BRAF Inhibitor Vemurafenib Improves the Antitumor Activity of Adoptive Cell Immunotherapy

Richard C. Koya, Stephen Mok, Nicholas Otte, Kevin J. Blacketer, Begonya Comin-Anduix, Paul C. Tumeh, Aspram Minasyan, Nicholas A. Graham, Thomas G. Graeber, Thinle Chodon, and Antoni Ribas

Abstract

Combining immunotherapy with targeted therapy blocking oncogenic BRAFV600E may result in improved treatments for advanced melanoma. In this study, we developed a BRAFV600E-driven murine model of melanoma, SM1, which is syngeneic to fully immunocompetent mice. SM1 cells exposed to the BRAF inhibitor vemurafenib (PLX4032) showed partial in vitro and in vivo sensitivity resulting from the inhibition of MAPK pathway signaling. Combined treatment of vemurafenib plus adoptive cell transfer therapy with lymphocytes genetically modified with a T-cell receptor (TCR) recognizing chicken ovalbumin (OVA) expressed by SM1-OVA tumors or pmel-1 TCR transgenic lymphocytes recognizing gp100 endogenously expressed by SM1 resulted in superior antitumor responses compared with either therapy alone. T-cell analysis showed that vemurafenib did not significantly alter the expansion, distribution, or tumor accumulation of the adoptively transferred cells. However, vemurafenib paradoxically increased mitogen-activated protein kinase (MAPK) signaling, in vivo cytotoxic activity, and intratumoral cytokine secretion by adoptively transferred cells. Taken together, our findings, derived from 2 independent models combining BRAF-targeted therapy with immunotherapy, support the testing of this therapeutic combination in patients with BRAFV600E mutant metastatic melanoma.

Introduction

Targeted therapies that block driver oncogenic mutations in BRAFV600E result in unprecedentedly high response rates and improved overall survival in patients with advanced melanoma (1–4). However, these responses are usually of limited durability, which is a common feature of most oncogene-targeted therapies for cancer. Conversely, many tumor immunotherapy strategies induce low-frequency, but extremely durable, tumor responses, frequently lasting years (5–7). The ability to combine both treatment approaches could merge the benefits of high response rates with targeted therapies and durable response rates with immunotherapies.

Authors' Affiliations: 1Department of Surgery, Division of Surgical Oncology; 2Department of Medicine, Division of Hematology/Oncology; 3Department of Molecular and Medical Pharmacology, Crump Institute for Molecular Imaging; 4Department of Medicine, Division of Dermatology; 5UCLA Biomedical Physics Interdepartmental Graduate Program, Los Angeles; and 6the Jonsson Comprehensive Cancer Center, University of California Los Angeles, California

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

R.C. Koya and S. Mok contributed equally as first authors.

Corresponding Authors: Richard C. Koya, Division of Surgical Oncology, 54-140 CHS, Los Angeles, CA 90095-1782. Phone: 310-825-2676; Fax: 310-825-2403; E-mail: rkoya@mednet.ucla.edu; and Antoni Ribas, Division of Hematology-Oncology, 11-934 Factor Building, UCLA Medical Center, 10833 Le Conte Avenue, Los Angeles, CA 90095-1782. Phone: 310-206-3928; Fax: 310-825-2493; E-mail: aribas@mednet.ucla.edu.

doi: 10.1158/0008-5472.CAN-11-2837
©2012 American Association for Cancer Research.
Immunosensitization with BRAF Inhibitors

described. We derived such a cell line from mice transgenic for the BRAFV600E mutation with restricted expression in melanocytes, resulting in a murine melanoma model syngeneic to C57Bl/6 mice. This model allowed us to test the concept of immunosensitization (16) by combining the vemurafenib-induced inhibition of driver oncogenic BRAFV600E signaling with adoptive cell transfer (ACT) immunotherapy. Vemurafenib meets most of the criteria as an immune-sensitizing agent (16). In humans, it selectively inhibits a driver oncogene in cancer cells (17), which is neither present nor required for the function of lymphocytes (9). It results in rapid melanoma cell death in humans as evidenced by a high frequency of early tumor responses in patients (1, 18). The antitumor activity may increase the expression of tumor antigens directly by tumor cells (8), or enhance the cross-presentation of tumor antigens from dying cells to antigen-presenting cells. In addition, the profound and selective antitumor effects of vemurafenib against BRAFV600E mutant melanoma cells may result in a more permissive tumor microenvironment allowing for an improved effector function of CTLs, which may be further enhanced by a direct effect of paradoxical MAPK activation. Using 2 different TCR transgenic cell ACT models, we tested the concept of immunosensitization with vemurafenib, showing an improvement of the antitumor effects using the combination over either single-agent therapy alone.

