Journal of Clinical & Cellular Immunology

Furness et al., J Clin Cell Immunol 2015, 6:4
http://dx.doi.org/10.4172/2155-9899.1000347

Review Article Open Access

Biomarkers of Response to Immune Modulatory Therapies in Cancer

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Received date: April 10, 2015, **Accepted date:** July 19, 2015, **Published date:** July 26, 2015

Abstract

Immune modulatory antibody-based therapies serve to augment and direct the endogenous immune response against cancer. Significant efficacy has been demonstrated in multiple subtypes of solid and haematological malignancies. Despite great promise, responses are limited to a fraction of treated patients highlighting the need to decipher underlying mechanisms of response and resistance. Here we review progress in this area with a focus on the identification of candidate predictive biomarkers of response.

Keywords: Antibodies; Biomarkers; Cancer; CTLA-4; Immune modulation; PD-1

Introduction

The development and clinical application of immune modulatory antibody-based therapies continues to generate much excitement. In contrast to monoclonal antibodies (mAb) targeting cancer cells directly, immune modulatory mAb serve to direct and augment the endogenous immune response against cancer. This has translated into significant clinical activity in multiple subtypes of solid and haematological malignancies within randomised clinical trials [1-13]. At present, three therapeutic agents have received US Food and Drug Administration (FDA) approval. Ipilimumab, a humanised IgG1 monoclonal antibody (mAb), targeting cytotoxic T lymphocyte antigen-4 (CTLA-4), was the first drug to prolong survival in advanced melanoma, whilst nivolumab and pembrolizumab, both IgG4 mAbs targeting programmed cell death-1 (PD-1), have demonstrated significant clinical activity across multiple solid and, most recently, haematological tumour subtypes [3,5-7,9-13]. For responding patients, these agents offer the potential for durable remission and even cure. Response rates are, however, modest, engendering significant efforts to decipher underlying mechanisms of response and resistance. Significant evolution in the understanding of immune checkpoint modulation at both a cellular and molecular level has led to promising advances in the identification of candidate predictive biomarkers. Here we review this progress, with a focus on the current FDA-approved therapies employed in clinical practice.

CTLA-4 and PD-1 - Key Regulators of T cell Response and Function

CTLA-4 was first described as a novel B7 family member nearly three decades ago [14]. Highly conserved between species [15], it plays a critical role in immune regulation supported by the death of knockout (KO) mice by 3-4 weeks of age secondary to lymphoproliferative disease and associated multi-organ failure [16]. It is a co-inhibitory cell surface molecule, closely related to CD28, also interacting with B7 molecules (CD80 and CD86) expressed on antigen presenting cells (APCs), but with greater affinity and avidity than CD28, thus negatively regulating T cell activation. T cell receptor (TCR) signalling in the presence of CTLA-4 inhibits T cell clonal expansion and initiation of effector functions such as IL-2 production [17]. It is thus a powerful negative regulator of T cell activation, recognised as an attractive therapeutic target on tumour-infiltrating lymphocytes (TILs).

PD-1 is also a B7 family member, related to CD28 and CTLA-4, demonstrated to negatively regulate TCR signalling upon engagement of its ligands programmed cell death ligand-1 (PD-L1) and/or programmed cell death ligand-2 (PD-L2) [18-23]. The PD-1 receptor was initially discovered as an upregulated gene in a T cell hybridoma undergoing cell death [24]. Signalling through PD-1 exerts its effects on cellular differentiation and survival through inhibition of the cell cycle and lymphocyte effector function and/or promotion of apoptosis; cellular events that are positively regulated by CD28 or interleukin-2 [25]. C57BL/6 and Balb/c mice KO for PD-1 develop late-onset glomerulonephritis and antibody-mediated cardiomyopathy respectively [26,27] Furthermore, PD-1 loss in non-obese diabetic mice mediates accelerated insulinitis and pro-inflammatory T cell cytokine production [28]. Together, these findings demonstrate a key role of PD-1 in down-modulating immune responses and maintaining peripheral T cell tolerance.

Anti-CTLA-4 Therapy

Defining the mechanisms underlying the activity of anti-CTLA-4 therapy

Despite a number of elegant pre-clinical studies, a comprehensive understanding of the mechanisms underlying the activity of anti-CTLA-4 mAb has been lacking until recently. The initial hypothesis was that mAb targeting CTLA-4 would act to block co-inhibitory signals at the immune synapse, 'taking the brakes off' effector CD8 T

cell (Teff) responses. The subsequent demonstration that CTLA-4 is constitutively expressed on regulatory T cells (T_{reg}) raised the possibility of an additional impact on the T_{reg} compartment [29-32]. It was later demonstrated that for maximal anti-tumour activity, blockade of both Teff and T_{reg} compartments is required [33], although the specific impact of anti-CTLA-4 mAb on the T_{reg} compartment remained unclear. A consistent observation associated with anti-CTLA-4-mediated tumour rejection had been a positive shift in the intra-tumoural ratio of Teff to T_{reg}. A number of studies had demonstrated that anti-CTLA-4-mediated expansion of both Teff and T_{reg} in the blood and secondary lymphoid organs of mice [34-36]. It was therefore unclear how anti-CTLA-4 mAb act to preferentially expand Teff in the tumour whilst simultaneously expanding both populations in the periphery. Three pre-clinical studies subsequently demonstrated that anti-CTLA-4 mAb serve to preferentially deplete intra-tumoural T_{reg} leading to a shift in the Teff/T_{reg} ratio correlating with tumour rejection [37-39]. Preferential depletion of T_{reg} occurs secondary to a higher relative density of expression of CTLA-4 on the T_{reg} versus Teff and a tumour microenvironment enriched for activatory Fc gamma receptor (FcγR) expressing tumour-associated macrophages (TAMs) with capacity for antibody-dependent cellular cytotoxicity (ADCC). These studies highlighted a previously unrecognised importance of antibody isotype, target molecule density and FcγR-expressing innate effector cells in dictating the final outcome of immune modulatory therapies.

Insights from clinical trials

In keeping with the described pre-clinical studies, Hodi and colleagues identified a striking linear relationship between the extent of tumour necrosis in post-treatment biopsies and the ratio of intra-tumoural $CD8^+$ T cells and $FoxP3^+$ T_{reg} in six patients with advanced melanoma and ovarian cancer undergoing CTLA-4 blockade with ipilimumab following GVAX therapy [40]. This raised the possibility of selectively targeting T_{reg} as a complementary strategy for combination therapy. In the same year, Sharma and colleagues conducted a neoadjuvant clinical trial of ipilimumab in patients with localised bladder cancer [41]. The study design allowed analysis of surgical specimens and peripheral blood mononuclear cells (PBMCs) prior to and following three infusions of ipilimumab. Anti-CTLA-4 therapy resulted in a consistent increase in $CD4^+ICOS^+$ T cells in both the periphery and tumour as well as a reduction in $CD4^+FoxP3^+$ cells in the tumour in all patients. A significant population of $CD4^+ICOS^+$ cells were identified as FoxP3-, IFN-γ-producing cells, demonstrated to recognise the tumour-associated antigen NY-ESO-1. The authors therefore described the observed shift in $CD4^+ICOS^+$ and $CD4^+FoxP3^+$ populations as a change in the balance of effector to regulatory T cells. Owing to the neoadjuvant nature of the study, the clinical relevance of the described immunological findings, specifically their predictive value, is yet to be determined.

