particular.

Dr. Sosman: Do you treat only patients with the target or do you go in with an open mind but make sure you treat patients with the target?

Dr. Flaherty: Failing a compelling treatment for the other group, we feel you should keep it open and then make sure that you're able to determine who the people are who have the target and who don't. Since it's common to have a BRAF mutation, you can interrogate that population with 400 patients on one arm of the study. Understanding the relevance of the mitogen-activated protein kinase pathway inhibition and BRAF wild-type melanoma needs to be the focus.

Dr. Atkins: What do you think is the mechanism of the increased sensitivity to chemotherapy in the b-RAF wild-type versus a b-RAF mutant melanoma population?

Dr. Flaherty: Gavin Robertson has published data suggesting that BRAF mutations (this is, again, in cell lines and so has yet to be validated) would be associated with a greater angiogenic drive. There is higher vascular endothelial growth factor production *in vitro*. We would like to determine in our tumor samples if there is a relationship between BRAF mutation and higher immature fraction of vessels and other things that we think are more believable statements of angiogenesis. I would have expected the BRAF mutant tumors and probably the NRAS mutant tumors as well to have more robust responses, but maybe not if they've got such a drive to angiogenesis.

Dr. Atkins: Todd Golub's model had two different classes of melanomas; one of these groups was microphthalmia-associated transcription factor driven. I'm not sure how that would relate to mutations in BRAF. If you could analyze and divide into those two subsets, would you think there would be one that would be more sensitive to BRAF inhibition than the other? Would that be worth looking at in the context of these targeted therapies, as well as immunotherapy?

Dr. Haluska: That is a specific weakness of array experiments. They look at expression patterns but that is relatively disconnected from either activating mutations or tumor suppressor loss. Neither of those is shown in the analyses. Often, there is no independent classic genetics that allows one to look at the known involved genes in concert. Curiously, in Franco Marincola's fine needle aspirations, one of the prominently identified genes was *PTEN*, which usually doesn't come up with the others. But the short answer to your question is, I don't know.

Dr. Elder: One mechanism for activating the pathway is extracellular autocrine loop receptor tyrosine kinase signaling. There are data from Meenhard Herlyn's lab that this is actually active in the mutated state as well (38).

References

1. Atkins MB, Lee S, Flaherty LE, et al. A prospective randomized phase III trial of concurrent biochemotherapy (BCT) with cisplatin, vinblastine, dacarbazine (CVD), IL-2 and interferon α-2b (IFN) versus CVD alone in patients with metastatic melanoma (E3695): an ECOG-coordinated intergroup trial [abstract 2847]. Proc Am Soc Clin Oncol 2003;22:708.
2. Sun W, Schuchter LM. Metastatic melanoma. Curr Treat Options Oncol 2001;2:193–202.
3. Tang L, Tron VA, Reed JC, et al. Expression of apoptosis regulators in cutaneous malignant melanoma. Clin Cancer Res 1998;4:1865–71.
4. Jansen B, Wacheck V, Heere-Ress E, et al. Chemosensitisation of malignant melanoma by *BCL2* antisense therapy. Lancet 2000;356:1728–33.
5. Millward MJ, Bedikian AY, Conry RM, et al. Randomized multinational phase 3 trial of dacarbazine (DTIC) with or without Bcl-2 antisense (oblimersen sodium) in patients (pts) with advanced malignant melanoma (MM): analysis of long-term survival. J Clin Oncol 2004;22:7505.
6. Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. Nature 2002;417:949–54. Epub 2002 Jun 9.
7. Tsao H, Zhang X, Fowlkes K, Haluska FG. Relative reciprocity of NRAS and PTEN/MMAC1 alterations in cutaneous melanoma cell lines. Cancer Res 2000;60:1800–4.

8. Gartside MG, Curtis A, Yudt L, et al. Identification of FGFR2 as a novel melanoma oncogene [abstract 2588]. Proc Am Assoc Cancer Res 2005.

9. Garraway LA, Widlund HR, Rubin MA, et al. Integrative genomic analyses identify MITF as a lineage survival oncogene amplified in malignant melanoma. Nature 2005;436:117–22.

10. Uribe P, Wistuba II, Gonzalez S. BRAF mutation: a frequent event in benign, atypical, and malignant melanocytic lesions of the skin. Am J Dermatopathol 2003;25:365–70.

11. Johnston SR. Farnesyl transferase inhibitors: a novel targeted therapy for cancer. Lancet Oncol 2001;2:18–26.

12. Alsina M, Fonseca R, Wilson EF, et al. Farnesyltransferase inhibitor tipifarnib is well tolerated, induces stabilization of disease, and inhibits farnesylation and oncogenic/tumor survival pathways in patients with advanced multiple myeloma. Blood 2004;103:3271–7. Epub 2004 Jan 15.

13. Cohen SJ, Ho L, Ranganathan S, et al. Phase II and pharmacodynamic study of the farnesyltransferase inhibitor R115777 as initial therapy in patients with metastatic pancreatic adenocarcinoma. J Clin Oncol 2003;21:1301–6.

14. Smalley KS, Eisen T. Farnesyl transferase inhibitor SCH66336 is cytostatic, pro-apoptotic and enhances chemosensitivity to cisplatin melanoma cells. Int J Cancer 2003;105:165–75.

15. Wilhelm SM, Carter C, Tang L, et al. BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. Cancer Res 2004;64:7099–109.

16. Hingorani SR, Jacobetz MA, Robertson GP, Herlyn M, Tuveson DA. Suppression of BRAF(V599E) in human melanoma abrogates transformation. Cancer Res 2003;63:5198–202.