Materials and Methods

Mice, cell lines, and reagents
Breeding pairs of C57BL/6 (Thy1.2, Jackson Laboratories, Bar Harbor, ME), pmel-1 (Thy1.1) transgenic mice (kind gift from Dr. Nicholas Restifo, Surgery Branch, National Cancer Institute, Bethesda, MD), NOD/SCID/γ chainnull (NSG) mice (NOD.Cg-Prkd−/−Il2rg−/−C2, Jackson Laboratory, Bar Harbor, ME), and mice transgenic for BRAFV600E mutation expression in melanocytes (kind gift from Drs. Philip Hinds and Frank Haluska, Tufts Medical Center, Boston, MA) were bred and kept under defined-flora pathogen-free conditions at the Association for the Assessment & Accreditation of Laboratory Animal Care-approved animal facility of the Division of Experimental Radiation Oncology, UCLA, and used under the UCLA Animal Research Committee protocol #2004-159. The SM1 murine melanoma was generated from a spontaneously arising tumor in BRAFV600E mutant transgenic mice. The tumor was minced and first implanted into NSG mice, and then serially implanted into C57BL/6 mice for in vivo experiments. Part of the minced tumor was plated under tissue culture conditions for deriving the SM1 cell line. When used in vitro, SM1 was maintained in RPMI (Mediatech) with 10% fetal calf serum (Omega Scientific), 2 mmol/L L-glutamine (Invitrogen), and 1% (v/v) penicillin, streptomycin, and amphotericin (Omega Scientific). Sequencing for BRAFV600E mutation was carried out as previously described (19). SM1-OVA was generated by stable expression of OVA through lentiviral transduction as previously described (20). A plasmid expressing the 2 chains of an MHC-I-restricted TCR specific for OVA (OT-1) was a kind gift of David Baltimore (California Institute of Technology, Pasadena, CA; ref. 21). The plasmid was recloned to express a F2A picornavirus sequence between the TCR chains for high expression upon transduction of murine splenocytes using a murine stem cell virus retroviral vector as previously described (22, 23). M202, M229, and M233 are previously described human melanoma cell lines (19). Vemurafenib (also known as PLX4032, RG7204, or RO5185426) was obtained under a materials transfer agreement with Plexxikon Inc. (Berkeley, CA). Vemurafenib was dissolved in dimethylsulfoxide (DMSO; Fisher Scientific) and used for in vitro studies as previously described (19). For in vivo studies, vemurafenib was dissolved in DMSO, followed by PBS (100 µL), which was then injected daily intraperitoneally (i.p.) into mice at a dose of 10 mg/kg. Because the original formulation of vemurafenib is poorly bioavailable (1, 15), we used a dosing regimen i.p. that has been shown to have adequate pharmacokinetic parameters in blood (24).

SM1 oncogenic analysis
BRAFV600E sequencing was carried out as previously described (19). Copy number analysis was conducted using a mouse high-density genotyping array (The Jackson Laboratory, Bar Harbor, Maine) and data was genotyped with the R MouseDivGeno (v1.03) software package (25). To detect regions of copy number alteration, we selected the subset of “chippable” invariant genomic probes (i.e., exon 1 probes; ref. 25). Normalized and log2 transformed data was segmented using circular binary segmentation algorithm in the R DNA copy package (26). The minimum number of markers for a changed segment was set at 5, with a 0.0005 significance level to accept a change-point. The segmented data was visualized in the Integrative Genomics Viewer (27). For comparison to human melanoma, we compared this data to copy number alterations observed in 108 human melanoma short-term cultures and cell lines (28).