In another small cohort of patients, Ribas and colleagues determined the impact of tremelimumab, an IgG2 mAb targeting CTLA-4, on the tumour microenvironment (TME) of patients with advanced melanoma [42]. Fifteen biopsies were performed in seven patients at variable timepoints pre- and post-therapy including both responding and non-responding lesions. Immunohistochemical analysis of the TME was performed with focus on $CD8^+$ T cells, T_{reg} and the immunosuppressive enzyme indoleamine 2,3-dioxygenase (IDO). Responding patients were found to have a striking increase in granzyme B^+ $CD8^+$ T cells, these were absent at baseline and distributed throughout the tumour areas rather than in the periphery as observed in pre-dosing biopsies. In contrast to the findings of Hodi and colleagues, no impact on T_{reg} or change in IDO expression by $CD1a^+$ dendritic cells (DC's) was observed. This analysis was expanded within the setting of an early clinical trial, evaluating pre and post treatment biopsies in 32 patients with advanced melanoma undergoing therapy with tremelimumab [43]. $CD8^+$ T cell infiltration was observed in response to therapy in both responding and non-responding patients. Functional analyses of HLA-DR, CD45RO and Ki67 on the described $CD8^+$ TILs failed to differentiate between responders and non-responders. Analysis of $FoxP3^+$ T_{reg} identified a trend towards higher infiltrates in responding lesions but nil significant.

In a similar manner, again within the context of a phase II trial, Hamid and colleagues attempted to prospectively identify candidate biomarkers from the TME associated with clinical response to ipilimumab in 82 patients with advanced melanoma [44]. Candidate biomarkers were evaluated in tumour biopsies collected pre-treatment and 24-72 hours after the second ipilimumab dose. In contrast to the findings of Huang et al., significant associations were observed between clinical activity and high baseline levels of FoxP3 and IDO. Baseline TIL scores did not significantly correlate with outcome, however, the increase in TILs between baseline and week 3 met significance. Based on the pre-clinical data, one might hypothesise that the observed relationship between baseline $FoxP3^+$ cells and clinical outcome could be explained by depletion of T_{reg} by ipilimumab and consequent shift in the ratio of Teff to T_{reg}. The lack of similar findings with tremelimumab43 may be explained by its IgG2 isotype and resulting low affinity for FcγRs on innate effector cells. With a lack of ADCC capacity, tremelimumab may therefore only benefit those with an existing, favourable baseline ratio of Teff to T_{reg}. Nevertheless, in the human setting, ipilimumab-mediated depletion of T_{reg} is yet to be demonstrated, although studies to date have largely focused on peripheral blood rather than tumour infiltrating lymphocytes [45].

Longitudinal sampling studies in patients with advanced cancer are notoriously difficult. Evaluation of mechanism is often less challenging in the neoadjuvant setting where samples are guaranteed before and after therapy. The drawback of these studies is the inability to determine the predictive value of any observed findings where surgical intervention is curative. A calculated approach was adopted by Tarhini and colleagues in evaluating the mechanistic activity of ipilimumab in patients with operable, regionally advanced melanoma [46]. Blood and tumour was assessed at baseline and then again at week 6 following two infusions of ipilimumab and surgical resection of disease. In keeping with pre-clinical studies, a significant increase in the percentage of circulating T_{reg} was observed and associated with improved progression free survival (PFS). Moreover, relative to baseline, an increase in activated ($CD69^+$) tumour-infiltrating $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells was observed. There was also a trend towards an inverse association between the change in intra-tumoural T_{reg} and clinical benefit, again in keeping with pre-clinical studies. Circulating and tumour-associated monocytic myeloid-derived suppressor cells (mMDSCs) were also assessed. Treatment was observed to mediate a reduction in both circulating and intra-tumoural mMDSCs associated with improved PFS.

Despite the enrolment of 35 patients, only 24 and 10 specimens were available for immunohistochemical and flow cytometric analysis respectively. This once again highlights the difficulties in tumour sampling in patients with advanced disease. In contrast to haematological malignancies, patients with advanced solid cancers

often have little or no readily accessible disease for biopsy. This group of patients are also commonly highly symptomatic of their disease and unfit to undergo more invasive sampling. Despite known dissociation in immune responses between tumour and peripheral blood [47], such difficulties have prompted efforts to identify biomarkers through non-invasive sampling.

Circulating biomarkers of response

Owing to the described mechanism of anti-CTLA-4 mAb, a number of studies have set out to evaluate the impact and possible predictive value of anti-CTLA-4 on circulating T lymphocytes. Ku and colleagues evaluated the change in peripheral absolute lymphocyte count (ALC) in relation to ipilimumab therapy in 53 patients with advanced melanoma [48]. Baseline ALC counts failed to predict response, however, an ALC of ≥ 2000/uL following two doses of ipilimumab (week 7) was associated with a significantly higher clinical benefit rate and median OS compared to those with an ALC of <1000/uL. Santegoets and colleagues observed similar findings in ALC counts within a phase I/II dose escalation/expansion trial of GVAX plus ipilimumab in 28 patients with advanced prostate cancer. On further evaluation, however, no differences in overall frequencies of circulating $CD3^+CD4^+/CD8^+$ T lymphocytes or $CD3^-CD56^+$ NK cells were observed [49]. A detailed analysis of pre-treatment frequencies of Teff and T_{reg} subsets was performed as well as longitudinal analysis through therapy and correlation with clinical outcome. A number of findings were observed to correlate with median OS including baseline non-naïve $CD8^+$, $CD4^+PD\text{-}1^+$ and $CD4^+$ T cells. The strongest predictor of outcome, identified by unsupervised clustering, was baseline frequency of $CD4^+CTLA\text{-}4^+$ effector T cells. Interestingly, intracellular CTLA-4 was virtually undetectable in $CD4^+$ T cells of healthy donors but abundant in both T_{reg} and CD4 effector T cells in prostate cancer patients. These data, in part, complement the described findings of Sharma et al. highlighting further a potential role for CD4 effector T cells in dictating the outcome of anti-CTLA-4 therapy. The study shed no further light on the predictive value of baseline or on therapy $FoxP3^+$ T_{reg}, these were observed steadily to rise in response to therapy, with increases of greater than 50% associated with shorter OS.

