BRAF(V600) Inhibitor GSK2118436 Targeted Inhibition of Mutant BRAF in Cancer Patients Does Not Impair Overall Immune Competency

David S. Hong1, Luis Vence2, Gerald Falchook1, Laszlo G. Radvanyi2, Chengwen Liu2, Vicki Goodman3, Jeffery J. Legos3, Sam Blackman3, Antonio Scarmadio1, Razelle Kurzrock1, Gregory Lizee2, and Patrick Hwu2

Abstract

Purpose: An intact immune system likely contributes to the outcome of treatment and may be important for clearance of drug-resistant tumor cells and for prevention of recurrence. Although pharmacologic inhibition of BRAF(V600E) in melanoma patients, which is linked to immune suppression, results in an initial response rate, these responses are typically of limited duration. Combining immunotherapeutic drugs with kinase-targeted agents is an attractive strategy to increase clinical efficacy. Evidence suggesting that mitogen-activated protein kinase pathway activation in tumor cells contributes to immune suppression suggests that the two approaches may be synergistic, provided that BRAF(V600E) inhibitors are nontoxic to immune cells.

Methods: To assess effects of mutant BRAF inhibition on systemic immunity, we studied 13 patients with tumors carrying a BRAF mutation who underwent treatment with GSK2118436, a V600 mutant BRAF-specific inhibitor. We carried out peripheral blood immunomonitoring before and following one or two 28-day cycles of treatment.

Results: GSK2118436 treatment had no detectable impact on most immune parameters tested, including serum cytokine levels, peripheral blood cell counts, leukocyte subset frequencies, and memory CD4+ and CD8+ T-cell recall responses. A slight increase in serum TNF-α over the course of treatment was observed. In addition, three of the four human leukocyte antigen-A2–positive patients experienced a modest increase in circulating tumor antigen–specific CD8+ T cells following BRAF(V600) inhibitor therapy.

Conclusions: GSK2118436 treatment results in no detectable negative impact on existing systemic immunity or the de novo generation of tumor-specific T cells. These findings suggest that future trials combining specific BRAF(V600E) inhibition with immunotherapy should not impair immune response.

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Introduction

Metastatic melanoma (MM) is one of the deadliest of cancers, with stage IV patients facing a less than 10% five-year survival rate (1–3). The poor survival in the metastatic setting and its increasing annual incidence has created an urgent need for effective new therapies. Two of the most successful strategies have been to utilize immune-modulating drugs or pharmacologic agents that target oncogenic kinases that constitutively activate the mitogen—activated protein kinase (MAPK) pathway in tumor cells (4, 5). Both approaches have produced a clinical benefit in melanoma patients, and there is an emerging rationale that supports combining these 2 approaches in future clinical trials (6, 7).

Approximately 50% to 60% of melanomas express an activating mutation in the BRAF oncogene at amino acid position 600, with about 85% to 95% of the V600 being V600E mutations and 10% to 15% V600K mutations. These genetic alterations are also found, but to a lesser extent, in colorectal cancer and papillary thyroid carcinoma (8–10). The aberrations result in constitutive activation of the MAPK signaling pathway, which favors tumor cell growth through various mechanisms, including reduced apoptosis, increased metastatic potential, invasiveness, and immune suppression (11–16). New therapies that target the MAPK pathway have produced demonstrable efficacy in the clinic, supporting this approach. Recently, inhibitors targeting BRAF(V600E) have directly induced objective responses in

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).
The mutant BRAF-specific inhibitor, GSK2118436, is a potent antitumor agent beginning to be widely used in cancer patients harboring BRAF mutations at the valine 600 position (V600). However, little is known on whether this drug negatively affects lymphocyte subsets and memory T-cell responses in vivo during therapy. In this study, we measured a number of immune parameters in the peripheral blood of patients during mutant BRAF-specific inhibitor therapy and found no detectable negative impact on existing systemic immunity or the de novo generation of tumor-specific T cells. The study findings strongly suggest that future trials combining V600 mutant BRAF inhibition with immunotherapy will not impair immune response as was found with previous mitogen-activated protein kinase inhibitors that were less specific and often resulted in significant detrimental effects on the immune system of cancer patients undergoing therapy.