Cell viability assays
Murine and human melanoma cells, naïve C57BL/6 splenocytes, or activated pmel-1 splenocytes were seeded in 96-well flat-bottom plates (5 × 104 cells/well) with 100 µL of 10% fetal calf serum media and incubated for 24 hours. Graded dilutions of vemurafenib or DMSO vehicle control, in culture medium, were added to each well in triplicate and analyzed following the MTS assay (Promega).

ACT therapy in vivo models
For the OVA model, SM1-OVA tumors were implanted s.c. in C57BL/6 mice. When tumors reached 5 to 8 mm in diameter, mice were conditioned for ACT with a lymphodepleting regimen of 500 cGy of total body irradiation and then received 1 × 106 C57BL/6-derived splenocytes i.v. that had been genetically modified to express the OT-1 TCR by retroviral transduction as previously described (22, 23). For the pmel-1 model, C57BL/6 mice with previously implanted SM1 tumors were treated with lymphodepleting total body irradiation, i.v. injection of 1 × 106 gp10025-33 peptide-activated pmel-1 splenocytes and subcutaneous vaccination with gp10025-33 peptide-pulsed dendritic cells when tumors reached 5 to 8 mm in diameter as previously described (29, 30). In both cases, the ACT was followed by 3 days of daily i.p. administration of 50,000 IU of interleukin
2 (IL-2). Tumors were followed by caliper measurements 3 times per week.

**Flow cytometric analysis**

SM1 tumors harvested from mice were digested with collagenase and DNase (Sigma-Aldrich). Splenocytes and tumor-infiltrating lymphocytes (TIL), obtained from digested SM1 tumors, were stained with antibodies to CD8α (CalTag), CD3, CD4, Thy1.1 (Becton Dickinson Biosciences), OVA/H-2Kb tetramer, or gp10025-33/H-2Db tetramer (Beckman Coulter), and analyzed with LSR-II or FACSCalibur flow cytometers (Becton Dickinson Biosciences), followed by analysis using Flow-Jo software (Tree-Star) as previously described (30). Intracellular interferon gamma (IFN-γ) staining was done as previously described (30).

**Immunofluorescence imaging**

Staining was carried out as previously described (23). Briefly, sections of OCT (Sakura Finetek) cryopreserved tissues were blocked in donkey serum/PBS and incubated with primary antibodies to CD8α (CalTag), CD3, CD4, Thy1.1 (Becton Dickinson Biosciences), followed by secondary donkey anti-rat antibodies conjugated to DyLight 488 (Jackson Immunoresearch Laboratories) or streptavidin-conjugated FITC (Invitrogen). Negative controls consisted of isotype matched rabbit or rat immunoglobulin G in lieu of the primary antibodies listed above. Then, 4,6-diamidino-2-phenylindole (DAPI) was used for the visualization of nuclei. Immunofluorescence images were taken in a fluorescence microscope (Axioplan-2; Carl Zeiss Microimaging).

**In vitro cytotoxicity assay**

The assay was conducted as previously described (30). In brief, splenocytes from naïve WT C57BL/6 mice were pulsed with 50 μg/mL of gp10025-33 Peptide or the same amount of control OVA257-264 peptide. After 1 hour of incubation, gp10025-33-pulsed WT splenocytes were labeled with 6 nmol/L CFSE for 10 minutes at 37°C, whereas control OVA257-264-pulsed splenocytes were differentially labeled with a 10-fold dilution of CFSE (0.6 nmol/L). Cells were injected i.v. into experimental mice at 16 days after pmel-1 adoptive cell transfer. After 10 hours, 3 mice per group were sacrificed and their spleens examined for the presence of CFSE-labeled cells. Percent cytotoxic activity was calculated as number of live gp10025-33-pulsed splenocytes divided by the number of live OVA257-264 pulsed splenocytes, which were distinguished on the basis of the 10-fold difference in CFSE fluorescence by flow cytometry.

**Bioluminescence imaging**

Pmel-1 splenocytes were retrovirally transduced to express firefly luciferase as previously described (29), and used for ACT. Bioluminescence imaging was carried out with a Xenogen IVIS 200 Imaging System (Xenogen/Caliper Life Sciences) as previously described (22, 23).

