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Immunomodulatory Activity of Nivolumab in Metastatic Renal Cell Carcinoma

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Abstract

Purpose—Nivolumab, an anti-PD-1 immune checkpoint inhibitor, improved overall survival versus everolimus in a phase 3 trial of previously treated patients with metastatic renal cell carcinoma (mRCC). We investigated immunomodulatory activity of nivolumab in a hypothesis-generating prospective mRCC trial.

Experimental Design—Nivolumab was administered intravenously every 3 weeks at 0.3, 2, or 10 mg/kg to previously treated patients and 10 mg/kg to treatment-naïve patients with mRCC. Baseline and on-treatment biopsies and blood were obtained. Clinical activity, tumor-associated

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J.F. Kurland and J.S. Simon were employees of Bristol-Myers Squibb at the time the study was conducted and data analyzed.

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lymphocytes, PD-L1 expression (Dako immunohistochemistry; ≥5% vs. <5% tumor membrane staining), tumor gene expression (Affymetrix U219), serum chemokines, and safety were assessed.

Results—In 91 treated patients, median overall survival (95% CI) was 16.4 months (10.1–not reached [NR]) for nivolumab 0.3 mg/kg, NR for 2 mg/kg, 25.2 months (12.0–NR) for 10 mg/kg, and NR for treatment-naïve patients. Median percent change from baseline in tumor-associated lymphocytes was 69% ($CD3^+$), 180% ($CD4^+$), and 117% ($CD8^+$). Of 56 baseline biopsies, 32% had ≥5% PD-L1 expression, and there was no consistent change from baseline to on-treatment biopsies. Transcriptional changes in tumors on treatment included up-regulation of interferon-γ–stimulated genes (e.g., CXCL9). Median increases in chemokine levels from baseline to C2D8 were 101% (CXCL9) and 37% (CXCL10) in peripheral blood. No new safety signals were identified.

Conclusion—Immunomodulatory effects of PD-1 inhibition were demonstrated through multiple lines of evidence across nivolumab doses. Biomarker changes from baseline reflect nivolumab pharmacodynamics in the tumor microenvironment. These data may inform potential combinations.

Keywords

GU cancer-other; phase I-III trials-GU cancer-other; antibody immunotherapy; immunomodulation; immune responses to cancer; nivolumab; PD-1 inhibition

Introduction

Metastatic renal cell carcinoma (mRCC) is a heterogeneous disease that is highly resistant to chemotherapy (1). High-dose interleukin-2 produces durable, complete responses in a fraction of patients, providing proof of concept for the potential of immunotherapy in mRCC (2). Recently, targeted agents including tyrosine–kinase inhibitors, vascular endothelial growth factor inhibitors, and mammalian target of rapamycin inhibitors have become available for treatment of mRCC (3–6). Outcomes with these agents are improved, but therapeutic resistance is inevitable and median overall survival (OS) is limited (<20 months) (3–6).

Cellular immune responses may play a key role in modulating tumor progression in RCC and other cancers (7, 8). Tumors may co-opt immune checkpoint pathways to suppress the duration and amplitude of immune responses as a means of immune resistance (9). The introduction of the fully human programmed death-1 (PD-1) immune checkpoint inhibitor nivolumab has changed the therapeutic landscape for solid tumors, demonstrating durable responses in a subset of patients with multiple tumor types and improved OS in advanced melanoma and non–small-cell lung cancer to date (10–15). In mRCC, objective response rates (ORRs) of 20% to 22% and median OS of 18.2 to 25.5 months were reported in previously treated patients (12). A recent phase III study demonstrated significantly longer median OS with nivolumab versus everolimus (25.0 months vs. 19.6 months, respectively; HR = 0.73, $P = 0.002$) in patients with previously treated mRCC (16).

Examination of biomarkers may reveal prognostic or predictive factors relating to the disease or its treatment, which could provide the scientific rationale for combinations to

address resistance. In RCC, treatment decisions still depend primarily on clinical criteria only (17). For anti-PD-1–directed therapies, increased tumor or stromal PD-1 ligand 1 (PD-L1) expression is a logical candidate to predict therapeutic response, but does not appear to define the chance of response in a dichotomous way. Prior biomarker analysis, particularly in melanoma patients, provided further evidence that evaluating the tumor microenvironment could provide insights into the mechanism of tumor responses to immunotherapies (18–22). However, most studies evaluated biomarkers only in baseline biopsies. We hypothesize that examination of on-treatment biopsies provides additional insights into key changes in the tumor microenvironment that may contribute to or hinder immune response during treatment in mRCC.

In this hypothesis-generating exploratory analysis, we sought to investigate the immunomodulatory activity of nivolumab in patients with clear-cell mRCC. Here, we present an evaluation of changes in tumor-associated lymphocytes (e.g., $CD3^+$, $CD4^+$, and $CD8^+$ T cells), tumor PD-L1 expression, immune gene expression in the tumor, and serum-soluble factors (e.g., CXCL9 and CXCL10)—known markers of T-cell activation and migration in patients in response to immunotherapies (22–24).

Patients and Methods

Study design and treatment

This was an open-label, parallel, four-group, phase Ib study of nivolumab (Bristol-Myers Squibb, Lawrenceville, NJ; Ono Pharmaceutical Company Limited, Osaka City, Japan). Previously treated patients were randomized 1:1:1 to receive nivolumab 0.3, 2, or 10 mg/kg; treatment-naïve patients received nivolumab 10 mg/kg. Nivolumab was administered as an intravenous infusion on day 1 of the treatment cycle every 3 weeks until confirmed complete response, progressive disease, intolerable adverse events (AEs), or withdrawal of consent. Patients were permitted to continue nivolumab treatment beyond progression if the investigator noted clinical benefit and treatment tolerance.

Patients were required to provide fresh image-guided core needle biopsies of a soft-tissue site both at screening and on treatment as a condition of protocol participation. Tumor sites selected for biopsy must not have received previous radiation therapy. It was preferred that patients have at least one lesion large enough to undergo repeat core needle biopsies (one biopsy at baseline and one biopsy at C2D8). However, patients may also have had two distinct soft-tissue lesions eligible for core needle or excisional biopsies. One lesion was to be biopsied at baseline and the other was to be biopsied at C2D8.

Institutional review boards of participating institutions approved the protocol, and the study was conducted according to the Declaration of Helsinki and International Conference on Harmonisation. All patients gave written informed consent.

Patient population

Eligible patients had histologically confirmed mRCC with a clear-cell component, measurable disease defined by Response Evaluation Criteria in Solid Tumors (RECIST) v1.1, Karnofsky performance score of ≥70%, presence of soft-tissue tumor lesions that could

be biopsied at baseline and on treatment, and adequate organ and marrow function. To be eligible for the previously treated groups, patients must have been treated with between one and three previous systemic therapies for RCC, with progression following the most recent therapy within 6 months of study enrollment. For the treatment-naïve group, patients must not have received any previous systemic therapy in the metastatic or adjuvant setting. Exclusion criteria included active central nervous system metastases within 30 days of study enrollment; active or prior autoimmune disease; prior malignancy unless complete remission occurred ≥2 years prior to study enrollment; and previous treatment with anti–CTLA-4, anti–PD-1, anti–PD-L1, anti–PD-L2, anti-CD137, anti-CD40, or anti-OX40 antibodies.

Study assessments

The primary objective was to investigate the pharmacodynamic immunomodulatory activity of nivolumab on $CD4^+$ and $CD8^+$ tumor-associated lymphocytes and serum chemokines CXCL9 and CXCL10 in patients with clear-cell mRCC. Secondary objectives were to assess the activity and safety of nivolumab. Exploratory objectives included analyses of serum-soluble factors and gene expression profiling in the tumor and whole blood. Data cutoff for all analyses was January 12, 2015.