With a specific aim of identifying novel biomarkers associated with both clinical benefit and ipilimumab-mediated toxicity, Wang and colleagues assessed baseline characteristics and changes in $CD4^+$ and $CD8^+$ T cells sorted from the peripheral blood of advanced melanoma patients receiving ipilimumab [50]. Microarray analysis of purified $CD4^+$ and $CD8^+$ T cells was performed to assess gene-profiling changes induced by ipilimumab in 75 patients. Thereafter, to verify changes in selected molecules, a flow cytometric study was undertaken with pre-treatment, 3 month and 6 month post-ipilimumab PBMC samples from expanded groups of 55, 25 and 37 patients respectively. Analysis of candidate biomarkers at baseline revealed a low percentage of $Ki67^+EOMES^+CD8^+$ and $EOMES^+CD8^+$ T cells was significantly associated with relapse. Pre-therapy specimens were subsequently stratified by the median percentage of $EOMES^+CD8^+$ T cells. Patients with a higher baseline percentage of $EOMES^+CD8^+$ T cells had a significantly improved relapse-free survival; the same was true for $Ki67^+EOMES^+CD8^+$ T cells. The authors proposed further validation of the predictive value of these markers in a prospective manner.

Although studies evaluating mechanisms of response and resistance to anti-CTLA-4 have largely focused on T lymphocytes, mMDSCs have been identified as a suppressor subset with capacity to impact on outcome to anti-CTLA-4 therapy [51,52]. Meyer and colleagues collected peripheral blood samples from 49 patients with advanced melanoma undergoing ipilimumab therapy [51]. Lineage negative $CD14^+HLA\text{-}DR^-$ mMDSC were enriched in the peripheral blood of melanoma patients relative to healthy donors. A trend towards higher frequencies of mMDSCs was observed in patients with a high burden of metastatic disease. Interestingly, significantly lower percentages of $CD14^+HLA\text{-}DR^-$ mMDSCs were observed in patients responding to ipilimumab versus non-responders. Baseline values were therefore compared to mean values during and after treatment. A trend towards lower baseline values in responders was observed compared to non-responders. Patient numbers were small, possibly contributing to the lack of statistical significance. In parallel, Kitano and colleagues developed a computational algorithm-driven system for evaluation of mMDSC frequency for prediction of clinical outcomes. In a larger study of 68 patients with advanced melanoma treated with ipilimumab, a low pre-treatment mMDSC frequency, defined as less than 14.9%, was significantly associated with improved OS in both uni- and multi-variate analyses. In terms of mechanism, no relationship between ALC and mMDSC frequency was observed, however, a statistically significant inverse correlation between the percentage change in absolute $CD8^+$ T cell number and mMDSC frequency at week 6 was demonstrated, in keeping with the described suppressor function of this myeloid subset.

Lactate dehydrogenase (LDH) emerged as a candidate biomarker following subgroup analysis of overall survival in a landmark phase III trial of ipilimumab in advanced melanoma [1]. Interestingly, the hazard ratio for ipilimumab versus the control arm was only significant in patients with baseline serum LDH values within normal range. Based on these data, Kelderman and colleagues retrospectively correlated baseline LDH in two separate cohorts of 'real world' melanoma patients treated within an expanded access programme (EAP) [53]. In a multi-variate model, LDH was found to be the strongest predictive factor for OS. Patients with a baseline LDH two times the upper limit of normal were highly unlikely to derive benefit from ipilimumab with a significantly lower median OS observed in this group. The absence of a control arm precluded discrimination between the prognostic and predictive value of LDH as a marker, nevertheless, in the absence of more robust biomarkers it was highlighted as a readily available marker to guide clinical decision-making.

In addition to its role as a key regulator of angiogenesis, vascular endothelial growth factor (VEGF) is a potent inhibitor of DC maturation and T cell responses [54,55]. Serum VEGF levels are known to correlate with melanoma stage, moreover, high circulating serum VEGF is a poor prognostic marker in patients with melanoma [56,57]. The prognostic and/or predictive value of serum VEGF in relation to immune modulatory therapy had, however, remained undetermined. Yuan and colleagues retrospectively analysed serum VEGF levels in 176 patients with advanced melanoma, before and after therapy with ipilimumab [58]. Baseline VEGF levels were associated with clinical response, patients with a baseline serum VEGF value greater than 43pg/mL, treated with either 3 or 10mg/kg of ipilimumab were less likely to derive clinical benefit. In addition, higher baseline serum VEGF levels were associated with a significantly poorer OS. Prospective studies were therefore called for in order to determine the predictive value. Such findings suggest potential synergy between anti-CTLA-4 and anti-VEGF therapies, indeed, combination therapy with ipilimumab and bevacizumab appears promising in early clinical trials [59].

Genetic determinants of response to anti-CTLA-4 therapy

Although the prognostic significance of TILs has been demonstrated for a number of solid cancers [60-65], their predictive value in relation to immune modulatory therapies is unclear. Ji and colleagues performed gene expression profiling on tumour biopsies collected from 45 patients with advanced melanoma, three weeks before commencement of ipilimumab, within a phase II clinical trial [66]. High baseline expression of immune-related genes was associated with favourable clinical outcome to therapy. Genes involved in the immune response increased in expression whilst those for melanoma-specific antigens and cell proliferation decreased. These findings highlighted the potential importance of an existing, endogenous anti-tumour immune response in dictating outcome to immune modulation. Adding another layer of complexity, recent advances in next generation sequencing techniques have enabled more detailed characterisation or 'immunoprofiling' of both the periphery and the tumour microenvironment allowing quantitative assessment of the clonality and repertoire of T cell receptors. In two studies evaluating T cell repertoire in response to anti-CTLA-4 therapy, a diversification of the T cell repertoire in the peripheral blood with an increased number of unique T cell receptor β chain complementarity determining region 3 (CDR3) sequences was observed. In a separate study, improved overall survival was seen in patients who maintained peripheral T cell clones that were present in high frequencies prior to anti-CTLA-4 blockade [67,68].

It remains to be elucidated whether the maintenance of a specific T cell receptor clonotype can be used as a prognostic and/or predictive biomarker in patients treated with immune modulatory antibodies. Furthermore, although T cell receptor sequencing provides information on the diversity and clonality of the T cell repertoire, limitations exist in the ability of this technique to provide detail regarding the antigen specificity of infiltrating lymphocytes. A study using peptide/MHC multimers and a panel of melanoma-derived neo-epitopes demonstrated a broadening of the peripheral melanoma-specific CD8 T cell repertoire following anti-CTLA-4 therapy with ipilimumab [69]. Interestingly, ipilimumab therapy did not appear to significantly affect the magnitude of the pre-existing melanoma-specific T cell peripheral responses suggesting anti-CTLA-4 therapy serves to prime rather than enhance pre-existing immune responses.