Immunotherapeutic approaches that activate T-cell-mediated immune responses have also met with mixed clinical success, particularly in the melanoma setting (22). These include interleukin-2 (IL-2) therapy, adoptive transfer of expanded tumor-infiltrating lymphocytes (TIL), dendritic cell (DC)-based vaccines, and more recently, antibody-based therapies that target the negative regulatory molecules, cytotoxic T lymphocyte–associated antigen (CTLA)-4 or programmed death receptor (PD)-1 (23–27). Some of these therapies have mediated objective responses and extended survival in MM patients, but disease relapses nevertheless occur in most treated patients.

In light of these clinical findings, future approaches that combine immunotherapeutic drugs with kinase-targeted agents provide an attractive strategy that may potentially increase clinical response rates, with the ultimate goal of improved survival (28). Furthermore, accumulating evidence that MAPK pathway activation in tumor cells contributes to immune suppression suggests that these 2 approaches may be synergistic (16). However, because many immune cells use the MAPK pathway to mediate their own function and survival, they can be sensitive to the effects of MAPK pathway inhibitors. Indeed, previous generations of MAPK inhibitors have produced detrimental effects on systemic immunity, and some therapies have been shown to directly cause lymphopenia in treated patients (29–32). Some of these off-target effects can be attributed to the lack of specificity of the agent and/or the targeting of wild-type kinases that are essential for immune cell activity.

Newer compounds that specifically target mutated oncoproteins like BRAF(V600E) are theoretically less likely to be detrimental to the immune system because the mutation is limited to tumor cells. Laboratory studies have confirmed this notion, as exposure to BRAF(V600E)-specific inhibitors seemed to be much less toxic to human T lymphocytes in vitro than an MEK inhibitor (33, 34). However, due to the complexities of the immune system and issues related to pharmacokinetics and bioavailability, it is critical to examine the effects of mutant BRAF inhibition on systemic immunity in vivo using more direct ex vivo assays on patient blood cells. Here, we report the results of an immune monitoring study in which cancer patients were treated with GSK2118436 (35), a specific inhibitor of mutated BRAF at V600 capable of inhibiting BRAF(V600E), BRAF (V600K), and BRAF(V600G) mutations.

Materials and Methods

Patients

A companion blood collection protocol (local protocol # LAB09-0114) approved by the MD Anderson Cancer Center Institutional Review Board was introduced to patients enrolled on the GlaxoSmithKline study of a Phase I open-label, multi-dose, dose-escalation Study to Investigate the Safety, Pharmacokinetics and Pharmacodynamics of the BRAF inhibitor GSK2118436 in Subjects with Solid Tumors* (local protocol #2009-0142, NCTNCT00880321). Enrollment on the companion study was optional and only those patients who met eligibility criteria for a targeted therapy trial were approached. Inclusion criteria for the companion protocol included: patients with pathologically confirmed advanced or metastatic cancer (any tumor type); a blood sample adequate to determine baseline (pretreatment) immune response status; scheduled to receive a tyrosine kinase inhibitor regimen; and ability to understand and willingness to sign a written consent document. Exclusion criteria included: immunocompromised patients (i.e., receiving steroid or nonsteroidal anti-inflammatory medication); patients who had a bone marrow transplant at any time before the study, or any other type of transplant within 30 days before day 1 of study treatment; absolute neutrophil counts less than 1,500/mL; uncontrolled intercurrent illness including, but not limited to, ongoing or active infection requiring parenteral antibiotics; known history of HIV1 and HIV2, hepatitis B or hepatitis C; a known hypersensitivity to any of the components or metabolites of the drug products evaluated in this study; and psychologic, familial, sociologic, or geographic conditions that would not permit study follow-up and compliance with the study protocol.

Peripheral blood mononuclear cell collection and processing

Blood specimens (60 mL; 6 × 10 mL heparinized tubes) were taken for peripheral blood mononuclear cell (PBMC)
isolation and T-cell number determination. Samples were collected using standard techniques for "Green Top" Vacutainer™ Blood Collection Tubes. PBMCs were isolated with Ficoll-Hypaque gradient, frozen at −80°C overnight and then transferred to liquid nitrogen. This initial isolation, done by centrifugation (1500 to 1800 RCF, for 20 minutes in a swingout head horizontal rotor) was within 4 hours of blood collection. For analysis, PBMCs were removed from liquid nitrogen and thawed in a 37°C water bath. Thereafter, cells were washed with 20 mL of complete medium (RPMI 1640 plus 10% AB serum), centrifuged at 1,500 rpm for 10 minutes, and suspended in complete medium.