Patients were evaluated for response every 6 weeks for the first 12 months from randomization, then every 12 weeks until documented progression. Patients who discontinued treatment for reasons other than progression continued to have tumor assessments according to schedule.

Biopsies obtained from metastatic lesions at baseline (screening) and cycle 2 day 8 (C2D8) were used to evaluate tumor-associated lymphocytes, PD-L1 expression, and gene expression. Biopsies were divided in half, with half formalin-fixed and paraffin-embedded for immunohistochemistry analyses and half in RNAlater for gene expression analyses. Therefore, there was no need to prioritize tumor tissue sections for immunohistochemistry or gene expression analyses. The number and composition of tumor-associated lymphocytes were assessed using two validated multiplex immunohistochemistry assays provided by Mosaic Laboratories (Lake Forest, CA) on formalin-fixed, paraffin-embedded sections. Assays included one dual stain, $CD3^+/CD8^+$, and one triple stain, $CD3^+/CD4^+/FOXP3^+$. Each multiplex immunohistochemistry assay was designed and validated to be compatible with Clinical Laboratory Improvement Amendments guideline class I test validation. A representative 20× staining field was spectrally imaged using the Nuance Multispectral Imaging System with software v.2.4.0 (Caliper Life Sciences, Hopkinton, MA) attached to a Nikon 90i microscope. The multispectral image was acquired in a spectral range of 420 to 720 nm using 20-nm wavelength steps. Image cubes were analyzed using inForm software v1.2 (Caliper Life Sciences). Image cubes were unmixed using spectral absorbance patterns for each chromogen and hematoxylin.

PD-L1 expression on the tumor cell surface was assessed in fresh samples at a central laboratory using an automated immunohistochemistry assay (Bristol-Myers Squibb/Dako immunohistochemistry assay using the 28–8 antibody), as described previously (25). RNA was extracted from fresh biopsies in parallel to immunohistochemistry and from whole blood at C1D1 (prior to nivolumab infusion), C1D2, and C2D8. RNA was labeled by WT-

Pico Ovation (NuGEN, San Carlos, CA). Gene expression profiling was performed using the HG-U219 array plate on the GeneTitan platform (Affymetrix, Santa Clara, CA). The robust multi-array analysis algorithm (26) was used to establish intensity values for each of 18,562 loci (BrainArray v.10) (27). Data have been deposited in ArrayExpress (E-MTAB-3218 and E-MTAB-3219).

Assessment of serum chemokines (CXCL9, CXCL10) and other serum-soluble factors at baseline (C1D1 before treatment), C1D1 (after treatment), C2D1, C2D8, and C4D1 was performed for all treated patients for whom serum was available using a multiplex panel based on Luminex technology (Myriad Rules-Based Medicine, Austin, TX).

Safety assessments were conducted at every visit and evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0 (28). Patients who received at least one dose of study drug were included in the safety population.

Statistical analysis

Activity analyses included best overall response (complete response, partial response, stable disease, progressive disease), progression-free survival (PFS), and OS, as well as ORR, i.e., the proportion of patients whose best response was a complete response or partial response. The 95% confidence intervals (CIs) for ORR were estimated using the Clopper–Pearson method (29). PFS was defined as the time from first dose to first documented disease progression, or death. PFS and OS functions were estimated by the Kaplan–Meier method with 95% CIs estimated using Greenwood's formula (30).

This study was not designed to statistically test a specific hypothesis; therefore, the sample size was not based on statistical power calculations. Pharmacodynamic effects of nivolumab on tumor-associated lymphocytes were described using summary statistics and changes or percent changes from baseline tabulated by cycle, visit, and dose.

Pharmacodynamic analyses of gene expression were based on an extended linear model, fit by restricted maximum likelihood (NLME version 3.1-109 under R 3.0.1 for Linux) (31). For blood samples, the model included fixed effects of treatment group and time on study as categorical variables, and treatment-by-time on study interactions. For tumor biopsy samples, the model also included fixed effects of process batch and sex (the latter because women were not equally represented in samples from each trial treatment group). Within-patient correlations were modeled by a spatial exponential structure with Euclidean distance. A multi-contrast conditional F test was used to compare the null hypothesis that all time-related fixed-effect parameters were zero versus an alternative hypothesis that gene expression changed over time in at least one treatment group. The q value of each test (expected proportion of false positives incurred at that P value) was also estimated. Results presented are genes for which this null hypothesis was rejected (P for time on study < 0.01), and the change over time averaged over treatment groups was ≥1.3-fold (biopsy; 108 genes in Supplementary Table S1) or ≥1.2-fold (blood; 59 genes in Supplementary Table S2). Transcripts meeting these significance criteria for a test of time on study were evaluated for enrichment (32) (P values provided were Bonferroni-corrected) of 1,539 genes from immune cell lineages (33) and for biological impact (MetaCore; Thomson Reuters, New York, NY).

To examine possible treatment group-specific effects among genes where the null hypothesis was rejected (P for time on study, < 0.01), a second such multi-contrast test of all time-by-treatment interaction parameters was used to test whether the pattern of expression change differed between at least two treatment groups. If this null hypothesis was rejected ($P < 0.01$ for interaction between dose and time on study or between previous treatment status and time on study), then we examined the effect size in each treatment group. Genes for which the change over time for at least 1 treatment group was ≥1.3-fold (biopsy; 37 probesets) or ≥1.2-fold (blood; 24 probesets) are presented in Supplementary Tables S3 and S4. In all cases, the change over time for at least two of the other treatment groups did not meet those criteria.

Results

Patient population

Patients were enrolled from September 2011 to September 2012 at 14 participating international centers. Ninety-two patients were assigned to treatment, 91 of whom were treated (Supplementary Fig. S1). The median age was 61 years, 67% were male, and 66% had received previous therapy for metastatic disease (Table 1). Baseline characteristics were similar between previously treated ($n = 67$) and treatment-naïve ($n = 24$) patients (Table 1).

Clinical activity

Responses were evaluated in the 91 treated patients (Table 2). The ORR was 15% (95% CI, 8.7–24.5). PFS rates were 43% (95% CI, 32–53) at 24 weeks and 25% (95% CI, 16–35) at 48 weeks. OS rates were 75% (95% CI, 64–83) at 12 months and 58% (95% CI, 46–68) at 24 months. Median OS (95% CI) was 16.4 months (10.1–not reached [NR]), NR, and 25.2 months (12.0–NR) for the nivolumab 0.3, 2, and 10 mg/kg groups for previously treated patients, respectively, and NR for treatment-naïve patients.

Tumor-associated lymphocytes

Repeat core needle biopsies were obtained for the majority of patients (same lesion: 60/91; distinct or unknown second lesion: 13/91), with metastatic sites including liver (16/73), lymph nodes (12/73), kidney (9/73), lung (6/73), adrenal (4/73), pancreas (2/72), and other locations (soft tissue, 24/73). Immunohistochemical analysis showed enrichment of $CD3^+$, $CD4^+$, and $CD8^+$ cells from baseline to C2D8 (Figs. 1A, 1B). For all nivolumab-treated patients who had baseline and C2D8 values ($N = 36$), median increases from baseline to C2D8 in the proportion of $CD3^+$, $CD4^+$, and $CD8^+$ cells were 9.83%, 0.44%, and 2.64%, respectively. Median percent changes from baseline of $CD3^+$, $CD4^+$, and $CD8^+$ cells were 69%, 180%, and 117%, respectively, with most patients experiencing increases. Baseline percentages and increases from baseline were greater for $CD3^+$ and $CD8^+$ than for $CD4^+$ cells (Fig. 1B). Most patients had very low or undetectable levels of $CD4^+$ cells at baseline and only modest changes from baseline. Fourteen patients had both baseline percentage levels of $CD4^+$ cells and changes from baseline values of <0.45%. These results did not appear to vary with nivolumab dose or previous treatment status (data not shown). The proportion of $CD3^+$, $CD4^+$, and $CD8^+$ cells and their relationships to each other are shown in Supplementary Figure S2.

