CHECKPOINT BLOCKADE IN CANCER IMMUNOTHERAPY: SQUARING THE CIRCLE

***Maria A.V. Marzolini, Sergio A. Quezada, Karl S. Peggs**

UCL Cancer Institute, University College London, London, UK
**Correspondence to m.marzolini@ucl.ac.uk*

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ABSTRACT

Manipulating the complex interaction between the immune system and tumour cells has been the focus of cancer research for many years, but it is only in the past decade that significant progress has been made in the field of cancer immunotherapy resulting in clinically effective treatments. The blockade of co-inhibitory immune checkpoints, essential for maintaining lymphocyte homeostasis and self-tolerance, by immunomodulatory monoclonal antibodies has resulted in the augmentation of anti-tumour responses. The greatest successes so far have been seen with the blockade of cytotoxic T lymphocyte associated antigen-4, which has resulted in the first Phase III clinical trial showing an overall survival benefit in metastatic melanoma, and in the blockade of the programmed cell death protein-1 axis. This concise review will focus on the clinical advances made by the blockade of these two pathways and their role in current cancer treatment strategies.

Keywords: Cancer immunotherapy, cytotoxic T lymphocyte associated antigen-4 (CTLA-4), programmed cell death protein-1 (PD-1), programmed death ligand-1 (PD-L1).

INTRODUCTION

There were an estimated 14.1 million new cases of cancer diagnosed in 2012 worldwide[1] and, coupled with an increasingly ageing population, the significant global health burden of cancer has led to the search for additional anti-tumour therapeutic strategies to be undertaken. The ability to harness and amplify the immune system's response towards tumour cells has appeared an attractive option in the development of cancer therapies. The principle of the immune surveillance hypothesis, first suggested by Burnet and Thomas[2-4] in the 1950s, proposes that the host's immune system can identify nascent tumour cells and act to eradicate them. The ability of the immune system to recognise cells as tumour cells is essential to preventing the eradication of healthy cells, and is dependent on the cell expressing an identification marker or 'tumour-specific antigen' which elicits an immune response (IR). Lymphocytes were proposed to be the principle cell mediating the immune surveillance mechanism. Without this protective mechanism, the rates of carcinogenesis would be expected to be much higher than experienced. Although an attractive hypothesis, experimental evidence to support the theory was lacking until the late 1990s when, amongst other advances, research showed that lymphocytes and interferon-gamma work together to prevent the development of tumours in immunodeficient mice.[5]

The concept of cancer immunoediting developed from these initial theories and was proposed to describe the interaction between the immune system and cancer, whereby malignant cells become less immunogenic leading to immune escape by the tumour.[6] The cancer immunoediting theory has three phases: elimination, equilibrium, and escape. The stage of tumour elimination reflects the traditional immune surveillance concept whereby the immune system recognises and eliminates tumour cells. The second stage of equilibrium describes the process by which tumour cells can adapt and become progressively less immunogenic and resistant to the actions of effector cells whilst some tumour cells continue to be eliminated. Therefore, the tumour is not completely eradicated

and is kept in check by the immune system. The final escape phase occurs when tumour cells can adapt to develop strategies for evading or subverting a host's IR, for example by expressing ligands that can inhibit T cell activation and proliferation, thereby escaping from the immune system's effector mechanisms and enhancing their ability to proliferate in an unrestricted manner. The aim of immunotherapy is to alter the balance from tumour escape to tumour elimination.

Following the formation of these hypotheses, cancer immunotherapy was a theoretical possibility but over the subsequent decades it failed to translate into effective clinical therapies and therefore appeared to be an impossible feat. The failure of therapies was principally due to a lack of understanding of the immunosuppressive features of the local tumour microenvironment and the need for T cells to infiltrate the tumour to exert their anti-tumour effect. However, in recent years, major breakthroughs in both the understanding of the IR and in the generation of specific monoclonal antibodies (mAbs) aimed at immune checkpoints have led to effective cancer immunotherapies and the achievement of a metaphorical 'squaring of the circle'.