**Micro-PET/computed tomography imaging**

Mice were anesthetized with 2% isoflurane. PET was conducted 1 hour after intravenous administration of 200 μCi of 

**Statistical analysis**

Data were analyzed with GraphPad Prism (version 5) software (GraphPad Software). A Mann–Whitney U test or ANOVA with Bonferroni post-test was used to analyze experimental data. Survival curves were generated by actuarial Kaplan–Meier method and analyzed with the Jump-In software (SAS) with log-rank test for comparisons from the time of tumor challenge to when mice were sacrificed due to tumors reaching 14 mm in maximum diameter, or the end of the study period had been reached.

**Results**

**Derivation of a BRAFV600E mutated murine melanoma syngeneic to C57BL/6 mice**

The cell line SM1 was derived from a spontaneously arising melanoma from a mouse with the BRAFV600E oncogene specifically expressed by melanocytes. These mice had been generated by germline insertion of the BRAFV600E gene downstream of the murine tyrosinase locus control region (promoter enhancer) as described previously (31). These melanocytes-specific BRAFV600E transgenic mice had been backcrossed for more than 20 generations with C57BL/6 mice. It has been previously described that mice carrying transgenic BRAFV600E develop melanocytic hyperplasia histologically reminiscent of human nevi, and develop spontaneous melanomas with low penetrance due to the dominant oncogenic senescence effect of BRAFV600E (31). Cross-breeding them with CDKN2A or p53-deficient mice increases the frequency of melanoma development (31), but we found that the resulting tumors could not be grown in C57BL/6 mice (data not shown) possibly due to innate responses against mixed-background minor antigens from the 2 transgenic strains. To optimize the chances of establishing a progressively growing tumor, we first passaged the original SM1 cells in deeply immune-deficient NSG mice, and from there we were able to implant and develop progressively growing tumors in fully immunocompetent C57BL/6 mice.

**SM1 is a vemurafenib-moderately sensitive BRAFV600E mutant melanoma**

Sequencing of the hotspot T1799A mutation in BRAF showed the presence of the BRAFV600E transversion in SM1 cells (Fig. 1a). Whole-genome copy number analysis showed multiple genomic aberrations in SM1, with frequent deletions and amplifications (Supplementary Fig. S1A), which is a common finding in human melanomas. Among target genes of interest, SM1 has deletion of CDKN2A and amplification of BRAF and MITF genes (Supplementary Fig. S1B), events that are also frequently observed in human melanoma (Supplementary Fig. S1C). We tested the antitumor effects of single-agent vemurafenib against SM1 by in vitro MTS cell proliferation assay after 72 hours of treatment. The 50% inhibition concentration (IC50)
of vemurafenib was 14 μmol/L, which is approximately one log higher than the sensitivity of M229 (IC50 of 0.5 μmol/L), a BRAFV600E mutant human melanoma cell line highly sensitive to vemurafenib, and at a similar range as the relatively resistant BRAFV600E mutant human melanoma cell line M233 (IC50 of 15 μmol/L). SM1 was more sensitive to vemurafenib than the NRASQ61L mutant (and BRAF WT) M202 and M207 cell lines (IC50 >200 μmol/L; Fig. 1B). Despite its relative resistance in MTS assays, SM1 responded to vemurafenib in vitro as shown by a profound G1 arrest effect (Fig. 1C), and evidence of apoptotic cell death with increasing concentrations (Fig. 1D). Further, the exposure of SM1 to vemurafenib resulted in the expected effects of inhibiting downstream MAPK pathway signaling with additional inhibition of the PI3K/AKT signaling (Fig. 1E), similar to that previously described in BRAFV600E mutant human melanoma cell lines (19, 32). SM1 tumors established subcutaneously in C57BL/6 mice responded to single-agent vemurafenib with a growth delay compared with the progressive tumor growth in mice treated with vehicle control (Fig. 1F). As with our prior results in tests conducted on human lymphocytes (9), increasing concentrations of vemurafenib did not negatively alter the viability of murine lymphocytes (Fig. 2A). Further, analysis for pERK showed paradoxical MAPK activation, shown by increase in pERK, most notably at 1 and 5 μmol/L, when murine splenocytes were exposed to vemurafenib and assayed 24 hours later (Fig. 2B). Because the response to single-agent vemurafenib was not complete and this BRAF inhibitor did not negatively affect murine splenocytes, we hypothesized that SM1 would be a useful model to test the potential beneficial effects of adding an immunotherapy strategy to the treatment with vemurafenib.
Combined therapy with vemurafenib and ACT immunotherapy improves antitumor responses against SM1 tumors