**Multiplex cytokine assay**

We measured cytokine levels from sera using a solid phase–based multiplex platform from Meso Scale Discovery (MSD). Each well of the 96-well plate–based assays contained antibodies to GM-CSF, IL-1β, IL-2, IL-12p70, IFNγ, IL-6, IL-8, IL-10, TNF-α ("Proinflammatory 9-Plex MULTI-SPOT" 96-well plate kit), as well as VEGF and MCP-1 (both singleplex kits). The MSD platform used in this study consisted of a 96-well plate–based assay with electrochemiluminescence as the basis for detection. Following the capture of the cytokine by the spotted antibody, labeled secondary detection antibodies were bound to the antigen. The detection antibodies were coupled to an electrochemiluminescent label that emitted light when electrochemically stimulated via carbon-coated electrodes in the bottom of the array wells. In addition, a detection buffer that was added before reading the array incorporated reagents that enhanced the electrochemical signal. The resulting signal was read with a charge-coupled device (Sector Imager 2400, MSD).

Calibration curves were prepared in the supplied assay diluent for human serum, with a range of 2,500 to 0.2 pg/mL (Proinflammatory 9-Plex and MCP-1) or 100,000 to 10 pg/mL (VEGF). Arrays were preincubated with 25 μL per well of assay diluent for 30 minutes, with shaking at room temperature. Following the preincubation, 25 μL of sample serum or calibrator was added in duplicate to the appropriate wells. The array was then incubated at room temperature for 2 hours with shaking and washed with Dulbecco’s phosphate-buffered saline plus 0.05% Tween 20, and 25 mL of detection antibody reagent. Following a 2-hour room temperature incubation with shaking, the array was washed and detection buffer was added. The assay results were read with an MSD Sector Imager 2400 incorporating the CCD. Sample cytokine concentrations were determined with Discovery Workbench software.

**Recall antigen–specific ELISPOT assay**

To test patients’ immunocompetence, we measured the ability of CD8+ and CD4+ T cells in PBMCs to react against a series of common recall antigens using a quantitative enzyme-linked immunosorbent spot (ELISPOT) assay measuring the frequency of IFN-γ–releasing cells. IFN-γ ELISPOT assays were conducted at pretreatment, 3 weeks posttreatment initiation (at the end of cycle 1), and 6 weeks posttreatment initiation (at the end of cycle 2). Forty-three peptides derived from cytomegalovirus (CMV), Epstein-Barr virus (EBV), and influenza (designated as CEF), presented on a large array of human leukocyte antigen (HLA) class I subtypes covering more than 90% of the human population were used to detect CD8+ T-cell responses against the CEF peptide pool. A similar ELISPOT approach was used to measure patient CD4+ T-cell immunocompetence using a recall antigen response against a chemically inactivated tetanus toxoid (TT), an agent that essentially all humans have been exposed to through vaccination. Phorbol 12-myristate 13-acetate/ionsynycin was used as a positive control for IFN-γ release. In brief, cryopreserved PBMCs were cultured overnight at 5 × 10^5 cells per well in a 96-well ELISPOT plate for detection of spots with a pair of IFN-γ–specific antibodies (Mabtech) in medium containing the 43 CEF peptide pool (each at 2 μg/mL), or inactivated TT (4 μL/mL), or no peptide (negative control). ELISPOTs were developed, dried, and read with a CTL ImmunoSpot ELISPOT reader, using the software programs Image Acquisition 4.5 and Immunospot 5 Professional (Cellular Technology Ltd.). Peptide-specific immune reactivity was determined by subtracting the background spots in wells without peptide. A positive response was defined as peptide-specific spots that were statistically higher (triplicates) than control wells using a 2-tailed t test (P < 0.05). A zero response was assigned if the peptide-specific wells were not different than control wells. The counts for the 43 CEF peptide pool or TT were presented as the total CEF or tetanus–specific T cells per million PBMCs assessed at each time point, respectively.