RNA expression analysis from tumor biopsies obtained in parallel ($n = 114$) showed that mean levels of transcripts for subunits of CD3 and CD8, but not CD4, significantly increased on treatment (1.7-fold for 915_at/CD3D, $P = 0.006$; 1.7-fold for 925_at/CD8A, $P = 0.002$; 1.2-fold for 920_at/CD4, $P = 0.175$; Fig. 1C). These changes were not dependent on nivolumab dose or previous treatment status.

PD-L1 expression

PD-L1 expression on tumor cells was assessed by immunohistochemistry in fresh biopsies obtained at baseline and C2D8. Of 56 evaluable baseline biopsies, 18 (32%) had ≥5% PD-L1 expression. In patients with fresh matched biopsies at baseline and on treatment, there was no consistent change in tumor PD-L1 expression following nivolumab treatment relative to baseline (Supplementary Fig. S3).

Gene expression profiling

Expression profiling data were obtained from 59 tumor biopsies at baseline and 55 at C2D8. A total of 42 patients had samples at both timepoints, of which at least 34 were repeat biopsies of the same lesion. Statistical analysis identified 108 transcripts that changed over time (≥1.3-fold change in mean expression, $P < 0.01$; Supplementary Table S1). The 108 transcripts included 71 previously associated with immune lineages (33) ($P = 1.3\times10^{-76}$), all of which increased at C2D8. Of these 71 transcripts, 43 have been defined as lymphoid-lineage specific (e.g., GZMA/G/H and KLRB1/D1/G1) or myeloid-lineage specific (e.g., CXCL11, PD-L1, and IDO1) (Fig. 2A). A subset of the lymphoid-lineage transcripts are completely specific to the T-cell lymphoid subset (e.g., CTLA-4, CD8A/B, CD3D/E/G, and ICOS). Sixteen of the 108 transcripts were previously identified as interferon-regulated (34), including CXCL9 and CD274 (PD-L1). Interferon-γ was the only interferon represented in the 108 genes. Forty-seven of the transcripts were previously identified as showing differential expression in baseline biopsies of patients who subsequently responded to ipilimumab ($P = 2.7\times10^{-86}$).

To evaluate whether pharmacodynamic transcriptional changes were observed in the periphery, microarray analysis was performed on whole blood samples ($N = 82$ at C1D1 and 74 at C1D2, with 70 having matched samples; $N = 73$ at C2D8). Expression of 59 transcripts changed from baseline (C1D1) to C1D2 (≥1.2-fold change in mean expression, $P < 0.01$; Supplementary Table S2), including 30 previously associated with immune lineages (33) ($P < 0.001$; Fig. 2B). These included transcripts for T-cell receptor α and β subunits and the CD3 γ subunit, all of which decreased relative to baseline. The 59 transcripts included 29 previously identified as interferon-regulated (34), all of which increased. No transcripts from interferon genes were regulated or detectable in blood.

These transcriptional effects were generally similar between dose groups and between previously treated and treatment-naïve patients ($P > 0.01$ for interaction between time and dose group or previous treatment status). The analyses of pharmacodynamic transcriptional effects that differ between treatment groups are presented in Supplementary Table S3 and Supplementary Table S4.

Interferon-γ–related chemokines

As observed increases in interferon-γ–regulated chemokine transcripts in tumor could potentially result in an increase in circulating chemokines in the periphery, several serum-soluble factors were quantified (Supplementary Table S5). In serum, increases were noted in CXCL9 and CXCL10, with median changes of 1,861 pg/mL (range, −2,000 to 22,890) and 157 pg/mL (range, −398 to 3,930), respectively, from baseline (C1D1 before treatment) to C2D8 ($N = 83$). Median percent changes from baseline of these chemokines were 101% (range, 45–1,730%) and 37% (range, −30% to 936%), respectively. Most patients had increases in both CXCL9 and CXCL10, observed across all baseline values (Fig. 3A). Within-patient changes in CXCL9 tended to be greater than within-patient changes in CXCL10 (Fig. 3B), and changes in CXCL9 and CXCL10 were highly correlated. In tumor, mean levels of CXCL9 and CXCL10 transcripts increased from baseline to C2D8 (2.4-fold for 4283_at/CXCL9, $P < 0.001$; 2-fold for 3627_at/CXCL10, $P = 0.011$; Fig. 3C). These observed changes were not associated with nivolumab dose or previous treatment status (data not shown). Serum levels of CXCL9 and CXCL10 cytokines at C2D8 showed correlation with their transcript levels in the corresponding patient biopsy ($N = 54$; CXCL9: $r = 0.37$, $P = 0.006$; CXCL10: $r = 0.30$, $P = 0.029$; Fig. 3D). No correlation was observed between serum cytokine levels at C1D1 (before treatment) and transcript levels in the corresponding patient biopsy obtained at screening ($r < 0.21$, $P > 0.1$).

Safety

Treatment-related AEs of any grade occurred in all previously treated and treatment-naïve patients (Table 3). The most common AEs (all grades) were fatigue and nausea, mainly grade 1 or 2. Grade 3 to 4 AEs occurred in 54% of previously treated patients overall and 50% of treatment-naïve patients, mainly fatigue.

Categories of select AEs, or AEs with potential immunologic causes, were also assessed (Table 3). Of interest, select pulmonary AEs (all pneumonitis) were only reported in 3 patients in the previously treated group (1 in the 0.3 mg/kg group and 2 in the 10 mg/kg group) and 3 patients in the treatment-naïve group.

Discussion

This prospective exploratory study highlights the importance of these pharmacodynamic studies for future investigations of immunotherapeutic agents in mRCC. This is the first prospective translational study involving analysis of both baseline and on-treatment biopsies in RCC, aimed specifically at understanding the immunomodulatory activity of nivolumab. The immunomodulatory effect of PD-1 inhibition with nivolumab was demonstrated through multiple lines of evidence across all doses studied.

Clinical activity was observed in both treatment-naïve and previously treated patients at each dose. Median OS was between 16.4 and 25.2 months for previously treated patients, similar to that seen in a phase II, randomized, dose-ranging study of nivolumab (18.2–25.5 months) and in a recent phase III study of nivolumab versus everolimus (25.0 months) in advanced clear-cell RCC (12, 16). Median OS was not reached for treatment-naïve patients. The type

and frequency of AEs were similar in previously treated and treatment-naïve patients and consistent with previous reports of nivolumab in solid tumors; the frequency of severe AEs was low (10–12). As with the dose-ranging study of nivolumab (12), there was no obvious dose-response relationship.

The current study of the pharmacodynamic effects of nivolumab in RCC has important implications to further our understanding of its mechanism of action in this setting and to select combination therapies. We have demonstrated that nivolumab reverses T-cell exhaustion within the tumor microenvironment as hypothesized. Immunohistochemistry analysis of tumor-associated lymphocyte markers (i.e., $CD3^+$ and $CD8^+$) showed increased lymphocytic presence in biopsies at C2D8 in the majority of treated patients across all nivolumab doses. This was accompanied by significant increases in the expression of genes that are hallmarks of Th1 inflammatory response and cytotoxic T-cell function, such as ICOS, interferon-γ, granzymes, and perforin. Transcripts for T-cell receptor subunits (e.g., CD3γ, TCRα, TCRβ) rapidly and transiently decreased in whole blood after treatment with nivolumab, suggesting that nivolumab may prompt T cells to exit the periphery. These data suggest that nivolumab either increased tumor trafficking or infiltration of T cells, facilitated the expansion of T cells already within the tumor microenvironment, or both.