We generated a mouse model targeting the model tumor antigen OVA. We stably expressed OVA in SM1 cells to generate SM1-OVA for studies of ACT with splenocytes expressing a TCR specific for OVA (Fig. 3A and 3B). Lymphodepleted C57BL/6 mice with established subcutaneous SM1-OVA tumors received ACT of splenocytes obtained from C57BL/6 mice genetically modified with a retroviral vector expressing the 2 chains of the OVA-specific TCR (Fig. 3D). We titrated the conditions of this immunotherapy to provide a suboptimal antitumor activity (similar in range to single-agent vemurafenib) to allow the testing of the benefits of the combination. In 2 replicate experiments, the combined therapy of vemurafenib and OT-1 TCR-engineered splenocyte ACT was consistently superior to either therapy alone (Fig. 3E), and it improved survival (Fig. 3F; P = 0.0004 by log rank test).

As the OVA model is based on the recognition of a foreign antigen, we decided to confirm the results in the pmel-1 ACT model (Fig. 4A). The pmel-1 model is based on T cells transgenic for a TCR recognizing the murine melanosomal antigen gp100 (33), which is endogenously expressed by SM1 (Fig. 4B). In 3 replicate experiments, the combined therapy with pmel-1 ACT and vemurafenib provided superior antitumor activity compared with either therapy alone, which had been titrated to provide a suboptimal response against established SM1 tumors (Fig. 4C). As with the OVA model, Kaplan–Meier analysis of actuarial survival curves generated with the combined data from 3 replicate experiments showed that the combined therapy improved survival compared with single therapies (Fig. 4D; P < 0.0001 by log rank test).

Vemurafenib does not alter the tumor antigen or MHC expression by SM1 cells

A hypothesized mechanism of improved antitumor activity of combining BRAF targeted therapy with immunotherapy is an increase in tumor antigen or MHC expression by cancer cells (8). Therefore, we tested whether exposure to vemurafenib increased the expression of the gp100 melanoma tumor antigen or the expression of surface MHC molecules, as well as the recognition by TCR transgenic cells specific for gp100. However, vemurafenib did not significantly alter gp100 tumor antigen expression by SM1 cells (Fig. 4B). The baseline expression of the MHC molecule H2-Db was very low in cultured SM1 cells, and it did not significantly change on exposure to vemurafenib (Supplementary Fig. S2).

No difference in T-cell number, distribution, and tumor targeting of ACT therapy when combined with vemurafenib

It has been reported that biopsies of some patients treated with BRAF inhibitors have increased CD8 infiltrates (10). To analyze whether vemurafenib expanded or changed the distribution of adoptively transferred cells in vivo with higher accumulation in tumors, we analyzed their presence in spleens, tumor-draining lymph nodes, and tumors. However, in our model there was no evidence of either a systemic (spleen or lymph nodes) or local (tumor) increase in the quantity of adoptively transferred antitumor T cells with treatment with vemurafenib (Fig. 5A and 5B; Supplementary Fig. S3A and 3B). To rule out the possibility that we were missing an effect by not analyzing the whole animal, we genetically labeled the adoptively transferred cells with treatment with vemurafenib (Fig. 5A and 5B; Supplementary Fig. S3A and 3B). To analyze whether vemurafenib expanded or changed the distribution of adoptively transferred cells with the firefly luciferase transgene to allow their in vivo tracking using bioluminescence imaging. Again, there was no evidence of a differential expansion of in vivo distribution and tumor targeting by the adoptively transferred pmel-1 cells when mice were treated with vemurafenib (Fig. 5C). The quantitative analysis of luciferase activity over time in mice treated with pmel-1 ACT alone or pmel-1 ACT combined with vemurafenib showed similar in vivo distribution to lymphoid organs and to the antigen-matched tumors (Fig. 5D). Further, we employed a higher resolution method to visualize a differential systemic immune response using the PET probe [18F]FAC, which has preferential uptake by activated murine lymphocytes (34). Again, there was no difference in the PET scan images with or without systemic treatment with vemurafenib (Supplementary Fig. S4).