**Flow cytometric analysis**

Multiparameter flow cytometry analysis of different cell subsets was conducted with PBMCs from patients at different time points. Cells were stained with different antibody panels to study different lymphocyte populations. The LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (Invitrogen) was used in all tubes to exclude dead cells, except for the apoptosis panel. To study dendritic and natural killer (NK) cells, we used the following panel: CD11c-V450, CD303 (BDCA-2)-FITC, (FITC, fluorescein isothiocyanate), CD123-PE, CD3/CD14/CD19-PerCP-Cy5.5 (dump channel), CD86-APC, CD56-PE-Cy7, and CD16-APC-C7. For the T-regulatory cell panel, we stained for Foxp3-efluor450 (eBioscience), CD25-FITC, ICOS-PE (eBioscience), CD4-PerCP-Cy5.5, CD39-APC, CD45RA-PE-Cy7, and CD8-APC-H7. For the apoptosis panel, we stained for CD3-AmCyan, CD19-V450, CD4-FTC, Annexin V–PE, 7-AAD (PerCP-Cy5.5), CD8-APC, CD56-PE-Cy7, and CD16-APC-C7. The frequencies of the different populations were translated into cell numbers (cells/μL of blood) using lymphocyte absolute counts (LAC).

All patients were typed for HLA-A2 expression using an anti–HLA-A2-FITC antibody (BD Biosciences). All patients positive for HLA-A2 were tested for the presence of melanoma antigen–specific T cells specific for gp100, MART-1,
tyrosinase, NY-ESO1, or survivin. The cells were stained with CD62L-V450, CD45RA-FITC, CD3-PE, CD27- PerCP-Cy5.5, CD14/CD16/CD19-PE-Cy7 (dunk channel), and CD8-APC-H7. The APC channel was then used to accommodate the class I HLA-A2–restricted gp100 or tyrosinase APC–labeled tetramer (Beckman Coulter), class I HLA-A2–restricted survivin APC–labeled pentamer (Proimmune), or class I HLA-A2–restricted NY-ESO1 or MART-1 APC–labeled dextramer (Immudex). The frequency (baseline frequency–control tetramer/pentamer/dextramer increase) was calculated by subtracting the net baseline frequency (baseline frequency at any given time point. All antibodies are from BD Biosciences, unless otherwise indicated, and the acquisition was carried out on a FACS Canto II flow cytometer (BD Biosciences). All analysis was done with the software FlowJo (Tree Star). The frequencies of the multimer^+ CD8 T-cell populations were translated into cell numbers (# cells/mL of blood) using LAC.

Statistical considerations

Data were summarized descriptively as frequency counts, means, and fold change from baseline. The paired t test and/or the Wilcoxon signed-rank test were used to compare changes in quantitative outcomes from baseline to postbaseline assessments (cycle 1 or 2). All statistical tests were 2-sided and P < 0.05 was considered statistically significant. Statistical tests were conducted with Prism 4 software.

Results

Serum cytokine levels remained largely unaltered during GSK2118436 mutant BRAF inhibitor treatment

Thirteen patients had blood drawn before treatment with the GSK2118436 V600 mutant BRAF-specific inhibitor as well as at the end of each of two 21-day treatment cycles (cycles 1 and 2). Patient characteristics are listed in Table 1. All patient tumors contained the BRAF(V600E) mutation (GTG to GAG) that changes the encoding amino acid at position 600 from valine (V) to glutamic acid (E) except for 1 patient (05) who had a K601E mutation on exon 15 of the Raf gene. Of the 13 patients enrolled, 9 presented with melanoma, 2 had colorectal cancer, and 2 patients had papillary thyroid cancer. Six patients were treated at the 150 mg twice daily dose of GSK2118436, whereas 5 were treated at the 200 mg twice daily dose, and 2 at 100 mg 3 times a day. Six men and 7 women participated in the study, and the median age was 57 years. Similar to other BRAF (V600E)-specific inhibitors, there were significant clinical responses primarily in melanoma patients to drug treatment that will be reported separately.