The field of cancer immunotherapy has expanded in recent years, including adoptive cellular therapy, vaccine approaches, and T cell gene therapy. In this concise review, the focus will be on one major branch of cancer immunotherapy, namely the generation of immunomodulatory antibodies designed to manipulate the immune system's co-inhibitory receptors to augment T cell effector function and the anti-tumour response. In contrast to traditional cancer therapies, which have direct cytotoxic effects on the malignant cells, this branch of cancer immunotherapy relies on indirect methods of tumour attack by manipulating the IR in the tumour microenvironment. This indirect method has been postulated to reset the immune memory with potentially more durable responses.

CO-INHIBITORY RECEPTORS

Cytotoxic T Lymphocyte-Associated Antigen-4 (CTLA-4)

The major breakthrough in translational cancer immunotherapy, resulting in successful Phase III clinical trials, followed the development of mAbs against CTLA-4. CTLA-4 is a co-inhibitory receptor that is expressed on activated T lymphocytes and is constitutively expressed on regulatory T lymphocytes. It acts as an inhibitory checkpoint to restrict the magnitude and duration of the IR generated after antigen engagement with the T cell receptor. The immune system has inherent inhibitory checkpoints to limit the degree of immune system activation, thereby preventing collateral damage of surrounding normal tissue and the sequela of autoimmunity. Both CTLA-4 and CD28, a co-stimulatory receptor, are members of the immunoglobulin (Ig) superfamily of receptors. Following the presentation of antigen by major histocompatibility complex molecules on antigen-presenting cells (APCs), the second signal for T cell activation is provided by CD28, which resides on the T cell surface, as it interacts with its respective ligands. CTLA-4's function appears to counteract that of CD28, as they share the same ligands, CD80 (B7-1), and CD86 (B7-2), which are expressed on APCs. CTLA-4 has a higher affinity for these ligands, leading to the theory that CTLA-4 may out-compete CD28 for ligand engagement, resulting in the restriction of the co-stimulatory function of CD28.[7]

The essential role played by CTLA-4 in limiting the IR and maintaining lymphocyte homeostasis was aptly demonstrated by the observations that CTLA-4 knockout mice develop fatal lymphoproliferative disorders within 3-4 weeks of birth.[8,9] The blockade of CTLA-4 with an antagonistic antibody was postulated to increase immune stimulation by releasing the inhibitory brakes on the effector IR in the presence of tumour. Initial preclinical models confirmed this theory by showing that anti-CTLA-4 antibodies could reject tumours and also that this rejection resulted in persistent immunity when challenged for a second time with tumour cells.[10] Whilst the mechanism of action of anti-CTLA-4 antibodies is still being investigated, evidence derived from murine models has shown the blockade of CTLA-4 on both effector and regulatory T cells contributes to its anti-tumour effect. Anti-CTLA-4 antibodies act to deplete the number of regulatory T cells within tumours and the composition of the tumour microenvironment, in particular the presence of Fcγ receptor–expressing macrophages, is essential in enabling this depletion to occur.[11,12] The initial success in anti-CTLA-4 antibody therapy was shown in the treatment of advanced melanoma. The increasing incidence of melanoma and the poor prognosis of patients with metastatic melanoma (MM), with median overall survival (OS) rates of less than 1 year, had indicated that new effective therapies were greatly needed.[13]

There have been two mAbs to CTLA-4 which have been examined in Phase III clinical trials in patients with advanced melanoma, ipilimumab and tremelimumab. Ipilimumab is a fully human immunoglobin G1 (IgG1) mAb to CTLA-4. The landmark Phase III randomised controlled trial (RCT) by Hodi et al.[14] was the first to show an OS benefit for any therapy in the treatment of MM. The study compared ipilimumab with and without glycoprotein 100 (gp100) vaccine with a gp100-alone group in patients with previously treated advanced melanoma. Gp100 is a peptide vaccine originating from a melanosomal protein, and has shown enhanced anti-tumour activity in combination therapy, for example with interleukin 2.[15] There was a significant difference in OS between the ipilimumab/vaccine group when compared with the vaccine-alone group (10 months versus 6.4 months). There was no significant difference noted between either of the ipilimumab groups. The second Phase III trial, which demonstrated a survival advantage for ipilimumab therapy in patients with melanoma, was performed by Robert et al.[16] They compared patients, who had no previous treatment for melanoma, receiving ipilimumab plus dacarbazine with a group receiving dacarbazine plus placebo. Dacarbazine is an alkylating agent and is the most commonly used chemotherapy in the treatment of melanoma. There was a significant increase in median OS for those receiving ipilimumab with dacarbazine rather than dacarbazine and placebo (11.2 months versus 9.1 months). In contrast to ipilimumab, tremelimumab is a humanised IgG2 mAb to CTLA-4 and was studied in treatment-naïve patients with melanoma in a Phase III trial by Ribas et al.[17] Unlike the aforementioned ipilimumab trials, no significant difference in median OS was shown between tremelimumab-treated patients and those receiving standard chemotherapy despite the induction of initially durable responses in a subset of patients.