At the tumor site, increased transcription was observed for genes encoding CXCL9 and CXCL10, key interferon-γ–regulated chemokines that guide the trafficking behavior of T cells, and for which elevated expression is a favorable prognostic factor in RCC (35). Two-thirds of patients had repeat core needle biopsies of the same soft-tissue lesions at baseline and on treatment, but we do not feel that multiple biopsies at the same site affected the conclusions of our study. In fact, a number of studies have shown the feasibility of undertaking repeat biopsies for the pharmacodynamic evaluation of targeted agents (36). Clinical response to ipilimumab, which promotes proliferation and activation of peripheral T cells, has been associated with pretreatment levels of CXCL9 and CXCL10 transcripts at the tumor site (21). The concomitant increased serum concentrations of CXCL9 and CXCL10 at C2D8, in the absence of detectable transcripts in whole blood, are in agreement with a model in which nivolumab induces CXCL9 and CXCL10 production by myeloid cells at inflammatory sites in tissue in order to recruit immunocompetent T cells to the tumor microenvironment (37). Notably, nivolumab produces a pharmacodynamic increase in transcripts for CTLA-4 itself and for a significant number of genes whose expression is associated with clinical response to ipilimumab (21), providing support for combination with anti-CTLA-4 therapies.

Transcripts for PD-L1 (CD274) are among those showing a pharmacodynamic effect in tumor biopsies. This PD-L1 expression likely originates from myeloid cell infiltrates, because we show that nivolumab treatment does not appear to consistently modulate PD-L1 expression at the tumor cell surface. Nivolumab-induced T-cell reactivation was expected to increase interferon-γ–regulated transcripts and proteins due to initiation of adaptive immune response. Using gene expression profiling, interferon-γ transcripts and interferon-regulated transcripts were found to have increased in tumors, while only interferon-regulated transcripts were seen to rapidly and transiently increase in whole blood following nivolumab treatment. These effects are in line with observations from previous studies that

demonstrated enhanced T-cell activation and increased expression of interferon-γ at the tumor site after PD-L1/PD-L2 blockade or PD-1 inhibition (38–40).

Interestingly, the pharmacodynamic effects of nivolumab also include significant increases in the expression of genes linked to innate immunity and natural killer (NK) cell function (e.g., KLRB1/D1/G1, CD69, and NKG7), suggesting the testable hypothesis that nivolumab may boost T-cell–mediated antitumor immune activity through enhanced infiltration and/or stimulation of NK cells. Das et al. similarly found that nivolumab modulated genes involved in NK cell function (41). These data suggest that nivolumab may be effectively combined with NK cell–directed therapies, such as lirilumab, in metastatic RCC.

This analysis identified immune pharmacodynamic effects that are produced across nivolumab doses and in most patients. Additional analyses of these data are being conducted to investigate the relationship between the immunomodulatory activity of nivolumab and clinical outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Conflict-of-Interest Statement

T.K. Choueiri has served as a consultant/advisor for Pfizer, GlaxoSmithKline, Novartis, Merck, Bristol-Myers Squibb, Bayer, Eisai, Roche, and Prometheus Labs, Inc., and has received institutional research funding from Pfizer, Novartis, GlaxoSmithKline, Bristol-Myers Squibb, Merck, Exelixis, Roche, AstraZeneca, Peloton, and Tracon.

M.N. Fishman has participated in speakers bureaus/advisory boards for Prometheus, GlaxoSmithKline, Pfizer, and Eisai, and has received research funding from Prometheus, GlaxoSmithKline, Pfizer, Bristol-Myers Squibb, Eisai, Acceleron, Tracon, Bayer/Onyx, and Exelixis.

B. Escudier has received honoraria from and has served as a consultant for Novartis, Bayer, GlaxoSmithKline, Pfizer, and Exelixis.

D.F. McDermott has received honoraria from and served as a consultant/advisor for Bristol-Myers Squibb, Merck, and Genentech.

C.G. Drake has served as a consultant/advisor for Agenus, Bristol-Myers Squibb, Compugen, Dendreon, MedImmune, ImmunExcite, ImmuNext, NexImmune, Janssen, Lilly, Merck, Pierre Fabre, Novartis, Potenza Therapeutics, Roche/Genentech, and Vesuvius, owns stock in Compugen, NexImmune, Potenza, and Tizona, and holds patents with Bristol-Myers Squibb and Janssen.

H. Kluger has received honoraria from Merck, served as a consultant for Prometheus Pharmaceuticals, Bioclinica, and Regeneron, received research funding from Merck and Bristol-Myers Squibb, and received funding for travel expenses from Bristol-Myers Squibb.

W.M. Stadler has received research funding from Bristol-Myers Squibb.

J.L. Perez-Gracia has received research funding from Bristol-Myers Squibb.

D.G. McNeel has served as a consultant/advisor for, received funding for leadership, received travel expenses from, owns stock in, and hold patents with Madison Vaccines Inc., and received research funding from Madison Vaccines Inc, Dendreon Corp., Bristol-Myers Squibb, and Medivation.

B. Curti has received honoraria from Prometheus Pharmaceuticals, received research funding from Bristol-Myers Squibb, MedImmune, and Prometheus Pharmaceuticals, received funding for travel expenses from Bristol-Myers Squibb, MedImmune, and Prometheus Pharmaceuticals, and received support from Bristol-Myers Squibb and MedImmune.

M.R. Harrison has served as a consultant/advisor for Novartis, AVEO Pharmaceuticals, Exelixis, Bayer, Genentech, and Pfizer, has received honoraria from Novartis and

Prometheus Pharmaceuticals, and has received research funding from Bristol-Myers Squibb, Argos Therapeutics, Exelixis, Pfizer, Dendreon, Janssen, and Genentech.

E.R. Plimack has served as a consultant/advisor for and research funding from Bristol-Myers Squibb, Merck, Dendreon, GlaxoSmithKline, Pfizer, and Acceleron, research funding from AstraZeneca, Eli Lilly, and Acceleron, and served as a consultant/advisor for Astellas, Novartis, and Genentech.

L. Appleman has received research funding from Bristol-Myers Squibb.

L. Fong has received research funding from Bristol-Myers Squibb.

L. Albiges has received research funding from Novartis and Pfizer, has served as a consultant for Novartis, Pfizer, Amgen, Sanofi, Bayer, and Bristol-Myers Squibb, and served as advisor for Novartis, Pfizer, and Bristol-Myers Squibb.

L. Cohen, T.C. Young, S.D. Chasalow, P. Ross-Macdonald, S. Srivastava, and M. Jure-Kunkel are employees of Bristol-Myers Squibb.

S.D. Chasalow, J. F. Kurland and P. Ross-Macdonald own stock in Bristol-Myers Squibb.

S. Srivastava has received honoraria from Bristol-Myers Squibb.

M. Jure-Kunkel holds a “Use of nivolumab in clear cell RCC” patent (pending).

J.F. Kurland and J.S. Simon were employees of Bristol-Myers Squibb at the time of this study analysis.

M. Sznol has served as a consultant/advisor for Genentech-Roche, Bristol-Myers Squibb, Astra-Zeneca/Medimmune, Pfizer, Novartis, Kyowa-Kirin, Amgen, Merus, Seattle Genetics, Immune Design, Prometheus, Anaeropharma, Astellas-Agensys, Immunova, Nektar, Neostem, Pierre-Fabre, Eli Lilly, Symphogen, Lion Biotechnologies, Amphivena, and Adaptive Biotechnologies.