17. Tanami H, Imoto I, Hirasawa A, et al. Involvement of overexpressed wild-type BRAF in the growth of malignant melanoma cell lines. Oncogene 2004;23:8796–804.

18. Karasarides M, Chiloeches A, Hayward R, et al. B-RAF is a therapeutic target in melanoma. Oncogene 2004;23:6292–8.

19. Ahmad T, Marais R, Pyle L, et al. BAY 43-9006 in patients with advanced melanoma: The Royal Marsden experience. J Clin Oncol 2004;22:7506.

20. Yu C, Bruzek LM, Meng XW, et al. The role of Mcl-1 down-regulation in the proapoptotic activity of the multikinase inhibitor BAY 43-9006. Oncogene 2005;24:6861–9.

21. Flaherty KT, Brose M, Schuchter L, et al. Phase I/II trial of BAY 43-9006, carboplatin (C) and paclitaxel (P) demonstrates preliminary antitumor activity in the expansion cohort of patients with metastatic melanoma. Proc Am Soc Clin Oncol 2004;22:7507.

22. Flaherty KT, Redlinger M, Schuchter LM, Lathia CD, Weber BL, O'Dwyer PJ. Phase I/II, pharmacokinetic and pharmacodynamic trial of BAY 43-9006 alone in patients with metastatic melanoma. J Clin Oncol 2005;23. Abstract 3037.

23. O'Dwyer PJ, Rosen M, Gallagher M, Schwartz B, Flaherty KT. Pharmacodynamic study of BAY 43-9006 in patients with metastatic renal cell carcinoma. J Clin Oncol 2005;23:193s. Abstract 3005.

24. Eisen T, Ahmad T, Gore ME, et al. Phase I trial of BAY 43-9006 (sorafenib) combined with dacarbazine (DTIC) in metastatic melanoma patients. J Clin Oncol 2005;23:712s. Abstract 7508.

25. Zhang XD, Borrow JM, Zhang XY, Nguyen T, Hersey P. Activation of ERK1/2 protects melanoma cells from TRAIL-induced apoptosis by inhibiting Smac/DIABLO release from mitochondria. Oncogene 2003;22:2869–81.

26. Lorusso P, Krishnamurthi S, Rinehart JR, et al. A phase 1-2 clinical study of a second generation oral MEK inhibitor, PD 0325901 in patients with advanced cancer. J Clin Oncol 2005;23:16s. Abstract 3011.

27. Margolin KA, Longmate J, Baratta T, et al. CCI-779 in metastatic melanoma: a phase II trial of the California Cancer Consortium. J Clin Oncol 2004;22:7523.

28. DiPaola RS, Kuczynski WI, Onodera K, et al. Evidence for a functional kit receptor in melanoma, breast, and lung carcinoma cells. Cancer Gene Ther 1997;4:176–82.

29. Furuhashi M, Sjoblom T, Abramsson A, et al. Platelet-derived growth factor production by B16 melanoma cells leads to increased pericyte abundance in tumors and an associated increase in tumor growth rate. Cancer Res 2004;64:2725–33.

30. Eton O, Billings L, Kim K, et al. Phase II trial of imatinib mesylate (STI-571) in metastatic melanoma (MM). J Clin Oncol 2004;22:7528.

31. Furge KA, Kiewlich D, Le P, et al. Suppression of Ras-mediated tumorigenicity and metastasis through inhibition of the Met receptor tyrosine kinase. Proc Natl Acad Sci U S A 2001;98:10722–7. Epub 2001 Sep 4.

32. Stahl JM, Sharma A, Cheung M, et al. Deregulated Akt3 activity promotes development of malignant melanoma. Cancer Res 2004;64:7002–10.

33. Davol PA, Garza S, Frackelton AR, Jr. Combining suramin and a chimeric toxin directed to basic fibroblast growth factor receptors increases therapeutic efficacy against human melanoma in an animal model. Cancer 1999;86:1733–41.

34. Smith V, Sausville EA, Camalier RF, Fiebig HH, Burger AM. Comparison of 17-dimethylaminoethylamino-17-demethoxy-geldanamycin (17DMAG) and 17-allylamino-17-demethoxygeldanamycin (17AAG) *in vitro*: effects on Hsp90 and client proteins in melanoma models. Cancer Chemother Pharmacol 2005;56:126–37. Epub 2005 Apr 20.

35. Amiri KI, Horton LW, LaFleur BJ, Sosman JA, Richmond A. Augmenting chemosensitivity of malignant melanoma tumors via proteasome inhibition: implication for bortezomib (VELCADE, PS-341) as a therapeutic agent for malignant melanoma. Cancer Res 2004;64:4912–8.

36. Cusack JC, Jr., Liu R, Houston M, Abendroth K, Elliott PJ, Adams J, Baldwin AS, Jr. Enhanced chemosensitivity to CPT-11 with proteasome inhibitor PS-341: implications for systemic nuclear factor-κB inhibition. Cancer Res 2001;61:3535–40.

37. Sunwoo JB, Chen Z, Dong G, et al. Novel proteasome inhibitor PS-341 inhibits activation of nuclear factor-κ B, cell survival, tumor growth, and angiogenesis in squamous cell carcinoma. Clin Cancer Res 2001;7:1419–28.

38. Satyamoorthy K, Li G, Gerrero MR, et al. Constitutive mitogen-activated protein kinase activation in melanoma is mediated by both BRAF mutations and autocrine growth factor stimulation. Cancer Res 2003;63:756–9.

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