Immunotherapy: Enhancement the efficacy of this promising therapeutic in multiple cancers

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Abstract

Cancer treatments often reach a refractory period leading to treatment failure and patients developing disease recurrence. This can be due to tumour cells escaping the immune response and creating an immunosuppressive microenvironment enhancing cancer progression. Immunotherapy has become a promising tool for cancer treatment as it restores the anti-tumour response of the patient's immune system. Immune checkpoint inhibitors are the most widely studied immunotherapies worldwide and are now approved for multiple cancers. However, CAR-T cell therapy has also shown promise by targeting T-lymphocytes that are genetically modified *ex vivo* to expressed chimeric antigen receptors and this is now approved to treat some haematological cancers. Although immunotherapy has shown successful treatment outcomes in multiple cancers, some patients do not respond to this treatment. Therefore, approaches to enhance the efficacy of immunotherapies are likely to be the key to improving their effectiveness. Therefore, combination therapies of checkpoint inhibitors +/- chemotherapy are at the forefront of current research. Furthermore, biomarkers that predict treatment response are now beginning to emerge. Additionally, utilizing nanoparticles as a new-targeted drug delivery system to enhance CAR-T cell therapy may enhance the efficacy of the cells when re-infused within the patient. Even if efficacy is enhanced, severe immune-related adverse events (irAEs) occur that are life threatening and could lead to therapy being stopped. Therefore, predictive biomarkers for toxicity are also needed to improve both the patient's quality of life and treatment outcomes. This review will look at the current immunotherapies in clinical trials and discuss how to enhance their efficacy.

Keywords- immunotherapy, immune checkpoint inhibitor, biomarker, adoptive cell therapy, colorectal cancer

1. Introduction

Cancer therapy is mainly focussed on surgery, chemotherapy, radiotherapy, and endocrine therapy (1). However, these strategies frequently reach a refractory period leading to treatment failure and the patient developing disease recurrence (2, 3). One solution may be to focus on enhancing the patient's own immune system to attack the tumour; as once cancer is initiated, it can progress as a result of tumour cells escaping from the immune system. Tumour cells can escape the immune response in variety ways in order to survive and further progress without being attacked by immune cells (4). Additionally, tumour cells can prevent immune cell actions, with the support of multiple cell types to create an immunosuppressive microenvironment (5). Therefore, tumour escape from immunologic control is confirmed as one of the hallmarks of cancer (6).

The immune responses recognise tumour cells and eradicate them by multiple processes involving cooperation of both the innate and adaptive arms (7, 8). This process involves both positive and negative regulators. Positive regulators enhance anti-tumour activity, whereas negative regulators inhibit this killing process and enhance tumour growth instead. Therefore, immunotherapy that targets the negative regulators to enhance the anti-tumour responses could be a promising alternative treatment strategy and may be a powerful tool to treat cancer.

Immunotherapy is now a main focus for many cancer types; it works by restoring the patient's immune responses to eliminate tumour cells (9, 10). It can be classified as active or passive by assessing the mechanism by which the therapy activates an immune response (11). Active immunotherapy includes preventive and therapeutic vaccines, immunomodulatory monoclonal antibodies, i.e. immune checkpoint inhibitors, and immunostimulatory cytokines, that stimulate the host's adaptive immune response in situ. Passive immunotherapy focuses on activating the host's immune response *in vitro* and transfers it back to the host known as

adoptive cell transfer or cell-based therapy, i.e. chimeric antigen receptor CAR-T cell therapy.

This review summarizes two types of immunotherapy; immune checkpoint inhibitors, and adoptive T-cell transfer in detail within the cancer setting. Immune checkpoint inhibitors focuses on anti PD-1/anti PD-L1/anti CTLA-4; as the mechanism of immune checkpoint proteins on T-cells (PD-1, PD-L1, CTLA-4) is to bind to their receptors on tumour cells or antigen presenting cells, which causes a negative effect by inhibiting T-cells function. Therefore, blocking the binding of immune checkpoint protein and their receptors could restore T-cells function to kill tumour cells. Whereas adoptive T-cell transfer or CAR T-cells; utilise T-cells from patients that are engineered *in vitro* to express chimeric antigen receptors which induces binding to tumour cell antigens and enhance T-cells function, which causes a positive effect to kill tumour cells. The review will also discuss strategies to enhance immunotherapy efficacy and how they relate to the effectiveness of treatment outcomes.

