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SHORT COMMUNICATION

Assessment of association between BRAF-V600E mutation status in melanomas and clinical response to ipilimumab

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Abstract Ipilimumab, a fully human monoclonal antibody against cytotoxic T lymphocyte antigen-4, has demonstrated significant improvement in overall survival in previously treated advanced melanoma patients. The BRAF inhibitor, vemurafenib, has shown up to 78% objective response rates in melanoma patients harboring the BRAF-V600E mutation but not in patients lacking the mutation. As an immune potentiator, the mechanism of action of ipilimumab may not be dependent of the activity of the BRAF pathway. To test this, we investigated whether the clinical activity of ipilimumab would be affected by the BRAF-V600E mutation status of the tumors. Thus, this retrospective analysis was carried using a set of tumor biopsies from a completed phase II clinical trial. CA184004 was a randomized, double-blind, multicenter trial of 82 previously treated or untreated patients with unresectable stage III/IV melanoma. Patients received ipilimumab 3 or 10 mg/kg every 3 weeks for four doses followed by maintenance dosing in eligible patients. The BRAF-V600E mutation status for 80 patients was determined in tumor biopsies by PCR-based assays. Data on disease control were available for 69 patients with evaluated BRAF-V600E mutation status. Rates of objective responses and stable disease in patients with BRAF-V600E mutation positive tumors (30%) were comparable to those in patients with the wild-type gene (~33%). Eleven patients displayed Durable Disease Control (DDC) of which 55% had BRAF-V600E mutation positive tumors and 45% did not. In the 48 patients showing no DDC, the mutation frequency was 50%. In this study, no association between BRAF-V600E mutation status of melanoma tumors and DDC after treatment with ipilimumab was detected.

Keywords Ipilimumab · Vemurafenib · CTLA-4 · BRAF-V600E

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Introduction

Ipilimumab is a fully human monoclonal immunoglobulin (IgG1κ) that binds to the cytotoxic T lymphocyte antigen-4 (CTLA-4) molecule expressed on a subset of T cells and acts as a potentiator of T cell activity. Ipilimumab has been shown to prolong survival in patients with pre-treated metastatic melanoma with 1- and 2-year survival rates of 46 and 24.6%, respectively [1]. Durable responses (up to 46+ months in duration) were also observed.

Forty to 60% of patients with melanoma have tumors that carry a somatic mutation in the gene encoding the protein kinase BRAF and 90% of these harbor an activating point mutation at position 600 [2–10], which results in constitutive kinase activity and subsequent oncogenic potential through a variety of known mechanisms such as reduced apoptosis and increased invasiveness [11]. Recently, the BRAF inhibitor, vemurafenib, was approved by the Food and Drug Administration (FDA) for treatment of metastatic melanoma positive for the V600 mutation. The observed objective response rate to vemurafenib is

~78% in patients whose tumors have the BRAF-V600E mutation but not those with the wild-type protein [12, 13]. There is also some evidence suggesting that the use of BRAF inhibitors such as vemurafenib might in fact promote tumor growth in patients whose tumors lack the mutation [14–16].

Unlike vemurafenib or other kinase inhibitors, ipilimumab's mechanism of action is independent of the BRAF signaling pathway, as ipilimumab targets the tumors indirectly by activation of the immune system rather than directly targeting tumor cells. Thus, ipilimumab is likely to be efficacious in melanoma patients with and without the BRAF-V600E mutation. In addition, there is strong evidence suggesting that treatment with ipilimumab also leads to enhanced and durable immune responses toward tumor-derived antigens [17]. A combination of a BRAF inhibitor and ipilimumab might be considered as a potential treatment regimen that may increase efficacy and duration of response in melanoma patients.

We conducted a retrospective analysis of melanoma tumor biopsies from ipilimumab-treated patients to delineate the effect of BRAF-V600E mutation status in metastatic tumors on durable disease control (DDC) after treatment with ipilimumab.

Materials and methods

Patients and tumor biopsies

In the phase 2 biomarker-focused clinical trial, CA184004, 82 patients with stage III/IV advanced melanoma were treated with 3 or 10 mg/kg ipilimumab. Study design, response assessment and disease control assessment were described elsewhere [18]. At least one tumor biopsy was available for 80 of the 82 patients. For 53 patients, paired specimens at two time points were available: one prior to ipilimumab treatment and one 3 weeks after initiation of ipilimumab treatment. Tumor biopsies were snap-frozen and stored at −80°C. Data on disease control status were available for 69 of the 80 patients.