Until recently, identification of the molecular determinants driving tumour-infiltrating T cell responses has remained unclear. Based on the observations that somatic mutations can give rise to neo-epitopes and that these may serve as neoantigens [70], Snyder and colleagues conducted a study to determine whether the genetic landscape of a tumour impacts upon clinical benefit derived from anti-CTLA-4 therapies [71]. Whole exome sequencing was performed on pre-treatment tumour tissue and matched blood samples in 64 patients. With use of sequencing data and bioinformatic approaches, candidate neoantigens were identified. Thereafter, relevant mutated peptides were synthesized and tested for their ability to activate lymphocytes from ipilimumab-treated patients. Using a discovery set of 11 responding and 14 non-responding patients, a neo-antigenic repertoire, unique to responding patients, was defined and subsequently validated in a separate cohort of 39 patients. High mutational load was associated with a benefit from CTLA-4 therapy, however, this factor alone was not sufficient to impart a clinical benefit. Rather, there were specific somatic neo-epitopes shared by patients with a prolonged benefit and absent in non-responders. These observations require validation in a larger cohort of patients, however, this study represents a major step forward, not only in the quest for predictive biomarkers, but for the entire field of tumour immunotherapy.

Anti-PD-1 Therapy

Defining the mechanisms underlying the activity of anti-PD-1 therapy

The programmed cell death-1 (PD-1) receptor-ligand interaction is a major pathway hijacked by tumours, promoting immune evasion and tumour escape. PD-1 is expressed on a variety of cellular subsets including activated T and B lymphocytes, natural killer (NK) cells, monocytes and dendritic cells [72]. In health, PD-1, expressed on the surface of activated T cells, acts to down-modulate unwanted or excessive immune responses, preventing autoimmunity and maintaining immunological tolerance to self-antigens. Expression patterns of PD-L1 and PD-L2 vary. PD-L1 is highly expressed on monocytes, but also at low levels on plasmacytoid and myeloid dendritic cell subsets as well as activated T lymphocytes [72]. Furthermore, PD-L1 expression can be induced by inflammatory cytokines including type I and type II interferons in non-haematopoietic cells of epithelial and endothelial origin [73]. In contrast, PD-L2 is expressed selectively within the myeloid compartment on macrophages and dendritic cells [72]

PD-L1 expression has been demonstrated in multiple solid tumours [74-76]. Two key mechanisms of tumour PD-L1 up-regulation and immune resistance have been described. Innate immune resistance refers to the constitutive expression of PD-L1 by tumour cells secondary to increased signalling via oncogenic pathways, independent of the cytokine milieu of the tumour microenvironment [75,77]. On the contrary, adaptive immune resistance is a process whereby tumour cells adapt to the endogenous immune response through aberrant upregulation of PD-L1 in the context of interferon gamma release by tumour infiltrating lymphocytes [78,79]. This mirrors the physiological role of PD-L1 upregulation, which serves to prevent excessive immune-mediated damage as a result of the immune response to infection [75,77].

In mouse models of chronic viral infection, continuous exposure to lymphocytic choriomeningitis virus (LCMV) was found to mediate functional dysregulation or 'exhaustion' of viral-specific CD8 T cells with associated increased PD-1 cell surface expression [80]. In vivo administration of antibodies blocking the PD-1/PD-L1 pathway restored viral-specific T cell function, leading to a substantial reduction in viral burden. Early studies in animal models provide support for the integral role of the PD-1/PD-L1 axis in tumour immunity. PD-L1 expression on tumour cells has been shown to inhibit T cell activation and lysis of tumour cells with increased tumour-specific T cell death [20,81]. In BALB/c PD-1 knockout mice, the growth of PD-L1 expressing murine myeloma cell lines was completely suppressed in contrast to rapid tumour cell growth in PD-1 positive controls [82]. Furthermore, PD-L1 expression on immunogenic P815 tumour cells associated with resistance to anti-4-1BB therapeutic antibody treatment was restored with anti-PD-L1 therapy [81]. Collectively, these data demonstrate the critical role of the PD-1/PD-L1 pathway in tumour immune evasion, highlighting its therapeutic significance in the treatment of cancer.

Insights from clinical trials

Several trials of monoclonal antibodies targeting the PD-1/PD-L1 pathway have demonstrated unprecedented rates of success with durable responses and survival benefit in patients with a variety of cancers [3-8,12,13,83]. In 2014, pembrolizumab and nivolumab, both fully humanised IgG4 mAbs targeting PD-1, received FDA approval for the treatment of patients with ipilimumab-refractory advanced melanoma. In the same year, nivolumab and an anti-PD-L1 inhibitor, MPDL3280A, achieved 'breakthrough designation' status for the treatment of subsets of refractory Hodgkin's lymphoma and metastatic bladder cancer respectively.

Whilst there is evidence to support PD-L1 expression as a predictor of response to anti-PD-1/PD-L1 therapy [3,6,8,12,13,83-85], the observed clinical activity in patients with PD-L1 negative tumours has questioned this hypothesis [5,6,11]. Initial support for tumour PD-L1 expression as a predictive biomarker arose from data generated within a phase I trial of nivolumab [3]. A response rate of 36% was reported in patients with PD-L1 positive tumours with no responses demonstrated in those harbouring PD-L1 negative tumours. The utility of PD-L1 as a predictive biomarker was later questioned however, following observations of clinical responses to nivolumab in PD-L1 negative melanoma in 17% of patients [85]. Furthermore, in a phase 1 trial of pembrolizumab, responses were also seen in PD-L1 negative melanoma and non-small cell lung cancer (NSCLC), albeit significantly lower than the PD-L1 positive subgroups [86]. Subsequent comprehensive analysis of tumour specimens obtained from 41 patients with a variety of advanced solid tumours treated with nivolumab, demonstrated that tumour PD-L1 expression was the factor most closely correlated with response to anti-PD-1 therapy, in keeping with findings from previous studies [87]. PD-L1 expression was significantly associated with tumour subtypes in which the majority of responses to anti-PD-1 therapy have been reported thus far, including melanoma, NSCLC and renal cell carcinoma. In a combination trial of ipilimumab and nivolumab, objective responses were reported in approximately 40% of patients with advanced melanoma treated with concurrent immunotherapy, irrespective of baseline tumour PD-L1 status, suggesting that tumour PD-L1 expression may be less relevant as a predictive biomarker for combination immunotherapy [6].

The dynamic, inducible nature of tumour PD-L1 expression, largely related to interferon gamma release by tumour-infiltrating lymphocytes may indeed explain the lack of consistency in correlation between tumour PD-L1 expression and response to anti-PD-1 therapy demonstrated in some clinical trials. Although, based on these studies, tumour PD-L1 expression as a predictive biomarker requires further evaluation, the observed clinical successes have led to the clinical evaluation of these agents in other highly positive PD-L1 expressing tumours such as Hodgkin lymphoma in which overexpression of PD-L1 on Reed-Sternberg cells occurs constitutively as a result of PD-L1 and PD-L2 gene co-amplification [8].

Comparison between and interpretation of the described clinical studies is limited by the use of varied staining antibodies and thresholds for determining PD-L1 positivity. Moreover, the observation that PD-L1 is expressed on tumour-infiltrating immune cells in addition to tumour cells, with some clinical trials including immune infiltrate PD-L1 expression in the cut off for PD-L1 positivity may be highly relevant [3,6,8,12,13,83-86]. Observed responses in PD-L1 negative patients in early stage clinical trials highlight the limited negative predictive power of PD-L1 expression as a biomarker of response. Moreover, significant discordance in PD-L1 expression between primary tumours, metastases and intra-patient metastases was recently demonstrated in a study of advanced melanoma [88]. Discordance in PD-L1 expression in renal cell carcinoma between primary and metastatic lesions has also been described [89]. These findings may, at least in part, explain why PD-L1 is a poor negative predictor of response to treatment (Figure 1).