To detect potential changes in systemic immunity in response to GSK2118436 treatment, we assessed the levels of several proinflammatory cytokines (GM-CSF, IFN-γ, IL-2, IL-10, IL-12p70, IL-1β, IL-6, IL-8, and TNF-α), in addition to MCP-1 and VEGF, in the serum of all patients before and after 1 or 2 cycles of treatment. We found no significant differences over the course of treatment for all cytokines measured (Supplementary Fig. S1), with the exception of TNF-α. As shown in Fig. 1, serum levels of TNF-α increased slightly, during the course of treatment, with mean baseline levels of 9.1 pg/mL rising to 11.2 pg/mL by the end of cycle 1 (P = 0.003). The increase in serum TNF-α levels was quite modest for most of the patients, but 1 patient (patient 08) showed a sharp increase in serum TNF-α levels, particularly at the end of treatment cycle 2 (Fig. 1). Interestingly, this patient had a positive blood culture for a bacterial infection and was treated for it during the same period that the rise in TNF-α levels was seen, which may explain this rise. Without this patient, the slight increase in serum TNF-α levels between the basal level and cycle 1 is significant (from P = 0.003 to 0.004), and the borderline significant increase

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Abbreviations: CRC, colorectal cancer; PR, partial response; SD, stable disease; PD, progressive disease.
between the basal level and cycle 2 becomes significant (from $P = 0.061$ to 0.013). Overall, the results indicate that serum cytokine levels remained largely unaffected by the V600 mutant BRAF inhibitor.

Mutant BRAF inhibition with GSK2118436 did not significantly alter the cell number (or frequency) of most immune cell subsets in peripheral blood

Because many immune cell types require MAPK pathway signaling for normal function, we analyzed the peripheral blood frequencies of different immune cell types to determine whether any changes could be observed over the course of GSK2118436 treatment. As shown in Fig. 2, flow cytometric analysis showed that overall cell number of T cells (CD4$^+$ and CD8$^+$), B cells, NK cells (cytotoxic and cytokine producing), DC (mDC and pDC), monocytes, and regulatory T cells (naïve and effector regulatory T cells) in peripheral blood were not significantly altered following the initiation of mutant BRAF inhibitor therapy. Although fluctuations were observed in individual patients, as a group these changes were not significant and were not likely to be attributed to the effects of drug treatment. The frequency (percentage) of these lymphocyte subpopulations was also unchanged (Supplementary Fig. S2).

Mutant BRAF inhibition with GSK2118436 did not significantly impact T cell–mediated recall responses in peripheral blood

To assess the effects of GSK2118436 treatment on functional T cell–mediated immune responses, patient PBMC samples were analyzed with IFN-$\gamma$ ELISPOT assays to measure CD8$^+$ and CD4$^+$ T-cell responses against specific recall responses at baseline, 4 weeks and 8 weeks posttreatment initiation (Fig. 3A). Figures 3B and C depict the magnitude and specificity of the CD8$^+$ and CD4$^+$ IFN-$\gamma$ responses in response to the pooled 43 CEF peptide library, or inactivated TT, respectively. The results showed that the frequency and functionality of CEF-specific CD8$^+$ T cells was not significantly altered throughout the course of the treatment (Fig. 3B). Although some patients showed variability in the ratio of IFN-$\gamma$ spots per million PBMCs between cycle 1 or 2 versus baseline, as a group, the changes were not significant ($P = 0.25$). Likewise, the frequency and functionality of TT-specific CD4$^+$ T cells was not significantly affected over the course of treatment ($P > 0.2$; Fig. 3C). Collectively, these
results show that treatment with the V600 mutant BRAF inhibitor GSK2118436 has no significant negative impact on recall responses by both CD8\(^+\) and CD4\(^+\) T cells.