2. Immune checkpoints and their inhibitors

Active immunotherapy targets the host's immune response to induce activation and restore anti-tumour function, one example of this are checkpoint inhibitors. Immune checkpoints are negative regulators of the immune system and play a crucial role in limiting anti-tumour immune responses. In the normal anti-cancer immune response; antigens on the surface of tumour cells bind to checkpoint proteins on the surface of T-lymphocytes leading to decreased T-cell function. This controls the anti-tumour response and avoids T-cell exhaustion; however, the tumour can hijack this mechanism to suppress anti-tumour functions and promotes tumour progression. Therefore, these immune checkpoints are important immunotherapeutic targets, with checkpoint blockade inhibiting this immune system modulation resulting in increased effector T-cells that can coordinate an anti-tumour

response (12). Immune checkpoint inhibitors are the most widely studied active immunotherapy in cancer research leading to some having been approved for clinical use (13). Two types of co-inhibitory proteins that are widely studied are programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte antigen 4 (CTLA-4) (Figure 1).

2.1 PD-1 and PD-L1

PD-1, known as CD279, is a type I transmembrane protein, a member of the CD28 family which is expressed on activated and exhausted T- and B-lymphocyte. PD-1 is expressed during the effector phase in peripheral tissue and is upregulated in many tumours. It binds to specific ligands, called PD-L1 (the program cell death ligand-1) and PD-L2 (the program cell death ligand-2), on tumour cells.

PD-L1 is expressed on various types of solid tumours, but is not expressed in normal epithelial tissue, where, PD-L2 is the dominant form. When PD-1 binds to PD-L1 there is decreased cytokine production and inhibition of T-cells proliferation and function within peripheral tissues and tumours. This plays a key role in negative immune cell regulation resulting in tumour immunity balancing and attenuation of the T-lymphocyte response within the tumour microenvironment (14, 15).

2.2 CTLA-4

CTLA-4, also known as CD152, is a CD28 homolog membrane glycoprotein on T-lymphocyte, found during the priming phase in lymph nodes. CTLA-4 interacts with specific protein (B7) on antigen presenting cells (APC) to produce co-inhibitory signals to decrease T-lymphocyte anti-tumour responses (16). This was the first pathway for immune checkpoint regulation that was proposed in 1996 by Leach et al. (17).

2.3 Immune checkpoints inhibitors to block ligand binding that inhibits T-cell functions

Immune checkpoint interactions can be blocked with anti-PD-1/anti-PD-L1/anti-

CTLA-4 antibodies leading to immune cell re-activation and a coordinated anti-tumour response of T-cells. Currently, three anti-PD-1 immune checkpoint inhibitors, pembrolizumab, nivolumab, and cemiplimab are approved for use within the clinical setting. Three anti-PD-L1 antibodies were also approved; atezolizumab, durvalumab, and avelumab. Ipilimumab is the only anti-CTLA-4 approved for clinical use (Table 1).

2.3.1 Anti-PD-1 (Pembrolizumab, Nivolumab, and Cemiplimab)

Pembrolizumab has been studied in multiple solid tumours and showed anti-tumour activity in clinical trials. As a result, in 2014, it was approved to treat advanced melanoma based on a phase III studied comparing pembrolizumab and the anti-CTLA-4, ipilimumab; pembrolizumab demonstrated prolong overall survival and less toxicity than ipilimumab (18). In 2015, pembrolizumab was further approved to treat advanced non-small-cell lung cancer (NSCLC) based on the result that it showed anti-tumour activity with manageable side-effects (19). In 2016, pembrolizumab was approved for recurrent or metastatic head and neck squamous cell carcinoma patients (20). A study by Seiwert et al. showed that pembrolizumab has efficacy over standard therapy by cetuximab. However, the latest phase III trial by Merck and Co., showed that this drug did not result in improved overall survival as previously observed (21). This finding did not affect the previous approval but does show variability in results for this cancer type and suggests that a predictive biomarker is needed to select responsive patients.