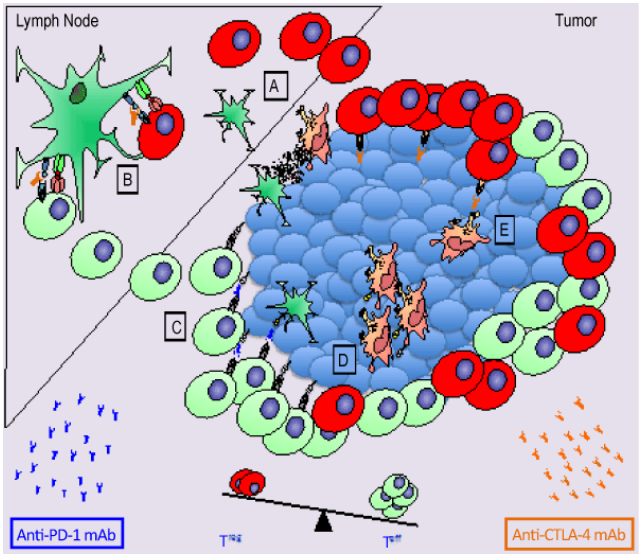

Figure 1: Mechanisms of response and resistance to immune modulatory therapies. [A] Professional antigen-presenting cells, dendritic cells (green) and macrophages (brown), engulf and process tumour cells and their products before migrating to lymph nodes. [B] Anti-CTLA-4 mAb enhance priming of Teff and T_{reg} leading to their expansion. [C] The activity of tumour-reactive Teff is limited by the engagement of PD-1 with its ligands PD-L1 (black/white) and PD-L2 (black/yellow) on tumour and myeloid cells respectively. [D] Tumour-associated macrophages are typically described as pro-tumour, promoting tumour progression and invasion. [E] Macrophage activity is, however, context dependent, in pre-clinical studies anti-CTLA-4 mAb act additionally in the tumour to deplete CTLA-4 expressing T_{reg} by ADCC, it is unclear whether this activity is common to ipilimumab.

Beyond tumour cell PD-L1 expression

In a series of recent publications, tumour-infiltrating immune cell rather than tumour PD-L1 expression was associated with clinical response to the anti-PD-L1 therapy MPDL3280A in patients with NSCLC and bladder cancer [83,90]. Responses were associated with baseline tumour gene expression of IFNγ, Granzyme-A, CD8 and EOMES, indicative of a Th1 type immune response. Increased CTLA-4 expression and the absence of chemokine CX3C motif ligand 1 (CX3CL1) expression was also seen in baseline tumour specimens of responding patients. Simultaneously, a study of pembrolizumab therapy in metastatic melanoma demonstrated higher numbers of $CD8^+PD\text{-}1^+$ and $PD\text{-}L1^+$ positive cells within the tumour and at the tumour invasive margin in baseline tumour specimens obtained from responding patients. A predictive model of response to pembrolizumab therapy was developed based on $CD8^+$ T cell

expression at the tumour invasive margin and was subsequently validated in a separate cohort of patients [84].

Collectively, these findings demonstrate the importance of acknowledging the interaction between multiple cellular subsets within the immune tumour microenvironment and the potential implications of these in dictating outcome. PD-L1 expression is likely only to form part of a predictive model or 'immunoscore' necessary for selecting patients expected to respond to anti-PD-1/PD-L1 therapy. In addition to PD-L1 expression, the geographical location and densities of various immune cell subsets appears to have predictive value [84,90]. Furthermore, the clinical significance of the relative expression of PD-L1 on tumour cells, myeloid cells and activated tumour-infiltrating lymphocytes is yet to be determined (Table 1).

Anti-CTLA-4 therapies
Shift in intra-tumoural CD8/T_{reg} ratio [34]
Increase in circulating $ICOS^+$ $CD4^+$ effector T cells [41]
Increase in Granzyme B^+ $CD8^+$ T cells [42]
Rise in absolute lymphocyte count [48,49]
Baseline FoxP3 and IDO expression [44]
Baseline 'immune active' tumour microenvironment [66]
CTLA-4^+ $CD4^+$ effector T cells [49]
$EOMES^+$ $CD8^+$ T cells [50]
Baseline circulating monocytic MDSCs [51]
Reduction in monocytic MDSCs in periphery and tumour [52]
Baseline serum VEGF [58]
Baseline serum LDH [53]
Maintenance of high frequency T cell clones in periphery [68]
Neo-antigenic repertoire [71]
Anti-PD-1/-PD-L1 therapies
Tumour PD-L1 expression [3,6,8,12,13,84-86]
Tumour infiltrating immune cell PD-L1 expression [83]
Increased tumoural IFNγ, Granzyme-A, CD8, EOMES and CX3CL1 gene expression [90]
Increased $CD8^+PD\text{-}1^+PD\text{-}L1^+$ cell density within tumour and at invasive tumour margin [84]
Increased clonality and reduced diversity of intra-tumoural T cell repertoire [84]
Higher somatic mutational burden and neo-antigenic repertoire [92]

Table 1: Candidate biomarkers of response to immune modulatory therapy.

Genetic determinants of response to anti-PD-1 therapy

In a study of pembrolizumab therapy in patients with advanced melanoma, baseline tumour specimens from responding patients were found to have a more clonal and less diverse T cell repertoire [84]. Furthermore, a ten-fold increase in the number of expanded clones following pembrolizumab therapy was seen in responding patients compared to those with disease progression [84]. The molecular determinants of relevant clonal T cell responses have, however, remained unclear until recently. Higher responses to anti-PD-1/PD-L1 therapy are typically seen in tumours associated with a high burden of somatic mutations such as melanoma, NSCLC and bladder cancer, highlighting the potential importance of the genomic landscape in predicting response [91]. Given the identification of a neo-antigenic repertoire, unique to responding patients undergoing CTLA-4 blockade, the same group studied patients with NSCLC undergoing pembrolizumab therapy [71,92]. Whole exome sequencing demonstrated a significant positive correlation between the level of somatic, non-synonymous mutational burden and improved response and progression-free survival [92]. The clinical efficacy of pembrolizumab was found to correlate with a higher burden of the identified neo-antigenic repertoire and a molecular signature characteristic of smoking-related mutagenesis. In a single patient with an observed rapid response to pembrolizumab, a $CD8^+$ T cell response against a neoantigen resulting from a HERC1 P3278S mutation was observed in peripheral blood. This was only detectable following commencement of therapy (0.005%), three weeks post initiation of therapy the magnitude of response was 0.040% of $CD8^+$T cells and this was maintained at day [44]. This study represents another key step in deciphering the mechanisms underlying response to immune modulatory therapies, furthermore, the observation that neo-antigen-reactive T cells could be detected in peripheral blood raises the possibility of blood-based/non-invasive sampling methods of monitoring response to therapy.