The objective responses reported with ipilimumab were durable, with 60% of patients, in a study by Hodi et al.,[14] maintaining their response for more than 2 years. Furthermore, in the ipilimumab/dacarbazine study, the median duration of response was 19.3 months (for those achieving a complete or partial response).[16] However, despite these durable responses, the clinical trials have shown that only a relatively small subset of patients derive benefit from ipilimumab therapy, with a reported overall response rate (RR) of 10.9–15.2%, irrespective of whether they were treatment naïve prior to receiving ipilimumab.[14,16] The ability to identify the group of patients who would benefit from ipilimumab therapy would limit the number of patients exposed to potentially harmful adverse events (AEs) and also would enable treatment to be tailored to those with the highest chance of success. The search for a predictive biomarker of ipilimumab response is currently ongoing but provisional studies have suggested that an initial high expression of FoxP3 may be a predictor of success.[18]

In this new era of immunotherapy agents, it has become apparent that the traditional disease response criteria, either using Response Evaluation Criteria in Solid Tumors or World Health Organization standards, may not be sufficient to assess disease responsiveness. Durable responses have been reported in patients who have initially developed new lesions shortly after commencing ipilimumab,[16] suggesting that the response may take longer to manifest itself when compared to directly cytotoxic traditional anti-tumour agents.[19] Immune-related response criteria have been proposed whereby total tumour burden is assessed, but further evaluations of these criteria are ongoing. In view of CTLA-4's function as a 'brake' on the duration and amplitude of T cell effector functions, it could be predicted that side-effects from therapies aimed at blocking CTLA-4 would manifest as autoimmune phenomena. The initial Phase I/II studies[20-22] identified that the majority of drug-related AEs were mostly inflammatory in nature (Table 1). Predominantly, these immune-mediated AEs affect the gastrointestinal tract, skin, liver, and endocrine systems, and the frequency of Grade 3-4 treatment-related AEs with ipilimumab were recorded as 10-15%[14] but much higher, at a rate of 56.3%, when ipilimumab was combined with dacarbazine,[16] potentially due to dacarbazine's known hepatotoxicity.

The majority of immune-mediated AEs can be treated with systemic glucocorticoid therapy and, in some rare steroid-resistant cases, with anti-tumour necrosis factor antibodies. The emphasis for successful management of these AEs is on active medical surveillance and prompt initiation of treatment which may result in the cessation of ipilimumab therapy and lead to prevention of life-threatening complications. The use of prophylactic systemic steroid therapy in combination with ipilimumab therapy has not been shown to be of benefit in reducing the incidence of severe cases of treatment-related colitis.[20] Furthermore, the

use of systemic steroids to treat immune-related AEs has not been shown to affect the efficacy of ipilimumab's anti-tumour response.[14,16] The success of ipilimumab in the treatment of melanoma has resulted in an examination of its function in other tumour types. A large Phase III trial[23] randomised 799 patients to receive either ipilimumab or placebo after receiving radiotherapy for castration-resistant prostate cancer (CRPC) that had progressed after docetaxel chemotherapy. No significant difference was found in median OS between the ipilimumab and placebo groups (11.2 months versus 10 months). As expected, Grade 3-4 treatment-related AEs were higher in the ipilimumab group (26% versus 3%). Further Phase III trials are ongoing to examine the role of ipilimumab in chemotherapy-naïve patients with prostate cancer. Anti-tumour responses have been reported in patients with metastatic renal cell carcinoma (RCC), with Phase II studies reporting a tumour RR of 12.5% in patients receiving 3 mg/kg of ipilimumab[24] and also in patients with Stage 3B/4 non-small cell lung cancer (NSCLC).[25]

Programmed Cell Death Protein-1/ Programmed Death Ligand-1 (PD-1/PD-L1)

PD-1 is also a co-inhibitory member of the Ig super family of receptors. Its prime function is to restrict T cell activation and effector function in the peripheral tissues at sites of inflammation and/or infection.