**Mutant BRAF inhibition with GSK2118436 increased the cell number (and frequency) of tumor antigen–specific T cells in a subset of treated patients’ peripheral blood**

In light of recent reports describing BRAF(V600E)-mediated upregulation of melanocyte differentiation antigens (MDA) expression in melanoma cell lines (33), we were interested to determine whether V600 mutant BRAF inhibition would impact the cell number and frequency of tumor antigen–specific CD8\(^+\) T cells in the peripheral blood of treated patients. To accomplish this, we screened all 13 study patients for expression of HLA-A2 and identified 4 such patients. To monitor the frequencies of tumor antigen–specific CD8\(^+\) T cells, we stained with tetramers, pentamers, or dextramers that were specific for HLA-A2–restricted epitopes derived from gp100, MART-1, tyrosinase, survivin, and NY-ESO1, and quantified their cell number and frequencies by flow cytometric analysis and the absolute lymphocyte count. The results showed that while most of the tumor antigen–specific CD8\(^+\) T cells levels screened were undetectable, 3 of the 4 patients did have detectable melanoma antigen–specific CD8\(^+\) T cells in their blood. Figure 4A shows the different tumor-specific multimer staining that show an increase in frequency with GSK2118436 treatment. The insert in each FACS plot shows the staining of that sample with a control multimer. As shown in Fig. 4B, 2 patients (001 and 014) showed discreet cell number increases in NY-ESO1–specific CD8\(^+\) T cells following GSK2118436 treatment. In both patients, these cells were not detectable before therapy, suggesting that they were generated de novo, potentially in response to tumor destruction and subsequent tumor antigen release.
Interestingly, patient 001 also showed a modest increase in MART-1–specific CD8+ T-cell number between pretreatment baseline levels and the end of cycle 1 (Fig. 4C). In addition, we found moderately amplified CD8+ T-cell response against survivin in patient 021, when baseline levels were compared with those at the end of cycle 2 (Fig. 4D). Collectively, these results suggest that the de novo generation of tumor antigen–specific immunity is not impaired by the systemic presence of the V600 mutant BRAF–specific inhibitor GSK2118436.

Discussion

It is now well established that immunotherapeutic interventions can extend survival in a subset of melanoma cancer patients. Such approaches include IL-2 therapy, adoptive T-cell transfer, and antibodies that target T cell co-inhibitory
molecules CTLA-4 and PD-1, all of which have been shown
to have varying degrees of clinical success (22, 36). How-
ever, strong evidence has been accumulating that the
immune system can make a critical contribution to addi-
tional antitumor responses even in the context of nonim-
munotherapeutic treatments (37, 38). For example, differ-
ent chemotherapeutic agents can either enhance or inhibit
antitumor immunity, depending on whether they induce
immunogenic or nonimmunogenic forms of tumor cell
death, and how harmful the agents are to immune cells in
vivo (39, 40). The emerging paradigm is that an intact
immune system contributes significantly to the outcome
of treatment and may be important for clearance of drug-
resistant tumor cells and for prevention of recurrence (41).

The emergence of kinase inhibitors that can target onco-
genic signaling pathways in tumors and induce tumor
growth arrest and/or apoptosis has provided another very
promising approach to fighting cancer (42). Early genera-
tions of tyrosine kinase inhibitors included dasatinib, sor-
afenib, and imatinib. Although these agents can induce
objective clinical responses in some cancer patients, they
also showed severe off-target cytotoxicity to immune cells
that resulted in lymphopenia and an increased frequency of
pathogen infections (30, 43, 44). These immunosuppres-
sive effects were likely due to the lack of specificity that
characterized the early generations of kinase inhibitors that
may have resulted in off-target MAPK pathway inhibition
compromising the function and survival of lymphocytes
and other immune cells (45, 46).

More recent generations of kinase-targeted agents have
shown a much higher degree of specificity, resulting in more
effective pathway-specific inhibition as well as a greater
avidity for the mutated forms of oncogenic kinases found
only in tumor cells. Inhibitors that specifically target mutat-
ed BRAF(V600E) have shown remarkable efficacy in the
clinic and in phase II/III trials, inducing responses in most
melanoma patients harboring the V600E mutation (5). The
therapeutic window of these specific inhibitors is much
higher than previous generations of kinase inhibitors,
showing comparatively little toxicity and few off-target
effects in vivo at doses that are clinically effective (35, 47).
However, despite these encouraging results, responses to BRAF(V600E)
inhibition are relatively short lived, and disease recurrence of inhibitor-resistant tumors
occurs in nearly all treated patients (20).