References

1. Buti S, Bersanelli M, Sikokis A, Maines F, Facchinetti F, Bria E, et al. Chemotherapy in metastatic renal cell carcinoma today? A systematic review. Anticancer Drugs. 2013; 24:535–54. [PubMed: 23552469]
2. Fyfe G, Fisher RI, Rosenberg SA, Sznol M, Parkinson DR, Louie AC. Results of treatment of 255 patients with metastatic renal cell carcinoma who received high-dose recombinant interleukin-2 therapy. J Clin Oncol. 1995; 13:688–96. [PubMed: 7884429]
3. Hutson TE, Escudier B, Esteban E, Bjarnason GA, Lim HY, Pittman KB, et al. Randomized phase III trial of temsirolimus versus sorafenib as second-line therapy after sunitinib in patients with metastatic renal cell carcinoma. J Clin Oncol. 2014; 32:760–7. [PubMed: 24297950]
4. Motzer RJ, Escudier B, Oudard S, Hutson TE, Porta C, Bracarda S, et al. Phase 3 trial of everolimus for metastatic renal cell carcinoma: final results and analysis of prognostic factors. Cancer. 2010; 116:4256–65. [PubMed: 20549832]
5. Motzer RJ, Porta C, Vogelzang NJ, Sternberg CN, Szczylik C, Zolnierek J, et al. Dovitinib versus sorafenib for third-line targeted treatment of patients with metastatic renal cell carcinoma: an open-label, randomised phase 3 trial. Lancet Oncol. 2014; 15:286–96. [PubMed: 24556040]

6. Motzer RJ, Escudier B, Tomczak P, Hutson TE, Michaelson MD, Negrier S, et al. Axitinib versus sorafenib as second-line treatment for advanced renal cell carcinoma: overall survival analysis and updated results from a randomised phase 3 trial. Lancet Oncol. 2013; 14:552–62. [PubMed: 23598172]
7. Drake CG, Lipson EJ, Brahmer JR. Breathing new life into immunotherapy: review of melanoma, lung and kidney cancer. Nat Rev Clin Oncol. 2014; 11:24–37. [PubMed: 24247168]
8. Harshman LC, Drake CG, Choueiri TK. PD-1 blockade in renal cell carcinoma: to equilibrium and beyond. Cancer Immunol Res. 2014; 2:1132–41. [PubMed: 25480555]
9. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012; 12:252–64. [PubMed: 22437870]
10. Brahmer JR, Drake CG, Wollner I, Powderly JD, Picus J, Sharfman WH, et al. Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. J Clin Oncol. 2010; 28:3167–75. [PubMed: 20516446]
11. Drake CG, McDermott DF, Sznol M, Choueiri TK, Kluger HM, Powderly JD, et al. Survival, safety, and response duration results of nivolumab (Anti-PD-1; BMS-936558; ONO-4538) in a phase I trial in patients with previously treated metastatic renal cell carcinoma (mRCC): Long-term patient follow-up. J Clin Oncol. 2013; 31(suppl) Abstract 4514.
12. Motzer RJ, Rini BI, McDermott DF, Redman BG, Kuzel TM, Harrison MR, et al. Nivolumab for metastatic renal cell carcinoma: results of a randomized phase II trial. J Clin Oncol. 2014; 33:1430–7. [PubMed: 25452452]
13. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. 2012; 366:2443–54. [PubMed: 22658127]
14. Topalian SL, Sznol M, McDermott DF, Kluger HM, Carvajal RD, Sharfman WH, et al. Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab. J Clin Oncol. 2014; 32:1020–30. [PubMed: 24590637]
15. McDermott DF, Drake CG, Sznol M, Choueiri TK, Powderly JD, Smith DC, et al. Survival, durable response, and long-term safety in patients with previously treated advanced renal cell carcinoma receiving nivolumab. J Clin Oncol. 2015; 33:2013–20. [PubMed: 25800770]
16. Motzer RJ, Escudier B, McDermott DF, George S, Hammers HJ, Srinivas S, et al. Nivolumab versus everolimus in advanced renal-cell carcinoma. N Engl J Med. 2015; 373:1803–13. [PubMed: 26406148]
17. Hernandez-Yanez M, Heymach JV, Zurita AJ. Circulating biomarkers in advanced renal cell carcinoma: clinical applications. Curr Oncol Rep. 2012; 14:221–9. [PubMed: 22434312]
18. Gajewski TF, Louahed J, Brichard VG. Gene signature in melanoma associated with clinical activity: a potential clue to unlock cancer immunotherapy. Cancer J. 2010; 16:399–403. [PubMed: 20693853]
19. Gajewski TF, Schreiber H, Fu YX. Innate and adaptive immune cells in the tumor microenvironment. Nat Immunol. 2013; 14:1014–22. [PubMed: 24048123]
20. Camisaschi C, Vallacchi V, Castelli C, Rivoltini L, Rodolfo M. Immune cells in the melanoma microenvironment hold information for prediction of the risk of recurrence and response to treatment. Expert Rev Mol Diagn. 2014; 14:643–6. [PubMed: 24914691]
21. Ji RR, Chasalow SD, Wang L, Hamid O, Schmidt H, Cogswell J, et al. An immune-active tumor microenvironment favors clinical response to ipilimumab. Cancer Immunol Immunother. 2012; 61:1019–31. [PubMed: 22146893]
22. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature. 2014; 515:568–71. [PubMed: 25428505]
23. Carthon BC, Wolchok JD, Yuan J, Kamat A, Ng Tang DS, Sun J, et al. Preoperative CTLA-4 blockade: tolerability and immune monitoring in the setting of a presurgical clinical trial. Clin Cancer Res. 2010; 16:2861–71. [PubMed: 20460488]