Table 1: The common immune-related adverse events associated with therapeutic immunomodulatory antibodies.

Immune-related adverse event
Dermatological • Rash • Pruritus • Vitiligo • Alopecia
Gastrointestinal • Diarrhoea • Colitis
Pulmonary • Pneumonitis
Endocrine • Hypothyroidism • Hyperthyroidism • Hypophysitis • Hypopituitarism
Hepatic • Hepatitis • Abnormal liver function tests

Its expression is induced upon activation of T cells, although it can also be expressed on B cells, natural killer cells, and monocytes. PD-1 exerts its function by interacting with its two known ligands, PD-L1 (PD-L1, also known as B7-H1 or CD274) and PD-L2 (also known as B7-DC or CD273). PD-L1 is expressed on activated T cells, B cells, and APCs, including tissue-associated macrophages. Furthermore, PD-L1 is expressed on some tumour cells allowing the tumour to circumvent T cell effector function by providing inhibitory signals to evade immune attack. PD-L1, as well as serving as PD-1's ligand, also interacts with CD80 and therefore any blocking of PD-1 does not make PD-L1 completely redundant. PD-1's second ligand, PD-L2, has a more restricted expression profile and is expressed on dendritic cells, mast cells, and macrophages.

The function of PD-1 in the maintenance of peripheral self-tolerance and the prevention of uncontrolled immune activation was established in preclinical models where it was firstly observed that PD-1 knockout mice developed autoimmune phenomenon including arthritis, glomerulonephritis, and autoimmune dilated cardiomyopathy.[26,27] Further preclinical models demonstrated apoptosis of activated T cells when exposed to tumour-associated PD-L1[28] and also that *in vivo* injection of anti-PD-L1 antibodies inhibited growth of tumours expressing PD-L1.[29] A number of mAbs targeting PD-1 have been examined in clinical trials. Nivolumab (also known as BMS-936558), a fully human IgG4 mAb to PD-1, was initially studied in a Phase I trial of 296 patients examining its safety profile and anti-tumour activity in melanoma, NSCLC, RCC, and prostate and colorectal cancer.[30] Objective responses were reported in NSCLC, melanoma, and RCC only and the disease responses observed were durable with 65% of evaluable patients maintaining their response for >1 year. Grade 3-4 treatment-related AEs were reported in 14% of patients and, in particular, drug-related pneumonitis was reported in 3% of treated patients with three drug-related deaths attributed to pneumonitis. Interestingly, when available tumour biopsies were examined for PD-L1 expression, 36% (9/25) of patients with positive biopsies had an objective response, compared to 0% of patients with PD-L1 negative tumours, suggesting that the expression of PD-L1 could be a possible biomarker for disease response to nivolumab. Further immunohistological examination of tumour biopsies taken prior to commencing nivolumab therapy

showed a significant association between PD-1 expression on tumour-infiltrating lymphocytes and PD-L1 expression by the tumour cells.[31] Maintenance of disease response after stopping nivolumab therapy has also been shown in the treatment of melanoma, suggesting that an immune memory is established resulting in durable responses.[32]

Pembrolizumab (previously known as lambrolizumab or MK-3475) is a humanised IgG4 kappa mAb against PD-1. Two different dosing regimens have been examined in patients with advanced melanoma, with the highest confirmed RR seen in 10 mg/kg (52%) when compared with 2 mg/kg and a reported combined confirmed RR across all doses of 38%.[33] It should be noted that there was a higher RR reported in this trial than in the Phase III RCTs of ipilimumab. The inclusion of patients who had previously received other immunotherapies, namely ipilimumab, allowed the study to show no significant difference in RR between those who were ipilimumab-naïve and those who had received prior ipilimumab therapy. An overall RR of 26% has been reported with pembrolizumab in patients with advanced melanoma who were ipilimumab refractory, indicating that the failure of one immunotherapy should not preclude treatment with another.[34] Interestingly, as with the reports from the ipilimumab clinical trials, delayed responses were noted, including some as late as 36 weeks after treatment initiation.

