Immunomodulatory Activity of Nivolumab in Metastatic Renal Cell Carcinoma


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-naive patients with mRCC. Baseline and on-treatment biopsies and blood were obtained. Clinical activity, tumor-associated 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% confidence interval (CI)] was 16.4 months [10.1 to not reached (NR)] for nivolumab 0.3 mg/kg, NR for 2 mg/kg, 25.2 months (12.0 to NR) for 10 mg/kg, and NR for treatment-naive 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 upregulation of IFNy-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.

Conclusions: 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. Clin Cancer Res; 22(22): 5461–71. © 2016 AACR.

Introduction

Metastatic renal cell carcinoma (mRCC) is a heterogeneous disease that is highly resistant to chemotherapy (1). High-dose IL2 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, VEGF inhibitors, and mTOR 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; refs. 3–6).

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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 (ORR) of 20% to 22% and median OS of 18.2 to 25.5 months were reported in previously treated patients (12). A recent phase 3 study demonstrated significantly longer median OS with nivolumab versus everolimus (25.0 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 patients with melanoma, 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 preliminary data on objective response rates, durable responses, and improved OS 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 (ORR) of 20% to 22% and median OS of 18.2 to 25.5 months were reported in previously treated patients (12). A recent phase 3 study demonstrated significantly longer median OS with nivolumab versus everolimus (25.0 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 patients with melanoma, 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).
Previous therapy, n (%) | Treatment-naive, Nivolumab 10 mg/kg (N = 24) | Treatment-naive, Nivolumab 10 mg/kg (N = 91) | Total (N = 91) |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgery</td>
<td>64 (96)</td>
<td>23 (96)</td>
<td>87 (96)</td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>25 (57)</td>
<td>5 (21)</td>
<td>30 (33)</td>
</tr>
<tr>
<td>Previous systemic therapy</td>
<td>67 (100)</td>
<td>0</td>
<td>67 (74)</td>
</tr>
<tr>
<td>Therapy for metastatic disease</td>
<td>60 (90)</td>
<td>0</td>
<td>60 (66)</td>
</tr>
</tbody>
</table>

Adjuvant therapy | 5 (7) | 0 | 5 (6) |
Nonadjuvant therapy | 5 (7) | 0 | 5 (6) |

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 immunohistochemical assay (Bristol-Myers Squibb/Dako immunohistochemical 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). Gene expression profiling was performed using the HG-U219 array plate on the GeneTitan platform (Affymetrix). The robust multiarray analysis algorithm (26) was used to establish intensity values for each of 18,562 loci (BrainArray v.10; ref. 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).

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, that is, the proportion of patients whose best response was a complete response or partial response. The 95% confidence intervals (CI) for ORR were estimated using the Clopper–Pearson method (29). PFS was defined as the time from first documented disease progression, or death.

### Table 2. Clinical activity

<table>
<thead>
<tr>
<th>ORR, n (%)</th>
<th>Treatment-naive, Nivolumab 10 mg/kg (N = 24)</th>
<th>Treatment-naive, Nivolumab 10 mg/kg (N = 91)</th>
<th>Total (N = 91)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previously treated (N = 67)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nivolumab 0.3 mg/kg (N = 22)</td>
<td>2 (9)</td>
<td>4 (18)</td>
<td>5 (22)</td>
</tr>
<tr>
<td>Nivolumab 2 mg/kg (N = 22)</td>
<td>11-29.2</td>
<td>5.2-40.3</td>
<td>7.5-43.7</td>
</tr>
<tr>
<td>Nivolumab 10 mg/kg (N = 23)</td>
<td>3 (13)</td>
<td>14 (53)</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>2.7-32.4</td>
<td>8.7-24.5</td>
<td></td>
</tr>
<tr>
<td>Best response, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete response</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Partial response</td>
<td>2 (9)</td>
<td>0</td>
<td>2 (8)</td>
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<tr>
<td>Stable disease</td>
<td>8 (36)</td>
<td>30 (46)</td>
<td>38 (48)</td>
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<tr>
<td>Progressive disease</td>
<td>9 (41)</td>
<td>6 (26)</td>
<td>7 (29)</td>
</tr>
<tr>
<td>Unable to determine</td>
<td>3 (14)</td>
<td>1 (4)</td>
<td>4 (8)</td>
</tr>
<tr>
<td>PFS rate, % (95% CI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 24 wks</td>
<td>NE</td>
<td>44 (23-63)</td>
<td>58 (35-76)</td>
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<tr>
<td>At 48 wks</td>
<td>NE</td>
<td>32 (13-52)</td>
<td>50 (28-68)</td>
</tr>
<tr>
<td>95% CI</td>
<td>39 (18-69)</td>
<td>43 (32-55)</td>
<td></td>
</tr>
<tr>
<td>OS rate, % (95% CI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 12 mo</td>
<td>71 (47-86)</td>
<td>74 (48-88)</td>
<td>81 (57-92)</td>
</tr>
<tr>
<td>At 24 mo</td>
<td>44 (22-64)</td>
<td>76 (51-89)</td>
<td></td>
</tr>
<tr>
<td>Median OS, mo (95% CI)</td>
<td>16.4 (10.1-NR)</td>
<td>25.2 (12.0-NR)</td>
<td>NR</td>
</tr>
</tbody>
</table>