Conclusion

The identification of biomarkers of response to immune modulatory therapies is an area of high scientific and clinical priority. While many studies have failed to conclusively identify predictive biomarkers, the insights provided have and continue to be instrumental in advancing understanding. Undoubtedly, the recent demonstration of a specific neo-antigenic repertoire underlying response to CTLA-4 and PD-1 blockade represents a major step forward. Predictably, these studies raise a number of further questions and therapeutic challenges. The observation that a high burden of somatic mutations might in fact be used for therapeutic gain with immune modulation is transforming approaches within the field. The prospect of sequencing tumours on an individual patient basis, developing truly bespoke cellular therapies and optimising activity with appropriate immune modulation now appears both attractive and achievable. The success of immune modulation is the result of sound basic science brought to the clinic in an intelligent manner. Pre-clinical and clinical studies have served to inform each other, expanding understanding of the mechanisms underlying response and resistance. The challenge now is to exploit this understanding, translating it in durable benefit for the majority rather than a selected few.

References

1. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, et al. (2010) Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 363: 711-723.
2. Robert C, Thomas L, Bondarenko I, O'Day S, Weber J, et al. (2011) Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med 364: 2517-2526.

3. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, et al. (2012) Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 366: 2443-2454.
4. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, et al. (2012) Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med 366: 2455-2465.
5. Hamid O, Robert C, Daud A, Hodi FS, Hwu WJ, et al. (2013) Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. N Engl J Med 369: 134-144.
6. Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, et al. (2013) Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med 369: 122-133.
7. Robert C, Ribas A, Wolchok JD, Hodi FS, Hamid O, et al. (2014) Anti-programmed-death-receptor-1 treatment with pembrolizumab in ipilimumab-refractory advanced melanoma: a randomised dose-comparison cohort of a phase 1 trial. Lancet 384: 1109-1117.
8. Ansell SM, Lesokhin AM, Borrello I, Halwani A, Scott EC, et al. (2015) PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. N Engl J Med 372: 311-319.
9. Armand P, Nagler A, Weller EA, Devine SM, Avigan DE, et al. (2013) Disabling immune tolerance by programmed death-1 blockade with pidilizumab after autologous hematopoietic stem-cell transplantation for diffuse large B-cell lymphoma: results of an international phase II trial. J Clin Oncol 31: 4199-206.
10. Motzer RJ, Rini BI, McDermott DF, Redman BG, Kuzel TM, et al. (2015) Nivolumab for Metastatic Renal Cell Carcinoma: Results of a Randomized Phase II Trial. J Clin Oncol 33: 1430-1437.
11. Robert C, Long GV, Brady B, Dutriaux C, Maio M, et al. (2015) Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med 372: 320-330.
12. Ribas A, Hodi FS, Kefford R, Hamid O, Daud A, et al. (2014) Efficacy and safety of the anti-PD-1 monoclonal antibody MK-3475 in 411 patients (pts) with melanoma (MEL). In ASCO Annual Meeting Proceedings 32: LBA9000.
13. Weber JS, Kudchadkar RR, Yu B, Gallenstein D, Horak CE, et al. (2013) Safety, efficacy, and biomarkers of nivolumab with vaccine in ipilimumab-refractory or -naive melanoma. J Clin Oncol 31: 4311-4318.
14. Brunet JF, Denizot F, Luciani MF, Roux-Dosseto M, Suzan M, et al. (1987) A new member of the immunoglobulin superfamily--CTLA-4. Nature 328: 267-270.
15. Harper K, Balzano C, Rouvier E, Mattéi MG, Luciani MF, et al. (1991) CTLA-4 and CD28 activated lymphocyte molecules are closely related in both mouse and human as to sequence, message expression, gene structure, and chromosomal location. J Immunol 147: 1037-1044.
16. Tivol EA, Borriello F, Schweitzer AN, Lynch WP, Bluestone JA, et al. (1995) Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. Immunity 3: 541-547.
17. Krummel MF, Allison JP (1996) CTLA-4 engagement inhibits IL-2 accumulation and cell cycle progression upon activation of resting T cells. J Exp Med 183: 2533-2540.
18. Agata Y, Kawasaki A, Nishimura H, Ishida Y, Tsubata T, et al. (1996) Expression of the PD-1 antigen on the surface of stimulated mouse T and B lymphocytes. Int Immunol 8: 765-772.
19. Dong H, Zhu G, Tamada K, Chen L (1999) B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. Nat Med 5: 1365-1369.
20. Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, et al. (2002) Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nat Med 8: 793-800.
21. Mazanet MM, Hughes CC (2002) B7-H1 is expressed by human endothelial cells and suppresses T cell cytokine synthesis. J Immunol 169: 3581-3588.
22. Petroff MG, Chen L, Phillips TA, Azzola D, Sedlmayr P, et al. (2003) B7 family molecules are favorably positioned at the human maternal-fetal interface. Biol Reprod 68: 1496-1504.
23. Ishida M, Iwai Y, Tanaka Y, Okazaki T, Freeman GJ, et al. (2002) Differential expression of PD-L1 and PD-L2, ligands for an inhibitory receptor PD-1, in the cells of lymphohematopoietic tissues. Immunol Lett 84: 57-62.
24. Ishida Y, Agata Y, Shibahara K, Honjo T (1992) Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. EMBO J 11: 3887-3895.
25. Carter L, Fouser LA, Jussif J, Fitz L, Deng B, et al. (2002) PD-1:PD-L inhibitory pathway affects both CD4(+) and CD8(+) T cells and is overcome by IL-2. Eur J Immunol 32: 634-643.
26. Nishimura H, Nose M, Hiai H, Minato N, Honjo T (1999) Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. Immunity 11: 141-151.
27. Nishimura H, Okazaki T, Tanaka Y, Nakatani K, Hara M, et al. (2001) Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice. Science 291: 319-322.
28. Wang J, Yoshida T, Nakaki F, Hiai H, Okazaki T, et al. (2005) Establishment of NOD-Pdcd1-/- mice as an efficient animal model of type I diabetes. Proc Natl Acad Sci U S A 102: 11823-11828.
29. Sakaguchi S, Sakaguchi N, Shimizu J, Yamazaki S, Sakihama T, et al. (2001) Immunologic tolerance maintained by CD25+ CD4+ regulatory T cells: their common role in controlling autoimmunity, tumor immunity, and transplantation tolerance. Immunol Rev 182: 18-32.
30. Read S, Malmström V, Powrie F (2000) Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of CD25(+)CD4(+) regulatory cells that control intestinal inflammation. J Exp Med 192: 295-302.
31. Read S, Greenwald R, Izcue A, Robinson N, Mandelbrot D, et al. (2006) Blockade of CTLA-4 on CD4+CD25+ regulatory T cells abrogates their function in vivo. J Immunol 177: 4376-4383.
32. Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T, Miyara M, et al. (2008) CTLA-4 control over Foxp3+ regulatory T cell function. Science 322: 271-275.
33. Peggs KS, Quezada SA, Chambers CA, Korman AJ, Allison JP (2009) Blockade of CTLA-4 on both effector and regulatory T cell compartments contributes to the antitumor activity of anti-CTLA-4 antibodies. J Exp Med 206: 1717-1725.
34. Quezada SA, Peggs KS, Curran MA, Allison JP (2006) CTLA4 blockade and GM-CSF combination immunotherapy alters the intratumor balance of effector and regulatory T cells. J Clin Invest 116: 1935-1945.
35. Schmidt EM, Wang CJ, Ryan GA, Clough LE, Qureshi OS, et al. (2009) Ctla-4 controls regulatory T cell peripheral homeostasis and is required for suppression of pancreatic islet autoimmunity. J Immunol 182: 274-282.
36. Kavanagh B, O'Brien S, Lee D, Hou Y, Weinberg V, et al. (2008) CTLA4 blockade expands FoxP3+ regulatory and activated effector CD4+ T cells in a dose-dependent fashion. Blood 112: 1175-1183.
37. Selby MJ, Engelhardt JJ, Quigley M, Henning KA, Chen T, et al. (2013) Anti-CTLA-4 antibodies of IgG2a isotype enhance antitumor activity through reduction of intratumoral regulatory T cells. Cancer Immunol Res 1: 32-42.
38. Simpson TR, Li F, Montalvo-Ortiz W, Sepulveda MA, Bergerhoff K, et al. (2013) Fc-dependent depletion of tumor-infiltrating regulatory T cells co-defines the efficacy of anti-CTLA-4 therapy against melanoma. J Exp Med 210: 1695-1710.
39. Bulliard Y, Jolicoeur R, Windman M, Rue SM, Ettenberg S, et al. (2013) Activating Fcα receptors contribute to the antitumor activities of immunoregulatory receptor-targeting antibodies. J Exp Med 210: 1685-1693.
40. Hodi FS, Butler M, Oble DA, Seiden MV, Haluska FG, et al. (2008) Immunologic and clinical effects of antibody blockade of cytotoxic T