Real-time chemistry methodology (RT-CM) for BRAF-V600E mutation genotyping

Genomic DNA from frozen tumor biopsies was purified using the AllPrep DNA/RNA Mini Kit according to manufacturer's instructions (Catalog No. 80204, Qiagen, Valencia, CA). All PCR reagents, equipment, and analytical software were purchased from Applied Biosystems (Foster City, CA) unless indicated. BRAF-V600E mutation was detected by real-time chemistry TaqMan MGB probes as described previously [19]. Primers and probes used were as follows: BRAF-51F (forward) 5′-TACTGTTTTCCTT TACTTACTACACCTCAGA-3′, BRAF-176R (reverse) 5′-ATCCAGACAACTGTTCAAACTGATG-3′, mutant probe 5′-FAM-CTACAGaGAAATCTC-3′, and wild-type probe 5′-VIC-AGCTACAGtGAAATC-3′.

BRAF-V600E genotyping using castPCR technology

In order to confirm the results from the genotyping method described above, we also determined the BRAF mutation status with a commercially available TaqMan® mutation detection assay (Life Technologies, Carlsbad, CA). These assays use competitive allele-specific TaqMan PCR (castPCR technology). Each wild-type or mutant allele assay was composed of a modified or unmodified allele-specific forward primer, locus-specific TaqMan® probe, locus-specific reverse primer, allele-specific MGB blocker, and TaqMan® Genotyping Master Mix (Catalog No. 4371355, Life Technologies). The assay was run according to manufacturer's instructions using 20 ng of genomic DNA.

Data analysis for BRAF-V600E genotyping

Results were analyzed with the Seq Detection System version 2.3. Control reference samples included a no DNA template control, a plasmid DNA containing the BRAF-V600E gene sequence (Life Technologies) and DNA isolated from either a BRAF-V600E positive cell line, COLO 201 (CCL-224, American Type Culture Collection, Manassas, VA) or a cell line containing the wild-type (WT) BRAF gene, SKNAS (CRL-2137, ATCC). A heterozygous reference sample was generated by mixing BRAF-V600E DNA and WT DNA 1:1. Results from BRAF-V600E mutation detected by real-time chemistry with TaqMan MGB probes were called either WT or mutant (Mut) manually in reference to the control samples. Results from the TaqMan® mutation detection assay were calculated as follows: The WT Cts were subtracted from the Mut Cts, generating ΔCt. Fold Change (FC) was calculated as $2^{\Delta Ct}$. Percent Mut was calculated by FC/(1 + FC). Reference WT control samples were all ≤1% Mut, so samples ≤1% were assigned as WT, and samples ≥1% were assigned as Mut. Percent Mut ranged from 99 to 7% and in those samples designated WT the percent Mut ranged from 0.84 to 0%.

Statistical methodology

Association with BOR and DDC

Best overall response (BOR) as assessed by the investigator was based on modified WHO criteria. Frequencies of BOR values were tabulated by BRAF-V600E mutation status.

Frequencies of DDC, defined as BOR of complete response (CR), partial response (PR), or stable disease (SD) lasting at least 24 weeks from first dose of ipilimumab, were tabulated by BRAF-V600E mutation status as well.

Pre- and post-treatment agreement on BRAF-V600E mutation status

Agreement on mutation status in paired tumor biopsies from patients was tabulated based on whether mutation calls were the same or not between pre-treatment and post-treatment biopsies.

Results

BRAF V600E mutation status and comparison in pre- and post-treatment tumors

We used two assays to determine the BRAF-V600E mutation status in 80 of the available tumor biopsies. In the RT-CM, TaqMan MGB probes with either a VIC or FAM reporter fluorophore were used to detect the wild-type (WT) or the mutant BRAF V600E sequences, respectively [19]. Using this assay, classification as either wild type (WT) or mutant (BRAF-V600E) was obtained for 59 of 80 specimens. Using castPCR technology, definitive genotyping results were obtained from 100% of the 80 specimens with complete agreement between the results of the two methods in the matching 59 tumor biopsies. Of the total 80 tumor biopsies, 40 were found to carry the BRAF-V600E mutation (50%) and 40 were found to be WT V600 (50%). In addition, the BRAF V600E mutation status in 53 paired tumor samples from pre- and post-treatment biopsies were in complete agreement.

Distribution of BRAF-V600E mutation in dose cohorts

In the CA184004 study, two different doses of ipilimumab were used, 3 and 10 mg/kg. BRAF-V600E mutation was detected in 23 out of 40 tumor biopsies (57.5%) in the 3 mg/kg cohort, and 17 out of 40 tumor biopsies (42.5%) in the 10 mg/kg cohort. Because the efficacy of ipilimumab did not differ significantly between the 3 and 10 mg/kg cohorts in this trial [20], the observed imbalance in BRAF-V600E mutation frequency between doses was not expected to lead to a bias in the analysis of the association between mutation status and efficacy measures.

Tumor biopsies used in this study originated from various metastatic sites, such as pancreas, breast, lymph nodes, and liver. No apparent associations between the pattern of BRAF mutations and the site of the tumor biopsy were observed (data not shown).