Abbreviation: NE, not evaluated.
*All treated patients were evaluated for response.
*Confirmed response only.

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PFS and OS functions were estimated by the Kaplan–Meier method with 95% CIs estimated using Greenwood’s formula (36).

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; ref. 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 (ref. 32; P values provided were Bonferroni–corrected) of 1,539 genes from immune cell lineages (33) and for biologic impact (MetaCore; Thomson Reuters).

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 multicontrast 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 to not reached (NR)], NR, and 25.2 months (12.0 to 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 of 91; distinct or unknown second lesion: 13 of 91), with metastatic sites including liver (16 of 73), lymph nodes (12 of 73; kidney (9 of 73), lung (6 of 73), adrenal (4 of 73), pancreas (2 of 72), and other locations (soft tissue, 24 of 73). Immunohistochemical analysis showed enrichment of CD3+, CD4+, and CD8+ cells from baseline to C2D8 (Fig. 1A and B). 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 was 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 Fig. 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
64 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 (ref. 33; mean expression, \( P = 1.3 \times 10^{-7} \)), all of which increased at C2D8. Of these 71 transcripts, 43 have been defined as lymphoid lineage-specific (e.g., GZMA/G/H and KLRC1/D1/G1) or myeloid-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 IFN-regulated (34), including CXCL9 and CXCL10 (PD-L1). IFNy was the only IFN 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 (\( P = 2.7 \times 10^{-86} \)). 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 (ref. 33; \( P < 0.001 \); Fig. 2B). These included transcripts for T-cell receptor \( \alpha \) and \( \beta \) subunits and the CD3\( \gamma \) subunit, all of which decreased relative to baseline. The 59 transcripts included 29 previously identified as IFN-regulated (34), all of which increased. No transcripts from IFN 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 Tables S3 and S4.

IFNy-related chemokines
As observed increases in IFNy-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 3627_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 2, randomized, dose-ranging study of nivolumab (18.2–25.5 months) and in a recent phase 3 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. Immunohistochemical 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, IFNγ, 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

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 IFN-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 CD12. Data are included from the 70 patients with measures at both time points, separated by treatment group. Genes labeled in orange are members of IFN-regulated transcription modules collated by the BRi2 consortium (34).
Figure 3.
Effect of nivolumab on chemokine markers. A, fold change from baseline at C2D8 versus baseline in serum concentrations of CXCL9 and CXCL10 in all treatment groups (N = 83). Both axes are on the log2 scale. B, scatter plot matrix of fold changes from baseline (log2 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 (log2) robust multiarray intensity for the treatment group and time point indicated. Error bars indicate 95% CIs estimated from the extended linear model. D, gene expression levels for CXCL9 (4283_at) and CXCL10 (3627_at) in biopsies obtained at C2D8 versus serum concentrations of CXCL9 and CXCL10 in the same patient at C2D8 (N = 54). Both axes are on the log2 scale. Shaded area represents 95% CI estimated from a linear model.
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 IFN-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 elevated expression is a favorable prognostic factor in RCC (35).

Clinical response to ipilimumab, which elevated expression is a favorable prognostic factor in RCC (35).

