CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING

This application claims the benefit of U.S. Provisional Patent Application No. 61/647,442, filed May 15, 2012, and U.S. Provisional Patent Application No. 61/790,747, filed March 15, 2013, the contents of which are hereby incorporated herein by reference in their entirety.

Throughout this application, various other publications are referenced in parentheses by author name and date, or by Patent No. or Patent Publication No. Full citations for these publications may be found at the end of the specification immediately preceding the claims. The disclosures of these publications are hereby incorporated in their entireties by reference into this application in order to more fully describe the state of the art as known to those skilled therein as of the date of the invention described and claimed herein. However, the citation of a reference herein should not be construed as an acknowledgement that such reference is prior art to the present invention.

FIELD OF THE INVENTION

This invention relates to methods for immunotherapy of a cancer patient comprising administering to the patient antibodies that disrupt the PD-1/PD-L1 signaling pathway. A biomarker may be used as part of this treatment for identifying suitable patients for immunotherapy and for predicting the efficacy of anti-PD-1 treatment.

BACKGROUND OF THE INVENTION

Human cancers harbor numerous genetic and epigenetic alterations, generating neoantigens potentially recognizable by the immune system (Sjoblom *et al.*, 2006). The adaptive immune system, comprised of T and B lymphocytes, has powerful anti-cancer potential, with a broad capacity and exquisite specificity to respond to diverse tumor antigens. Further, the immune system demonstrates considerable plasticity and a memory component. The successful harnessing of all these attributes of the adaptive immune system would make immunotherapy unique among all cancer treatment modalities. However, although an endogenous immune response to cancer is observed in preclinical models and patients, this response is ineffective, and established cancers are viewed as "self" and tolerated by the immune system. Contributing to this state of tolerance, tumors may exploit several distinct mechanisms to actively subvert anti-tumor immunity. These mechanisms include dysfunctional T-cell signaling (Mizoguchi *et al.*, 1992), suppressive regulatory cells (Facciabene *et al.*, 2012), and the co-opting of endogenous "immune

Table 3. Clinical Activity of Nivolumab in the Efficacy Population (N = 306)* as Assessed to March 2013

Tumor Type	ORRs[†] No. of patients/total no. of patients (%) [95% CI]	Median Duration of Response[‡], wk (range)	Stable Disease No. of patients/total no. of patients (%) [95% CI]		Median Progression-free Survival, mo (95% CI)	Median Overall Survival, mo (95% CI)	Overall Survival Rate, % (95% CI); patients at risk, n	
			≥ 24 wk	≥ 48 wk			1 yr	2 yr
Non-small cell lung cancer**	22/129 (17.1) [11.0, 24.7]	74.0 (6.1+, 133.9+)	13/129 (10.1) [5.5, 16.6]	6/129 (4.7) [1.7, 9.8]	2.3 (1.9, 3.7)	9.6 (7.8, 12.4)	42 (33, 51); 43	14 (4, 24); 5
Squamous	9/54 (16.7) [7.9, 29.3]	NR[§] (16.1, 133.9+)	8/54 (14.8) [6.6, 27.1]	3/54 (5.6) [1.2, 15.4]	3.7 (1.8, 7.2)	9.2 (7.3, 12.5)	39 (25, 53):16	¶
Non-squamous	13/74 (17.6) [9.7, 28.2]	63.9 (6.1+, 74.0+)	5/74 (6.8) [2.2, 15.1]	3/74 (4.1) [0.8, 11.4]	2.0 (1.8, 3.6)	10.1 (7.2, 13.7)	43 (31, 54): 26	¶
Melanoma	33/107 (30.8) [22.3, 40.5]	104 (18.4, 117.0+)	7/107 (6.5) [2.7, 13.0]	4/107 (3.7) [1.0, 9.3]	3.7 (1.9, 9.1)	16.8 (12.5, 31.6)	62 (53, 72); 55	43 (32, 53); 26
Kidney cancer	10/34 (29.4) [15.1, 47.5]	56.1 (36.6, 126.7+)	9/34 (26.5) [12.9, 44.4]	2/34 (5.9) [0.7, 19.7]	7.3 (3.7, 12.9)	>22[#] (13.6, NE^)	70 (55, 86); 23	50 (31, 70); 8

* No objective responses were seen in 19 patients with colorectal cancer or 17 patients with castration-resistant prostate cancer.

EXAMPLE 8

Correlation between Membranous PD-L1 Expression and Anti-PD-1 Response

IHC staining of PD-L1 was performed on pretreatment formalin-fixed paraffin-embedded (FFPE) tumor specimens using the murine anti-human PD-L1 mAb 5H1 (Dong *et al.*, 2002) in a standard IHC protocol (Taube *et al.*, 2012; Supp. Materials). Briefly, 5μm-FFPE sections mounted on glass slides were deparaffinized in xylene and antigen retrieval was performed using a Tris-EDTA buffer, pH 9.0 at 120°C for 10 min in a Decloaking Chamber (Biocare Medical). Endogenous peroxidase, biotin and proteins were blocked (CAS system K1500, Dako; Avidin/biotin Blocking Kit, SP-2001, Vector Laboratories; Serotec Block ACE), and the primary 5H1 Ab was added at a concentration of 2 μg/ml and allowed to incubate at 4°C for 20 h. Secondary Ab (biotinylated anti-mouse IgG1, 553441 BD) was applied at a concentration of 1 μg/ml for 30 min at room temperature (RT). The signal was then developed with amplification according to the manufacturer's protocol (CAS system K1500, Dako). Sections were counterstained with hematoxylin, dehydrated in ethanol and cleared in xylene, and a coverslip was applied.