Association of BOR and DDC after ipilimumab treatment and BRAF-V600E mutation status of melanoma tumors

Matching data for DDC and BRAF-V600E mutation status were obtained for 69 of the tumors isolated at pre-treatment (Table 1). Of the 11 patients who achieved DDC after ipilimumab treatment, 6 (54%) were BRAF-V600E and 5 (46%) were BRAF-V600 WT. Similarly, 24 (50%) of 48 patients in the No-DDC group were BRAF-V600E and 24 (50%) were WT. Thus, our present genotyping results did not detect any apparent association between DDC and BRAF V600E mutation status of melanoma tumors. Similarly, no apparent association was observed between BOR and BRAF V600E mutation status of the melanoma tumors (Table 1).

Discussion

In this study, we determined the BRAF-V600E mutation status in 80 tumor biopsies obtained from patients treated with ipilimumab monotherapy. Although the number of tumor biopsies was limited, specimens were obtained and analyzed from the majority of the patients enrolled in the trial (80 of the 82 patients). Forty of 80 (50%) of the tumors had the BRAF-V600E mutation and 40 (50%) of the tumors were BRAF V600 wild type. This was in agreement with published literature, where the mutation was detected in 40–60% of melanoma tumors [2–10].

Table 1 Association of BRAF-V600E mutation status with best overall response (BOR) and durable disease control (DDC)

Best overall response*	WT	BRAF-V600E
CR ($N = 1$)	1	0
PR ($N = 6$)	3	3
SD ($N = 13$)	7	6
PD ($N = 41$)	20	21
Unknown, ($N = 8$)	4	4
Total	35	34
Disease control status**	**WT**	**BRAF-V600E**
DDC, ($N = 11$)	5	6
Non-DDC, ($N = 48$)	24	24
Unknown, ($N = 10$)	6	4
Total	35	34

CR complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *N* total number of patients in row, *WT* wild-type BRAF-V600

* Assessed by modified WHO criteria

** Patients who underwent excision or resection of an index lesion were not included in the DDC group

BRAF-V600E mutation status was determined in 53 tumor biopsy pairs obtained either pre-treatment or 3 weeks after initiation of ipilimumab treatment. Although the biopsies harvested at different time points may have originated from different lesions, the BRAF-V600E mutation status was found to be in agreement within a patient; independent of timing or site of sample collection. This is consistent with the notion that the BRAF-V600E somatic mutation is an initiating event in the malignant transformation of melanomas and carried over from primary through metastatic disease [21, 22]. Although the number of paired tumors tested in this trial was limited, treatment with ipilimumab did not appear to affect the BRAF-V600E mutation status in melanoma tumors in the first 3 weeks. Furthermore, in this study, no clear evidence of an impact of the BRAF-V600E mutation on the clinical activity of ipilimumab was detected. In each of the response groups, the number of mutation positive patients was comparable to the number of WT patients.

In a previous publication, the investigators reported that non-self peptides presented by HLA-A*0201-positive melanoma cells harboring the BRAF-V600E mutation were able to induce T cell mediated cytolytic responses [23]. Therefore, theoretically ipilimumab might have provided a therapeutic advantage in this patient population over those without this mutation. However, in the current study, we did not detect any significant associations between BRAFV600E mutation status, the HLA-A*0201 status of the patients, and the DDC in ipilimumab-treated patients (data not shown). This might be due to the small sample size (only11 patients in DDC group) in the current study for performing this analysis. The development of ipilimumab for treatment of melanoma represents the first approach that has shown improvement of overall survival in advanced melanoma patients in the past few decades [24]. In addition, treatment with ipilimumab is associated with DDC in the majority of responding patients [25]. On the other hand, vemurafenib, a BRAF kinase inhibitor, shows objective responses in the majority of patients (up to 78%) but its inhibitory effects are limited to those tumors displaying the BRAF-V600E mutation and this response does not appear to be durable [12]. Recent data suggested that inhibition of BRAF in melanoma tumor cells that harbor the V600E mutation might increase expression of melanocyte antigens such as MART-1 and Gp100, which could confer improved recognition of the tumor cells by antigen-specific T cells [26]. Additionally, these researchers showed that selective inhibition of BRAF-V600E did not have deleterious effects on T cell proliferation or function. Based on this, it seems likely that the combination of a BRAF inhibitor and an immune potentiator such as ipilimumab could significantly improve anti-tumor effects and increase the frequency and/or duration of DDC. Future clinical trials are warranted to assess this hypothesis.

In summary, our data indicate that the efficacy of ipilimumab in treating melanoma tumors is not affected by the BRAF-V600E mutation status of the tumors. Ipilimumab appears to be equally effective in both the wild-type and BRAF-V600E-mutated melanoma patients.

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Conflict of interest Vafa Shahabi, Gena Whitney, Scott D. Chasalow, Suresh Alaparthy, and Jeffrey R. Jackson are employees of Bristol-Myers Squibb, the manufacturer of ipilimumab. Omid Hamid and Henrik Schmidt declare that they have no conflict of interest.

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