Table 3. Treatment-related AEs

<table>
<thead>
<tr>
<th>Event, n (%)</th>
<th>Previously treated</th>
<th>Treatment-naive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nivolumab 0.3 mg/kg</td>
<td>Nivolumab 2 mg/kg</td>
</tr>
<tr>
<td></td>
<td>Any grade</td>
<td>Grade 3/4</td>
</tr>
<tr>
<td>Fatigue</td>
<td>12 (55)</td>
<td>0</td>
</tr>
<tr>
<td>Nausea</td>
<td>8 (36)</td>
<td>0</td>
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<tr>
<td>Constipation</td>
<td>7 (32)</td>
<td>1</td>
</tr>
<tr>
<td>Cough</td>
<td>7 (32)</td>
<td>0</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>4 (18)</td>
<td>0</td>
</tr>
</tbody>
</table>

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) 0
Pruritus: 3 (14) 0 4 (18) 0 4 (17) 0 3 (13) 1 (4)
Rash: 5 (23) 0 2 (9) 0 2 (9) 0 2 (8) 0
Urticaria: 0 0 0 0 2 (9) 0 1 (4) 0
Gastrointestinal: 4 (18) 0 4 (18) 0 6 (26) 2 (9) 9 (38) 3 (13)
Diarrhea: 4 (18) 0 4 (18) 0 4 (17) 0 4 (17) 0
Hepatic: 3 (14) 2 | 3 (14) 0 5 (22) 3 (13) 2 (8) 0
ALT increased: 2 (9) 0 0 0 2 (9) 1 (4) 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 | 3 (14) 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 | 1 (5) 0 2 (9) 1 (4) 0 0
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.
Disclosure of Potential Conflicts of Interest
T.K. Choueiri reports receiving commercial research support from Bristol-Myers Squibb and Pfizer, and is a consultant/advisory board member for Bayer, Bristol-Myers Squibb, Merck, Novartis, and Pfizer. M.N. Fishman reports receiving commercial research support from Bristol-Myers Squibb, Exelixis, GlaxoSmithKline, Novartis, and Pfizer. D.F. McDermott is a consultant/advisory board member for Bristol-Myers Squibb, Genentech, Merck, Novartis, and Pfizer. C.G. Drake has ownership interest (including patents) in A2 Medimmune, Bristol-Myers Squibb, and Potenza, is a consultant/advisory board member for Agera, Bristol-Myers Squibb, Compugen, Eli Lilly, ImmunoGenex, Merck, Potenza Biotechtherapeutics, Roche/Genentech, and Tizona Biotechtherapeutics, and reports receiving commercial research grants from Aduro Biotech, Bristol-Myers Squibb, and Janssen. H. Kluger reports receiving commercial research support from Bristol-Myers Squibb. W.M. Stadler is a consultant/advisory board member for Genentech/Roche, and reports receiving commercial research grants from Bristol-Myers Squibb. J.L. Perez-Graza reports receiving commercial research support from Bristol-Myers Squibb. D.G. McNeel has ownership grants from Argenx, Bristol-Myers Squibb, Genentech, and Pfizer. E.R. Plimack is a consultant/advisory board member for Acceleron, Bristol-Myers Squibb, GlaxoSmithKline Novartis, and Pfizer. L. Applemann reports receiving commercial research grants from Astellas, Avera, Bristol-Myers Squibb, Exelixis, Medivation, Novartis, and Pfizer. L. Fong reports receiving commercial research grants from AbbVie, Bristol-Myers Squibb, Janssen, Merck, and Roche/Genentech. L. Albiges is a consultant/advisory board member for Amgen, Bayer, Bristol-Myers Squibb, Novartis, Pfizer, and Sanofi. S. Srinavasta has ownership interest (including patents) in Bristol-Myers Squibb. J.F. Kurland is an employee of MedImmune and has ownership interest (including patents) in Bristol-Myers Squibb. M. Sznol has ownership interest (including patents) in Adaptive Biotechnologies, and is a consultant/advisory board member for AstraZeneca, Bristol-Myers Squibb, Genentech/Roche, Immune Design, Kyowa-Kirin, Lion, Merck, Novartis, Pfizer, and Symphogen. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

References


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