The percentage of tumor cells exhibiting cell surface staining for PD-L1 was scored by two independent pathologists who were blinded to treatment outcomes. PD-L1 positivity was defined per specimen by a 5% expression threshold (Taube *et al.*, 2012; Thompson *et al.*, 2006), and in cases with multiple specimens, if any specimen met this criterion. A Fisher's exact test was applied to assess the association between PD-L1 expression and OR, noting, however, that this analysis was based in part on optional biopsies from a non-random subset of the population and testing of a statistical hypothesis was not pre-specified.

Sixty-one pretreatment tumor specimens from 42 patients (18 MEL, 10 NSCLC, 7 CRC, 5 RCC, and 2 CRPC) were analyzed for tumor cell surface PD-L1 expression (Figure 8). Biopsy specimens from 25 of 42 patients were positive for PD-L1 expression by IHC. A Fisher's exact test was applied to assess the association between PD-L1 expression and OR in a post-hoc analysis. Among the 42 surface-PD-L1^{+} patients, 9 (36%) achieved an OR , whereas among 17 patients with PD-L1^{-} tumors, none achieved an OR (Figure 8A). Thus, in a subset of patients cell surface expression of PD-L1 on tumor cells in pretreatment biopsies is associated with an increased rate of OR among patients treated with BMS-936558, while no patients with documented PD-L1-negative tumors experienced an OR. These data indicate that tumor PD-L1 expression is a

Table 10. Clinical Activity of Patients who Received the Concurrent Regimen of Nivolumab and Ipilimumab*

Cohort	Dose	Response-Evaluable Patients† n	CR n	PR n	uPR‡ n	irPR§ n	SD ≥24 wk n	irSD§ ≥24 wk n	Objective Response Rate¶ % [95% CI]	Aggregate Clinical Activity Rate‖ % [95% CI]	80% Tumor Reduction at 12 wk n (%)
1	0.3 mg/kg nivolumab + 3 mg/kg ipilimumab	14	1	2	0	2	2 (14)	0	21 [5-51]	50 [23-77]	4 (29)
2	1 mg/kg nivolumab + 3 mg/kg ipilimumab	17	3	6	0	0	0	2 (12)	53 [28-77]	65 [38-86]	7 (41)**
2a	3 mg/kg nivolumab + 1 mg/kg ipilimumab	15	1	5	2	1	2 (13)	0	40 [16-68]	73 [45-92]	5 (33)
3	3 mg/kg nivolumab + 3 mg/kg ipilimumab	6	0	3	0	1	0	1 (17)	50 [12-88]	83 [36-100]	0
All	Concurrent treatment	52	5	16	2	4	4 (8)	3 (6)	40 [27-55]	65 [51-78]	16 (31)

* CR denotes complete response, PR partial response, uPR unconfirmed partial response, irPR immune-related partial response, SD stable disease, irSD immune-related stable disease.

† Response-evaluable patients were those who received at least one dose of study therapy, had measurable disease at baseline, and one of the following: 1) at least one on-treatment tumor evaluation, 2) clinical progression, or 3) death prior to the first on-treatment tumor evaluation.

‡ Patients who had a PR after one tumor assessment but did not have sufficient follow-up time for confirmation of the initial PR.

Assessment of tumor PD-L1 expression and absolute lymphocyte count

Tumor PD-L1 expression and alterations in the peripheral blood ALC have been explored as biomarkers for nivolumab and ipilimumab monotherapy, respectively (Topalian *et al.*, 2012b; Berman *et al.*, 2009; Ku *et al.*, 2010; Postow *et al.*, 2012; Delyon *et al.*, 2013). Tumor PD-L1 expression was characterized via IHC staining and pharmacodynamic changes in peripheral blood ALC was analyzed. Using a ≥ 5% cut off to define PD-L1 positivity, tumor specimens from 21 of 56 (38%) patients were PD-L1-positive. ORs were seen in patients with either PD-L1-positive (6/13) or PD-L1-negative (9/22) tumors amongst patients treated with the concurrent regimen (post-hoc P-value > 0.99; Fisher's exact test). In sequenced-regimen cohorts, a numerically higher number of overall responses were seen in patients with PD-L1-positive tumor samples (4/8) compared with patients with PD-L1-negative tumors (1/13), but the numbers are small.

In contrast to observations with ipilimumab monotherapy, a consistent rise in ALC from baseline was not detected in patients treated with the concurrent combination or in patients treated with nivolumab following ipilimumab therapy. In the concurrent-regimen cohorts, patients with a low ALC at weeks 5 to 7 (< 1000 cells/μL) (Ku *et al.*, 2010) had similar OR (43%) compared with patients with a normal ALC at weeks 5 to 7 (40%). Likewise, in the sequenced-regimen cohorts, 17% of patients with low ALC had OR and 23% of patients with normal or high ALC had OR.

Sequence Listing Summary

SEQ ID NO:	Description
1	V_H amino acid sequence of 17D8
2	V_H amino acid sequence of 2D3
3	V_H amino acid sequence of 4H1
4	V_H amino acid sequence of 5C4
5	V_H amino acid sequence of 4A11
6	V_H amino acid sequence of 7D3
7	V_H amino acid sequence of 5F4
8	V_L amino acid sequence of 17D8
9	V_L amino acid sequence of 2D3
10	V_L amino acid sequence of 4H1
11	V_L amino acid sequence of 5C4