lymphocyte-associated antigen 4 in previously vaccinated cancer patients. Proc Natl Acad Sci U S A 105: 3005-3010.

41. Liakou CI, Kamat A, Tang DN, Chen H, Sun J, et al. (2008) CTLA-4 blockade increases IFNgamma-producing CD4+ICOShi cells to shift the ratio of effector to regulatory T cells in cancer patients. Proc Natl Acad Sci U S A 105: 14987-14992.
42. Ribas A, Comin-Anduix B, Economou JS, Donahue TR, de la Rocha P, et al. (2009) Intratumoral immune cell infiltrates, FoxP3, and indoleamine 2,3-dioxygenase in patients with melanoma undergoing CTLA4 blockade. Clin Cancer Res 15: 390-399.
43. Huang RR, Jalil J, Economou JS, Chmielowski B, Koya RC, et al. (2011) CTLA4 blockade induces frequent tumor infiltration by activated lymphocytes regardless of clinical responses in humans. Clin Cancer Res 17: 4101-4109.
44. Hamid O, Schmidt H, Nissan A, Ridolfi L, Aamdal S, et al. (2011) A prospective phase II trial exploring the association between tumor microenvironment biomarkers and clinical activity of ipilimumab in advanced melanoma. J Transl Med 9: 204.
45. Maker AV, Attia P, Rosenberg SA (2005) Analysis of the cellular mechanism of antitumor responses and autoimmunity in patients treated with CTLA-4 blockade. J Immunol 175: 7746-7754.
46. Tarhini AA, Edington H, Butterfield LH, Lin Y, Shuai Y, et al. (2014) Immune monitoring of the circulation and the tumor microenvironment in patients with regionally advanced melanoma receiving neoadjuvant ipilimumab. PLoS One 9: e87705.
47. Quezada SA, Peggs KS, Simpson TR, Shen Y, Littman DR, et al. (2008) Limited tumor infiltration by activated T effector cells restricts the therapeutic activity of regulatory T cell depletion against established melanoma. J Exp Med 205: 2125-2138.
48. Ku GY, Yuan J, Page DB, Schroeder SE, Panageas KS, et al. (2010) Single-institution experience with ipilimumab in advanced melanoma patients in the compassionate use setting: lymphocyte count after 2 doses correlates with survival. Cancer 116: 1767-1775.
49. Santegoets SJ, Stam AG, Lougheed SM, Gall H, Scholten PE, et al. (2013) T cell profiling reveals high CD4+CTLA-4 + T cell frequency as dominant predictor for survival after prostate GVAX/ipilimumab treatment. Cancer Immunol Immunother 62: 245-256.
50. Wang W, Yu D, Sarnaik AA, Yu B, Hall M, et al. (2012) Biomarkers on melanoma patient T cells associated with ipilimumab treatment. J Transl Med 10: 146.
51. Meyer C, Cagnon L, Costa-Nunes CM, Baumgaertner P, Montandon N, et al. (2014) Frequencies of circulating MDSC correlate with clinical outcome of melanoma patients treated with ipilimumab. Cancer Immunol Immunother 63: 247-257.
52. Kitano S, Postow MA, Ziegler CG, Kuk D, Panageas KS, et al. (2014) Computational algorithm-driven evaluation of monocytic myeloid-derived suppressor cell frequency for prediction of clinical outcomes. Cancer Immunol Res 2: 812-821.
53. Kelderman S, Heemskerk B, van Tinteren H, van den Brom RR, Hospers GA, et al. (2014) Lactate dehydrogenase as a selection criterion for ipilimumab treatment in metastatic melanoma. Cancer Immunol Immunother 63: 449-458.
54. Oyama T, Ran S, Ishida T, Nadaf S, Kerr L, et al. (1998) Vascular endothelial growth factor affects dendritic cell maturation through the inhibition of nuclear factor-kappa B activation in hemopoietic progenitor cells. J Immunol 160: 1224-1232.
55. Dikov MM, Ohm JE, Ray N, Tchekneva EE, Burlison J, et al. (2005) Differential roles of vascular endothelial growth factor receptors 1 and 2 in dendritic cell differentiation. J Immunol 174: 215-222.
56. Ugurel S, Rappl G, Tilgen W, Reinhold U (2001) Increased serum concentration of angiogenic factors in malignant melanoma patients correlates with tumor progression and survival. J Clin Oncol 19: 577-583.
57. Tas F, Duranyildiz D, Oguz H, Camlica H, Yasasever V, et al. (2006) Circulating serum levels of angiogenic factors and vascular endothelial growth factor receptors 1 and 2 in melanoma patients. Melanoma Res 16: 405-411.
58. Yuan J, Zhou J, Dong Z, Tandon S, Kuk D, et al. (2014) Pretreatment serum VEGF is associated with clinical response and overall survival in advanced melanoma patients treated with ipilimumab. Cancer Immunol Res 2: 127-132.
59. Hodi FS, Lawrence D, Lezcano C, Wu X, Zhou J, et al. (2014) Bevacizumab plus ipilimumab in patients with metastatic melanoma. Cancer Immunol Res 2: 632-642.
60. Ruffini E, Asioli S, Filosso PL, Lyberis P, Bruna MC, et al. (2009) Clinical significance of tumor-infiltrating lymphocytes in lung neoplasms. Ann Thorac Surg 87: 365-371.
61. Clarke B, Tinker AV, Lee CH, Subramanian S, van de Rijn M, et al. (2009) Intraepithelial T cells and prognosis in ovarian carcinoma: novel associations with stage, tumor type, and BRCA1 loss. Mod Pathol 22: 393-402.
62. Lee HE, Chae SW, Lee YJ, Kim MA, Lee HS, et al. (2008) Prognostic implications of type and density of tumour-infiltrating lymphocytes in gastric cancer. Br J Cancer 99: 1704-1711.
63. Sheu BC, Kuo WH, Chen RJ, Huang SC, Chang KJ, et al. (2008) Clinical significance of tumor-infiltrating lymphocytes in neoplastic progression and lymph node metastasis of human breast cancer. Breast 17: 604-610.
64. Pagès F, Berger A, Camus M, Sanchez-Cabo F, Costes A, et al. (2005) Effector memory T cells, early metastasis, and survival in colorectal cancer. N Engl J Med 353: 2654-2666.
65. Oble DA, Loewe R, Yu P, Mihm MC Jr (2009) Focus on TILs: prognostic significance of tumor infiltrating lymphocytes in human melanoma. Cancer Immun 9: 3.
66. Ji RR, Chasalow SD, Wang L, Hamid O, Schmidt H, et al. (2012) An immune-active tumor microenvironment favors clinical response to ipilimumab. Cancer Immunol Immunother 61: 1019-1031.
67. Robert L, Tsoi J, Wang X, Emerson R, Homet B, et al. (2014) CTLA4 blockade broadens the peripheral T-cell receptor repertoire. Clin Cancer Res 20: 2424-2432.
68. Cha E, Klinger M, Hou, Cummings C, Ribas A, et al. (2014) Improved survival with T cell clonotype stability after anti-CTLA-4 treatment in cancer patients. Sci Transl Med 6: 238ra70.
69. Kvistborg P, Philips D, Kelderman S, Hageman L, Ottensmeier C, et al. (2014) Anti-CTLA-4 therapy broadens the melanoma-reactive CD8+ T cell response. Sci Transl Med 6: 254ra128.
70. Segal NH, Parsons DW, Peggs KS, Velculescu V, Kinzler KW, et al. (2008) Epitope landscape in breast and colorectal cancer. Cancer Res 68: 889-892.
71. Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JM, et al. (2014) Genetic basis for clinical response to CTLA-4 blockade in melanoma. N Engl J Med 371: 2189-2199.
72. Keir ME, Butte MJ, Freeman GJ, Sharpe AH (2008) PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol 26: 677-704.
73. Eppihimer MJ, Gunn J, Freeman GJ, Greenfield EA, Chernova T, et al. (2002) Expression and regulation of the PD-L1 immunoinhibitory molecule on microvascular endothelial cells. Microcirculation 9: 133-145.
74. Zou W, Chen L (2008) Inhibitory B7-family molecules in the tumour microenvironment. Nat Rev Immunol 8: 467-477.
75. Taube JM, Anders RA, Young GD, Xu H, Sharma R, et al. (2012) Colocalization of inflammatory response with B7-h1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape. Sci Transl Med 4: 127ra37.
76. Lipson EJ, Vincent JG, Loyo M, Kagohara LT, Luber BS, et al. (2013) PD-L1 expression in the Merkel cell carcinoma microenvironment: association with inflammation, Merkel cell polyomavirus and overall survival. Cancer Immunol Res 1: 54-63.
77. Pardoll DM (2012) The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 12: 252-264.
78. Kim J, Myers AC, Chen L, Pardoll DM, Truong-Tran QA, et al. (2005) Constitutive and inducible expression of b7 family of ligands by human airway epithelial cells. Am J Respir Cell Mol Biol 33: 280-289.