Increased functional activation of intratumoral lymphocytes with exposure to vemurafenib

The in vivo cytotoxicity assay allowed testing of whether vemurafenib had a direct effect of enhancing lymphocyte cytotoxicity in vivo, independent of its effects on SM1 tumor cells, as the targets are syngeneic splenocytes devoid of the
BRAFV600E mutation. In 3 replicate experiments the ACT of pmel-1 cells induced potent cytotoxic effects against splenocytes pulsed with the gp100 peptide, but not against the control OVA peptide. The cytotoxicity increased with systemic treatment with vemurafenib when analyzed at limiting numbers of adoptively transferred pmel-1 cells (Fig. 6A), but not when the number of pmel-1 cells adoptively transferred was 1 log higher and the pmel-1 cells already had a very high lytic activity against gp100 peptide pulsed splenocytes (Supplementary Fig. S5). We then analyzed the activation state of TILs by detecting cytokine production. In 2 replicate experiments, TIL collected from mice treated with the combination showed a higher ability to respond to short-term ex vivo restimulation with the gp100 antigen, as assessed by IFN-γ secretion (Fig. 6B; Supplementary Fig. S6). Therefore, the addition of vemurafenib increased the functionality of adoptively transferred pmel-1 cells in terms of their ability to release an immune stimulating cytokine and intrinsic antigen-specific lytic activity.

Discussion

The two approaches with high response rates for the treatment of patients with metastatic melanoma are BRAF inhibitors and lymphocyte ACT therapies with ex vivo expanded melanoma-specific T cells (1, 18, 35). However, in both cases, tumors frequently relapse after an initial response (18, 36). Our data supporting the combination of ACT with vemurafenib provides a strong rationale to translate combined
immunotherapy and targeted therapy for patients with \(BRAF^{V600}\) mutant metastatic melanoma. The scientific rationale for combinations of targeted therapies and immunotherapy is based on the notion that pharmacologic interventions with specific inhibitors of oncogenic events in cancer cells could sensitize cancer cells to immune attack, which has been termed immunosensitization (16). An immune-sensitizing agent should ideally block key oncogenic events in cancer cells, resulting in an increase in cell-surface ligands for immune effector cells, and induce an intracellular pro-apoptotic cancer cell milieu, which would enhance the ability of immune effector cells such as CTLs and natural killer cells to recognize and kill cancer cells. At the same time, immune-sensitizing agents should not impair the viability or function of immune effector cells (16). Most of these desired features could be fulfilled by specific BRAF inhibitors currently used in patients with \(BRAF^{V600}\) mutant metastatic melanoma (8–10).

We explored the potential mechanisms by which vemurafenib could improve the antitumor activity of adoptively transferred T cells in 2 animal models. Our studies show that this BRAF inhibitor does not change the cell expansion or distribution of adoptively transferred cells by morphologic and molecular imaging studies. However, lymphocytes exposed to vemurafenib have higher pERK, which is a key feature of an activated MAPK signaling pathway. Further, we noted an immune cell-intrinsic ability to increase the cytotoxic function of antigen-specific T cells, and TILs from vemurafenib-treated mice had higher functional activation with increased ability to release the immune-stimulating cytokine IFN-\(\gamma\) following antigen re-exposure. These immune-activating effects of vemurafenib can be explained by the ability of RAF inhibitors to paradoxically activate the MAPK pathway in cells that are WT for BRAF but have strong upstream signaling (12–15). Therefore, it is possible that, in this model with a moderately
sensitive tumor target, the main beneficial effects of vemurafenib are derived from the ability of this agent to directly improve immune effector functions independent of the effects against the BRAFV600E mutant tumor.