24. Bedognetti D, Spivey TL, Zhao Y, Uccellini L, Tomei S, Dudley ME, et al. CXCR3/CCR5 pathways in metastatic melanoma patients treated with adoptive therapy and interleukin-2. Br J Cancer. 2013; 109:2412–23. [PubMed: 24129241]
25. Sznol M, Kluger HM, Callahan MK, Postow MA, Gordon RA, Segel NH, et al. Survival, response duration, and activity by BRAF mutation (MT) status of nivolumab (NIVO, anti-PD-1, BMS-936558, ONO-4538) and ipilimumab (IPI) concurrent therapy in advanced melanoma (MEL). J Clin Oncol. 2014; 32(suppl) Abstract LBA9003.
26. Irizarry RA, Bolstad BM, Collin F, Cope LM, Hobbs B, Speed TP. Summaries of Affymetrix GeneChip probe level data. Nucleic Acids Res. 2003; 31:e15. [PubMed: 12582260]
27. Dai M, Wang P, Boyd AD, Kostov G, Athey B, Jones EG, et al. Evolving gene/transcript definitions significantly alter the interpretation of GeneChip data. Nucleic Acids Res. 2005; 33:e175. [PubMed: 16284200]
28. National Cancer Institute. Common Terminology Criteria for Adverse Events (CTCAE) v4.0. CTEP website [Internet]. Available from: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40
29. Clopper CJ, Pearson ES. The use of confidence or fiducial limits illustrated in the case of the binomial. Biometrika. 1934; 26:404–13.
30. Brookmeyer R, Crowley J. A confidence interval for the median survival time. Biometrics. 1982; 38:29–41.
31. Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. the R Development Core Team. R package version 3.1-109. Feb 20. 2013 Linear and non-linear mixed effects model.
32. Tilford, CA., Siemer, NO. Gene set enrichment analysis. In: Nikolsky, Y., Bryant, J., editors. Protein Networks and Pathways. New York: Humana Press; 2009. p. 99-122.
33. Abbas AR, Baldwin D, Ma Y, Ouyang W, Gurney A, Martin F, et al. Immune response in silico (IRIS): immune-specific genes identified from a compendium of microarray expression data. Genes Immun. 2005; 6:319–31. [PubMed: 15789058]
34. Chaussabel D, Quinn C, Shen J, Patel P, Glaser C, Baldwin N, et al. A modular analysis framework for blood genomics studies: application to systemic lupus erythematosus. Immunity. 2008; 29:150–64. [PubMed: 18631455]
35. Kondo T, Nakazawa H, Ito F, Hashimoto Y, Osaka Y, Futatsuyama K, et al. Favorable prognosis of renal cell carcinoma with increased expression of chemokines associated with a Th1-type immune response. Cancer Sci. 2006; 97:780–6. [PubMed: 16863511]
36. Dowlati A, Haaga J, Remick SC, Spiro TP, Gerson SL, Liu L, et al. Sequential tumor biopsies in early phase clinical trials of anticancer agents for pharmacodynamic evaluation 1. Clin Cancer Res. 2001; 7:2971–6. [PubMed: 11595684]
37. Gasperini S, Marchi M, Calzetti F, Laudanna C, Vicentini L, Olsen H, et al. Gene expression and production of the monokine induced by IFN-gamma (MIG), IFN-inducible T cell alpha chemoattractant (I-TAC), and IFN-gamma-inducible protein-10 (IP-10) chemokines by human neutrophils. J Immunol. 1999; 162:4928–37. [PubMed: 10202039]
38. Brown JA, Dorfman DM, Ma FR, Sullivan EL, Munoz O, Wood CR, et al. Blockade of programmed death-1 ligands on dendritic cells enhances T cell activation and cytokine production. J Immunol. 2003; 170:1257–66. [PubMed: 12538684]
39. Rodig N, Ryan T, Allen JA, Pang H, Grabie N, Chernova T, et al. Endothelial expression of PD-L1 and PD-L2 down-regulates CD8+ T cell activation and cytolysis. Eur J Immunol. 2003; 33:3117–26. [PubMed: 14579280]
40. Peng W, Liu C, Xu C, Lou Y, Chen J, Yang Y, et al. PD-1 blockade enhances T-cell migration to tumors by elevating IFN-gamma inducible chemokines. Cancer Res. 2012; 72:5209–18. [PubMed: 22915761]
41. Das R, Verma R, Sznol M, Boddupalli CS, Gettinger SN, Kluger H, et al. Combination therapy with anti-CTLA-4 and anti-PD-1 leads to distinct immunologic changes in vivo. J Immunol. 2015; 194:950–9. [PubMed: 25539810]

Translational Relevance

This is the first prospective translational study of the programmed death-1 (PD-1) immune checkpoint inhibitor nivolumab involving analysis of both baseline and on-treatment biopsies in metastatic renal cell carcinoma. It was conducted to understand the immunomodulatory activity of nivolumab through the evaluation of key cellular immune responses and changes in the tumor microenvironment. In addition to demonstrating the immunomodulatory effect of PD-1 inhibition with nivolumab through multiple lines of evidence, this study identified immune pharmacodynamic effects that were shared by the majority of patients, irrespective of the dose administered. Transcriptional changes induced by nivolumab in the tumor microenvironment may provide the rationale for additional immune therapies in combination with nivolumab.

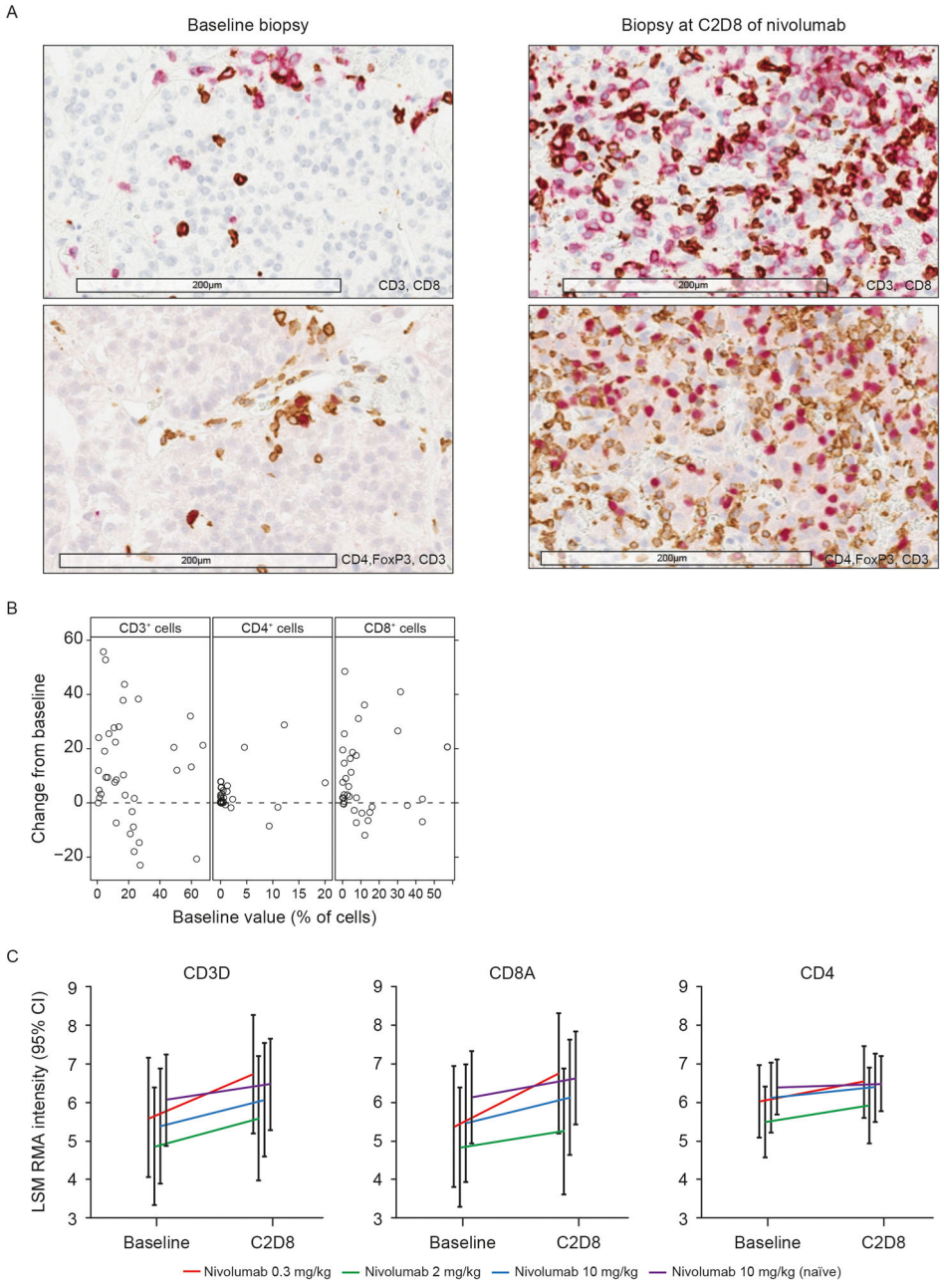

Figure 1.