The development of effective immunotherapeutic
interventions and more effective kinase inhibitors have
led to the idea of combining these therapies, with the
rationale being that the 2 treatments each use
different cytotoxic mechanisms, combination therapies
will show additive effects and will increase the duration of
responses (28). Interestingly, some studies have shown
that constitutive activation of the MAPK pathway by BRAF
(V600E) induces the downstream production of immu-
nosuppressive cytokines IL-6, IL-10, and VEGF (16).
Furthermore, another study showed that BRAF(V600E)
inhibition leads to the upregulation of MDA, leading to
increased recognition and killing of tumor cells by MDA-
specific CTL (33). This implied connection between onco-
genic BRAF signaling and immune suppression suggests
that combination therapies may, in fact, be synergistic,
provided that BRAF(V600E) inhibitors are nontoxic to
immune cells. Two recent studies are consistent with this
notion, both showing that BRAF(V600E) inhibition does
not significantly affect T-cell viability, proliferation, or
function at physiologically relevant concentrations in
vivo, in contrast to earlier generations of less specific
MAPK pathway inhibitors (33, 34).

One caveat of these in vivo studies is that although serum
concentrations of BRAF inhibitors can be accurately mea-
sured in pharmacokinetic studies in treated patients, much
of the drug may not be biologically active due to being
bound to serum proteins. Therefore, it is also critical to test
immune function in patients exposed to inhibitors, as we
have done in this study. By carrying out immune monitor-
ning on BRAF inhibitor-treated patients before and during
therapy, we show that GSK2118436 has negligible effects on
preexisting immunity and likely does not negatively impact
de novo antitumor immune responses, and on the contrary,
would seem to enhance it, as shown by the increase of tumor
antigen–specific T cells. For the most part, serum cytokine
levels, immune cell subsets, and memory CD4+ and CD8+
T-cell function remained unaffected. In fact, there was a
small but significant increase in the serum concentration of
TNF-α, and some patients even experienced an increase in
melanoma antigen–specific CD8+ T cells. In addition, the
frequency of apoptotic immune cells in the blood was not
affected during the course of treatment (data not shown).
Although these observations do not provide strong evidence
of an enhanced immune response, they do support the
notion that specific BRAF(V600E) targeting does not harm
the immune system, unlike many other more nonspecific
kinase-targeted agents. One weakness to this study was the
analysis was only conducted in peripheral blood, and not in
pre- and on-treatment tumor biopsies. Additional studies
on such biopsy samples or on TIL generated from pre- and
on-treatment lesions is warranted.

Because immunotherapy and BRAF inhibition each
induce substantial but incomplete clinical responses in
melanoma patients, it is hoped that future therapies com-
bining these 2 approaches will result in higher response
rates. We found that BRAF(V600E) kinase inhibitors such as
GSK2118436 do not adversely affect the frequencies of
different lymphocyte and antigen-presenting cell subsets
in the blood and do not affect serum cytokine levels.
Moreover, we found some evidence of increased melanoma
antigen–specific T cells in some patients during therapy.
These results suggest that BRAF(V600E) inhibition is well
poised as a combinatorial agent with immune therapies to
boost antitumor responses. Carrying out immune moni-
toring studies will be critical to analyze how the 2 therapies
work together in vivo; in particular, to determine whether
such combinations prove to be additive in their effects,
which could result in extension of patient survival, or
whether they are synergistic, in which case a higher rate of
clinical benefit may conceivably be achieved.
Disclosure of Potential Conflicts of Interest

D.S. Hong is an employee and has received a commercial research grant from GlaxoSmithKline; he has an ownership interest and is also a shareholder of GlaxoSmithKline. G.S. Falchook and R. Kurzrock have received a commercial research grant from GlaxoSmithKline. V.L. Goodman has ownership interest (including patents) from GlaxoSmithKline. J.J. Legos and S.C. Blackman have employment as Director of Cancer Research in GlaxoSmithKline. No potential conflicts of interest were disclosed by the other authors.

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References


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Correction: BRAF(V600) Inhibitor GSK2118436 Targeted Inhibition of Mutant BRAF in Cancer Patients Does Not Impair Overall Immune Competency

In this article (Clin Cancer Res 2012;18:2326–35), which was published in the April 15, 2012, issue of Clinical Cancer Research (1), there were several errors in the Disclosure of Potential Conflicts of Interest section. Dr. Hong is not an employee of GlaxoSmithKline, nor does he hold stock or ownership in the company. The author regrets these errors.

Reference

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