The third mAb to PD-1, pidilizumab, is a humanised IgG1-kappa mAb to PD-1 which has been studied in combination with rituximab (an anti-CD20 mAb) in patients with relapsed follicular lymphoma in a non-randomised Phase II trial.[35] An objective RR of 66% (16/29) was achieved with no reported Grade 3-4 treatment-related AEs, but further randomised trials are required to test its efficacy. Many tumours have been found to express PD-L1 and, in patients with RCC, high intratumoural levels of PD-L1 expression have been associated with more aggressive tumours.[36] Moreover, in ovarian cancer, a significantly poorer prognosis was reported in patients with a high intratumoural level of PD-L1 expression.[37] In view of the observation that many tumour types express PD-L1 as an escape mechanism to avoid immune effector functions, mAbs to PD-L1 have also been developed in an attempt to manipulate the PD-1/PD-L1 axis. Brahmer et al.[38] performed a Phase I trial of 207 patients with a variety of solid-organ malignancies who received BMS-936559, a fully human IgG4 mAb to PD-L1. This antibody inhibits the binding of PD-L1 to both PD-1 and CD80. There were no objective responses reported in colorectal or pancreatic cancers but objective responses were seen in melanoma, RCC, NSCLC, and ovarian cancer. For those patients with at least 1 year of follow-up, 50% had a durable response lasting for a minimum of 1 year. The percentage of objective responses to this anti-PD-L1 antibody (only 17% for those patients with melanoma) appeared to be lower than for anti-PD-1 therapies. However, the frequency of treatment-related AEs of Grade 3-4 severity was reported as only 9% in those patients treated with anti-PD-L1 with no reported cases of Grade 3-4 colitis.[38] In the clinical trials examining anti-PD-1 mAbs, Grade 3-4 AEs were reported in 12-22% of patients.[30,32-34] Treatment-related pneumonitis has been identified as a severe AE in anti-PD-1 trials, with reported frequencies of 3-4%[30,32-33] and a small number of deaths reported as a consequence of pneumonitis. High clinical suspicion for pneumonitis and prompt initiation of steroid therapy has been recommended in those patients receiving anti-PD1 or anti-PD-L1 therapy.[39]

COMBINATION THERAPY

Combination therapy has appeared attractive in the study of immunomodulatory antibodies as it may potentially allow for a lower dose of each antibody to be used, thus harnessing both of their immunomodulatory functions. Preclinical studies have shown that the blockade of both CTLA-4 and PD-1 pathways resulted in a more marked anti-tumour effect than blocking either pathway alone, suggesting that combination therapy may be a more effective therapeutic approach.[40] A Phase I study examining the role of combination therapy with nivolumab and ipilimumab in patients with melanoma has reported objective responses in 53% of patients with substantial tumour reductions in excess of 80%.[41] Predictably, the frequency of treatment-related AEs of Grade 3-4 in patients receiving concurrent therapy was high at 53% but these events were generally reversible in nature. The combination of radiotherapy with immunomodulatory antibodies has also been examined, with a Phase III trial investigating patients with CRPC receiving radiotherapy followed by either ipilimumab or placebo reporting no significant difference in OS between either group.[23] Further collaborative Phase III RCTs are required but the high objective RRs initially reported with immunomodulatory antibody combination therapy are encouraging.

CONCLUSION

In conclusion, immunomodulatory mAbs, aimed at blocking immune checkpoints, have given rise to a new era of cancer immunotherapy. Their impact on the treatment of MM has resulted in durable responses and improvements in OS, and they have also demonstrated anti-tumour activity in a variety of other solid organ malignancies. The discovery of biomarkers to predict those patients who are more likely to respond to immunomodulatory therapy will allow for a more tailored approach to treatment, with a reduction in the number of patients exposed to potentially severe immune-mediated AEs. The need to redefine criteria for disease response has also been identified, as the pattern of objective responses differs when compared to conventional, directly cytotoxic cancer therapies. Future clinical studies examining the combination of immunomodulatory antibodies with conventional anti-cancer therapies (e.g. radiotherapy), their role in treatment naïve patients, and the efficacy of manipulating the PD-1/PD-L1 pathway in those patients who are ipilimumab refractory will further define the role of these agents in cancer therapy. Combinations of different immunotherapies may hold the key to maximising RRs, although this will only be determined by further collaborative clinical trials.

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