Evaluation of tumor-associated lymphocytes in tumor biopsies obtained at baseline and at C2D8 of nivolumab treatment. A. Immunohistochemistry for $CD3^+$, $CD4^+$, and $CD8^+$ cells. Scale bars are denoted on the images. Top two panels: CD3 (red), CD8 (brown); bottom two panels: CD3 (brown), CD4 (purple), FoxP3 (nuclei, red). B. Change from baseline in percentage of cells that are $CD3^+$, $CD4^+$, or $CD8^+$ in tumor biopsies. Data are included for patients with immunohistochemistry data at both baseline and C2D8 in all treatment groups combined ($N = 36$). C. Expression levels for genes CD3D (915_at), CD8A (925_at), and CD4 (920_at) in tumor biopsies. Values presented are least squares means of the (log-2)

robust multi-array intensity for the treatment group and time point indicated. Error bars indicate 95% confidence intervals estimated from the extended linear model.

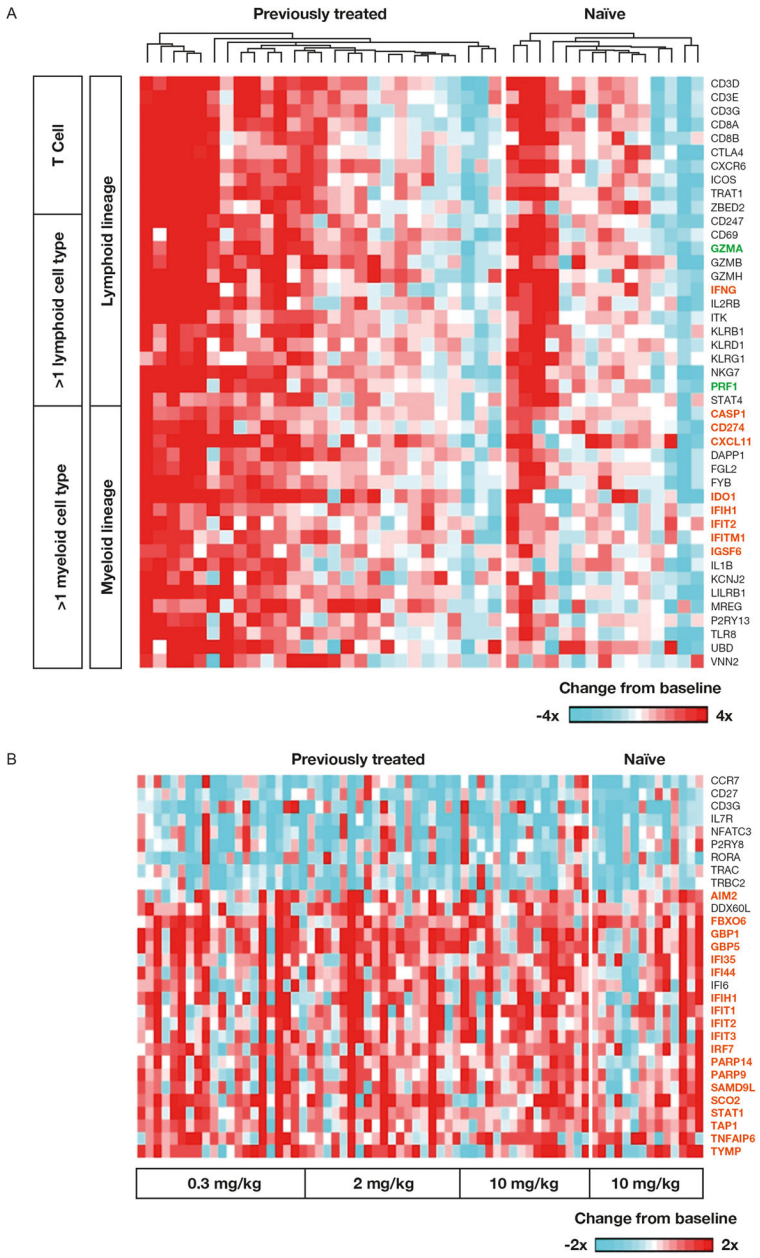

Figure 2.

Change from baseline tumor gene expression for immune lineage-specific transcripts and 24-hour change from baseline in peripheral blood immune-specific transcripts. A. Change of the expression level in tumor biopsies of the 43 regulated transcripts (>1.3 fold, $P < 0.01$) that are specifically associated with either the lymphoid or myeloid immune lineage. Within the lymphoid lineage, the 10 transcripts indicated are specific to T cells. Data are included from the 42 patients with measures at both time points, separated by their previous treatment status. Genes labeled in orange are members of interferon-regulated transcription modules collated by the BRi2 consortium (34). Markers of immune cytolytic activity are labeled in green. B. Change of 30 transcripts in peripheral blood associated with immune lineages and

significantly regulated (≥1.2-fold, $P < 0.01$) in all treatment groups at cycle 1 day 2. Data are included from the 70 patients with measures at both time points, separated by treatment group. Genes labeled in orange are members of interferon-regulated transcription modules collated by the BRi2 consortium (34).

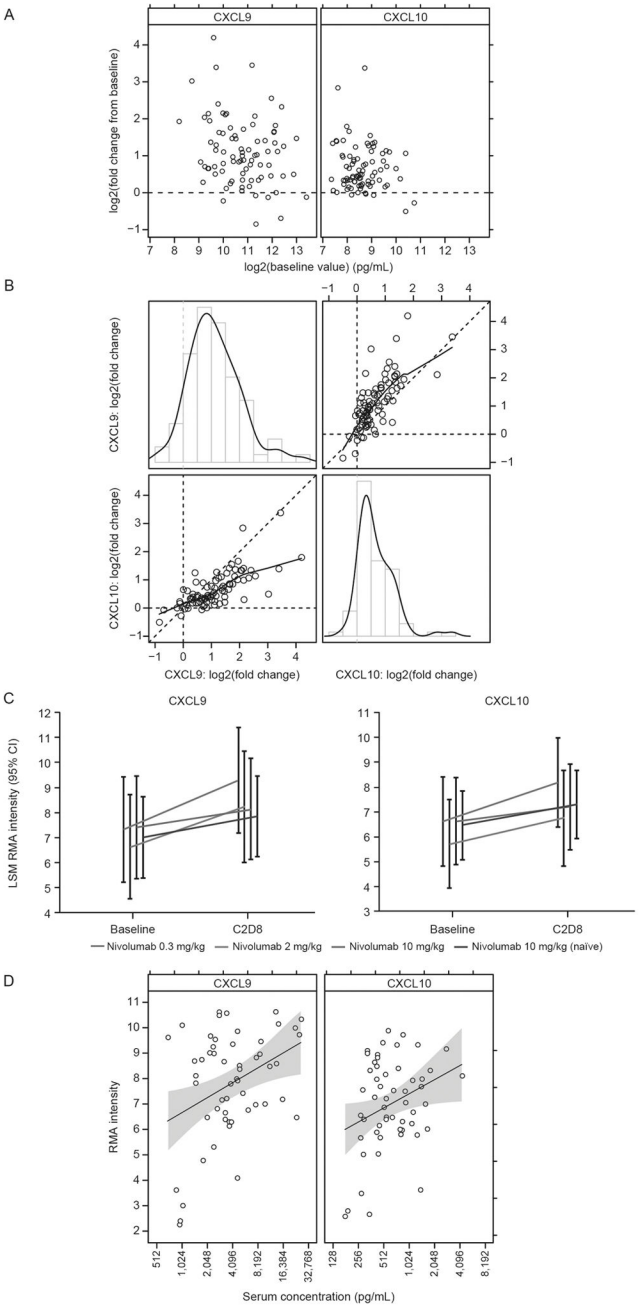

Figure 3.