One of the potential mechanisms of combinatorial activity of tumor-damaging agents and immunotherapy, leading to increased TIL activation, is an increased antigen presentation by the tumor cells themselves (8). However, in our studies we could not readily detect an increase in tumor antigen or MHC molecule expression by SM1 cells exposed to vemurafenib. An alternative approach leading to increased antigen presentation would be an increased tumor antigen cross-presentation by host antigen-presenting cells picking up antigen released by dying cancer cells. However, it is hard to develop direct evidence of tumor antigen cross-presentation in these animal models, which may be further explored. In addition, it is possible that vemurafenib could alter the tumor microenvironment inhibiting the production of immune suppressive factors by the melanoma cells, leading to increased adaptively transferred lymphocyte activation without increasing antigen cross-presentation. A slower tumor growth and blocking the onco-genic MAPK pathway signaling would favorably modulate the tumor microenvironment allowing antitumor lymphocytes to be better activated and produce IFN-γ as we have detected.

It is possible that the mechanism of improved combinatorial effects may be different in a BRAFV600E mutant tumor with higher sensitivity to vemurafenib. In our models based on the SM1 cell line, single-agent vemurafenib had a mainly anti-proliferative effect in vivo, as opposed to the induction of rapid tumor regression. SM1 is relatively resistant to single-agent vemurafenib in vitro and in vivo, possibly because of the multiple genomic alterations in this cell line, including deletion of CDKN2A and amplification of BRAF and MITF. In fact, amplification of BRAFV600E is a bona fide mechanism of resistance to BRAF inhibitors in the clinical setting (37), and possibly the main reason why SM1-established tumors in mice do not regress with the treatment with vemurafenib. If new murine melanoma cell lines driven by BRAFV600E are developed in the future with higher in vitro and in vivo sensitivity to BRAF inhibitors, it is possible that even more synergistic effects of BRAF inhibitors with immunotherapy may be detected. A rapid tumor response may be more likely to induce tumor antigen-specific T-cell activation by antigen cross-presentation, or inhibition of the immunosuppressive
tumor microenvironment, and the responding tumor may enlist inflammatory cells producing chemokine attractants for lymphocytes, resulting in increased intratumoral infiltration.

In conclusion, combined therapy with the BRAFV600E-specific inhibitor vemurafenib and TCR-engineered ACT resulted in superior antitumor effects against a fully syngeneic BRAFV600E mutant melanoma. Although the absolute number of T cells infiltrating the tumor was not increased by vemurafenib, the combination increased the functionality of antigen-specific T lymphocytes. Therefore, our studies support the clinical testing of combinations of BRAF targeted therapy and immunotherapy for patients with advanced melanoma.

Disclosure of Potential Conflicts of Interest
A. Ribas has received honoraria from consulting with Roche-Genentech, which is the maker of vemurafenib. No potential conflicts of interest have been disclosed by the other authors.

Acknowledgments
The authors thank Ashley Cass for assistance with bioinformatic analyses.

Vemurafenib (PLX4032) was generously provided by Dr. Gideon Bollag from Plexxikon Inc.

Grant Support
This work was funded by the NIH grants P50 CA086306 and P01 CA132681, The Seaver Institute, the Louise Belley and Richard Schnarr Fund, the Wesley Coyle Memorial Fund, the Garcia-Corsini Family Fund, the Fred L. Hartley Family Foundation, the Ruby Family Foundation, the Jonsson Cancer Center Foundation, and the Caltech-UCLA Joint Center for Translational Medicine (all to A. Ribas). N.A. Graham was supported by the UCLA Tumor Biology Program, US Department of Health and Human Services, and Ruth L. Kirschstein Institutional National Research Service Award T32 CA009056. A. Minasyan was supported by the Eugene V. Cota-Robles Fellowship and the Competitive Edge Fellowship funded by NSF. A.G.E.P. P.C. Tumeh was supported by K08 AI091663.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received August 21, 2011; revised April 28, 2012; accepted May 19, 2012; published OnlineFirst June 12, 2012.
Immunosensitization with BRAF Inhibitors

References


20. Koya RC, Kasahara N, Pullarkat V, Levine AM, Stripecke R. Transduc-

tion of acute myeloid leukemia cells with third generation self-inacti-

vating lentiviral vectors expressing CD80 and GM-CSF: effects on proliferation, differentiation, and stimulation of allogeneic and autolo-


34. Radu CG, Chu CJ, Nair-Gill E, Shelly SM, Barrio JR, Satyamurthy N, et al. Molecular imaging of lymphoid organs and immune activation by positron emission tomography with a new [18F]-labeled 2-deoxy-