79. Lee SK, Seo SH, Kim BS, Kim CD, Lee JH, et al. (2005) IFN-gamma regulates the expression of B7-H1 in dermal fibroblast cells. J Dermatol Sci 40: 95-103.

80. Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, et al. (2006) Restoring function in exhausted CD8 T cells during chronic viral infection. Nature 439: 682-687.

81. Hirano F, Kaneko K, Tamura H, Dong H, Wang S, et al. (2005) Blockade of B7-H1 and PD-1 by monoclonal antibodies potentiates cancer therapeutic immunity. Cancer Res 65: 1089-1096.

82. Iwai Y, Ishida M, Tanaka Y, Okazaki T, Honjo T, et al. (2002) Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. Proc Natl Acad Sci U S A 99: 12293-12297.

83. Powles T, Eder JP2, Fine GD3, Braiteh FS4, Loriot Y5, et al. (2014) MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. Nature 515: 558-562.

84. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, et al. (2014) PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature 515: 568-571.

85. Grosso J, Horak CE, Inzunza D, Cardona DM, Simon JS, et al. (2013) Association of tumor PD-L1 expression and immune biomarkers with clinical activity in patients (pts) with advanced solid tumors treated with nivolumab (anti-PD-1; BMS-936558; ONO-4538). J Clin Oncolo 31: 15.

86. Daud AI, Hamid O, Ribas A, Hodi S, Hwu W, et al. (2014) Antitumor activity of the anti-PD-1 monoclonal antibody MK-3475 in melanoma (MEL): Correlation of tumor PD-L1 expression with outcome. In Proceedings of the 105th Annual Meeting of the American Association for Cancer Research (pp. 5-9).

87. Taube JM, Klein A, Brahmer JR, Xu H, Pan X, et al. (2014) Association of PD-1, PD-1 ligands, and other features of the tumor immune microenvironment with response to anti-PD-1 therapy. Clin Cancer Res 20: 5064-5074.

88. Madore J, Vilain RE, Menzies AM, Kakavand H, Wilmott JS, et al. (2015) PD-L1 expression in melanoma shows marked heterogeneity within and between patients: implications for anti-PD-1/PD-L1 clinical trials. Pigment Cell Melanoma Res 28: 245-253.

89. Begg CB, Seshan VE, Zabor EC, Furberg H, Arora A, et al. (2014) Genomic investigation of etiologic heterogeneity: methodologic challenges. BMC Med Res Methodol 14: 138.

90. Herbst RS, Soria JC, Kowanetz M, Fine GD, Hamid O, et al. (2014) Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature 515: 563-567.

91. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, et al. (2013) Signatures of mutational processes in human cancer. Nature 500: 415-421.

92. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, et al. (2015) Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science 348: 124-128.

This article was originally published in a special issue, entitled: **"Tumor Biology",** Edited by Xiaozhou Fan, The University of Texas M. D. Anderson Cancer Center, USA