Effect of nivolumab on chemokine markers. A. Fold change from baseline at cycle 2 day 8 vs. baseline in serum concentrations of CXCL9 and CXCL10 in all treatment groups (N = 83). Both axes are on the log base-2 scale. B. Scatter plot matrix of fold changes from baseline (log base-2 scale) in CXCL9 and CXCL10 among 83 patients who had serum data at baseline and C2D8. The diagonal panels give kernel density estimates and histograms summarizing the univariate distributions of CXCL9 and CXCL10 individually. C. Gene expression levels for CXCL9 (4283_at) and CXCL10 (3627_at) in fresh tumor tissue samples. Values presented are least squares means of the (log-2) robust multi-array intensity

for the treatment group and time point indicated. Error bars indicate 95% confidence intervals estimated from the extended linear model. D. Gene expression levels for CXCL9 (4283_at) and CXCL10 (3627_at) in biopsies obtained at cycle 2 day 8 versus serum concentrations of CXCL9 and CXCL10 in the same patient at cycle 2 day 8 ($N = 54$). Both axes are on the log base-2 scale. Shaded area represents 95% confidence interval estimated from a linear model.

Table 1

Baseline patient characteristics and demographics

	Previously Treated, Nivolumab 0.3, 2, and 10 mg/kg (*N* = 67)	Treatment-naïve, Nivolumab 10 mg/kg (*N* = 24)	Total (*N* = 91)
Median age, years	61.0	63.5	61.0
Sex, n (%)			
Male	46 (69)	15 (63)	61 (67)
Female	21 (31)	9 (38)	30 (33)
Previous therapy, n (%)			
Surgery	64 (96)	23 (96)	87 (96)
Radiotherapy	25 (37)	5 (21)	30 (33)
Previous systemic therapy	67 (100)	0	67 (74)
Therapy for metastatic disease	60 (90)	0	60 (66)
Adjuvant therapy	5 (7)	0	5 (6)
Neoadjuvant therapy	5 (7)	0	5 (6)

Table 2

Clinical activity

	Previously Treated (*N* = 67)			Treatment-naïve	
	Nivolumab 0.3 mg/kg (*N* = 22)	**Nivolumab 2 mg/kg (*N* = 22)**	**Nivolumab 10 mg/kg (*N* = 23)**	**Nivolumab 10 mg/kg (*N* = 24)**	**Total (*N* = 91)[a]**
Objective response rate, n (%)[b]	2 (9)	4 (18)	5 (22)	3 (13)	14 (15)
95% CI	1.1, 29.2	5.2, 40.3	7.5, 43.7	2.7, 32.4	8.7, 24.5
Best response, n (%)					
CR	0	0	0	2 (8)	2 (2)
PR	2 (9)	4 (18)	5 (22)	1 (4)	12 (13)
Stable disease	8 (36)	10 (46)	11 (48)	13 (54)	42 (46)
Progressive disease	9 (41)	5 (23)	6 (26)	7 (29)	27 (30)
Unable to determine	3 (14)	3 (14)	1 (4)	1 (4)	8 (9)
Progression-free survival rate, % (95% CI)					
At 24 weeks	NE	44 (23, 63)	58 (35, 76)	50 (28, 68)	43 (32, 53)
At 48 weeks	NE	NE	32 (13, 52)	39 (18, 59)	25 (16, 35)
Overall survival rate, % (95% CI)					
At 12 months	71 (47, 86)	72 (48, 86)	74 (48, 88)	81 (57, 92)	75 (64, 83)
At 24 months	44 (22, 64)	61 (36, 78)	51 (27, 71)	76 (51, 89)	58 (46, 68)
Median overall survival, months (95% CI)	16.4 (10.1, NR)	NR	25.2 (12.0, NR)	NR	–

Abbreviations: CI, confidence interval; CR, complete response; NE, not evaluated; NR, not reached; PR, partial response.

[a]All treated patients were evaluated for response.

[b]Confirmed response only.

Table 3

Treatment-related adverse events

	Previously Treated						Treatment-naïve	
	Nivolumab 0.3 mg/kg (*N* = 22)		Nivolumab 2 mg/kg (*N* = 22)		Nivolumab 10 mg/kg (*N* = 23)		Nivolumab 10 mg/kg (*N* = 24)	
Event, n (%)	**Any Grade**	**Grade 3/4**	**Any Grade**	**Grade 3/4**	**Any Grade**	**Grade 3/4**	**Any Grade**	**Grade 3/4**
Total	22 (100)	15 (68)	22 (100)	8 (36)	23 (100)	13 (57)	24 (100)	12 (50)
Occurring in ≥15% of patients in all treatment groups (any grade)								
Fatigue	12 (55)	0	13 (59)	2 (9)	15 (65)	0	13 (54)	1 (4)
Nausea	8 (36)	0	7 (32)	0	6 (26)	0	10 (42)	0
Constipation	7 (32)	1 (5)	6 (27)	1 (5)	4 (17)	0	6 (25)	0
Cough	7 (32)	0	8 (36)	0	4 (17)	0	5 (21)	0
Diarrhea	4 (18)	0	4 (18)	0	5 (22)	1 (4)	9 (38)	1 (4)
Select AEs (occurring in ≥2 patients in any treatment group for preferred term)								
Skin	9 (41)	0	5 (23)	0	9 (39)	1 (4)	10 (42)	
Pruritus	3 (14)	0	4 (18)	0	4 (17)	0	4 (17)	0
Rash	5 (23)	0	2 (9)	0	2 (9)	0	2 (8)	0
Rash pruritic	1 (5)	0	0	0	2 (9)	0	1 (4)	0
Urticaria	0	0	0	0	2 (9)	0	1 (4)	0
Palmar-plantar erythrodysesthesia	0	0	0	0	0	0	2 (8)	0
Gastrointestinal	4 (18)	0	4 (18)	0	6 (26)	2 (9)	9 (38)	3 (13)
Diarrhea	4 (18)	0	4 (18)	0	5 (22)	1 (4)	9 (38)	1 (4)
Colitis	0	0	0	0	2 (9)	1 (4)	2 (8)	2 (8)
Hepatic	3 (14)	2 (9)	3 (14)	0	5 (22)	3 (13)	2 (8)	0
AST increased	1 (5)	1 (5)	0	0	3 (13)	2 (9)	2 (8)	0
ALT increased	2 (9)	1 (5)	0	0	2 (9)	1 (4)	2 (8)	0
Blood bilirubin increased	0	0	0	0	2 (9)	1 (4)	2 (8)	0
Renal	3 (14)	2 (9)	4 (18)	0	3 (13)	1 (4)	2 (8)	0
Blood creatinine increased	2 (9)	0	4 (18)	0	2 (9)	0	2 (8)	0
Acute renal failure	1 (5)	1 (5)	1 (5)	0	2 (9)	1 (4)	0	0

	Previously Treated						Treatment-naïve	
	Nivolumab 0.3 mg/kg (*N* = 22)		Nivolumab 2 mg/kg (*N* = 22)		Nivolumab 10 mg/kg (*N* = 23)		Nivolumab 10 mg/kg (*N* = 24)	
Event, n (%)	**Any Grade**	**Grade 3/4**	**Any Grade**	**Grade 3/4**	**Any Grade**	**Grade 3/4**	**Any Grade**	**Grade 3/4**
Endocrine	1 (5)	0	3 (14)	0	4 (17)	0	3 (13)	1 (4)
Hypothyroidism	1 (5)	0	1 (5)	0	3 (13)	0	2 (8)	0
Pulmonary	1 (5)	1 (5)	0	0	2 (9)	1 (4)	3 (13)	0
Pneumonitis	1 (5)	1 (5)	0	0	2 (9)	1 (4)	3 (13)	0
Hypersensitivity/infusion reaction	1 (5)	0	2 (9)	0	5 (22)	0	6 (25)	1 (4)
Infusion-related reaction	1 (5)	0	1 (5)	0	4 (17)	0	5 (21)	1 (4)

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.