Over the past two years, pembrolizumab has been approved for multiple types of cancer including classical Hodgkin lymphoma (cHL), metastatic urothelial carcinoma, gastric cancer or gastroesophageal junction (GEJ) adenocarcinoma, and colorectal cancer (CRC). In cHL, pembrolizumab was approved based on the study that treated both adults and pediatric patients with resistance to current therapy. The result showed a 69% overall response rate with 11.1 months of median response duration until reaching unacceptable toxicity (22). In

advanced metastatic urothelial carcinoma (mUCC), it was approved based on the phase II trial that compared pembrolizumab to chemotherapy. Pembrolizumab showed a median overall survival of 10.3 months, whereas chemotherapy only showed a median survival of 7.4 months (23). In the same year, pembrolizumab was also approved for non-resectable or metastatic microsatellite instability-high (MSI-H)/mismatch-repair deficient (dMMR) solid tumours including colorectal cancer (20, 24-27). This was the first approval based on the specific biomarkers regardless of the origin of tumour.

In gastric cancer or GEJ adenocarcinoma, pembrolizumab was approved based on the phase II KEYNOTE-059 study (28). From the 143 patients, the result showed 13.3% objective response rate and response duration of 2.8-19.4 months. MSI-H was observed in 7 patients showing a 57% objective response rate and response duration from 5.3-14.1 months suggesting MSI status could predict response to pembrolizumab as seen in other cancers. Recently, pembrolizumab was further approved for treatment of recurrent or metastatic cervical cancer for patients who express PD-L1 on tumour cells (29). In the same month, it was further approved to treat resistant primary mediating large B-cell lymphoma (PMBCL) in adults and pediatrics and showed a 45% response rate (30). However, 26% of patients did developed serious adverse effects suggesting further work is still needed in the cancer type.

Nivolumab, a second PD-1 inhibitor, has also been investigated in several tumours and shown anti-tumour activity. In 2014, it was approved for metastatic melanoma treatment (31, 32), and in 2015, nivolumab was further approved for metastatic NSCLC after a study demonstrated improved overall survival compered to docetaxel therapy (33). Nivolumab was also approved for use in advanced metastatic renal cell carcinoma (RCC) as it showed improved overall survival over everolimus (Afinitor) (34). In 2016, nivolumab was approved for recurrent or metastatic head and neck squamous cell carcinoma (HNSCC) after a study showed longer overall survival when compared to chemotherapy (35).

Recently, in September 2018, cemiplimab (REGN2810) is approved to treat patients with metastatic cutaneous squamous cell carcinoma (CSCC) or locally advanced CSCC who are not candidates for curative surgery or curative radiation (36). There are also many other anti-PD-1 antibodies, for example, pidilizumab (a humanized anti-PD-1), AMP-224, MEDI0680, PDR001, CT-001, in clinical trials for several tumour types (37).

2.3.2 Anti-PD-L1 (Atezolizumab, Durvalumab, and Avelumab)

PD-L1 inhibitors have also been approved for use in solid tumours. Atezolizumab was approved for advanced bladder cancer (38) and metastatic NSCLC in 2016 (39, 40). In 2017, avelumab was approved for merkel cell cancer, an aggressive skin cancer, and UCC. In merkel cell cancer, avelumab was the first targeted therapy approved for this disease, the phase II trial studied avelumab in stage IV chemotherapy refractory disease via an international multicenter trial across North America, Europe, Asia, and Australia (41). The result showed a 31% response rate and 10.4 months response duration. In UCC, a study in mUCC patients showed 11.4 weeks median response rate. However, almost all patients developed adverse events (42). Overall, PD-1 and PD-L1 inhibitors have shown good response rates across a variety of cancers but further work is needed to enhance their efficacy and decrease toxicity. In addition, durvalumab is approved for advanced or mUCC in 2017 and NSCLC in 2018 (43, 44).

2.3.3 Anti-CTLA-4 (Ipilimumab)

The main CTLA-4 inhibitor studied to date is ipilimumab, a monoclonal antibody that was the first checkpoint inhibitor the FDA-approved for advanced melanoma in 2014. It was shown to increase T-lymphocyte proliferation and restore the anti-tumour immune response (45). Based on the phase III clinical study in unresectable stage III or IV melanoma that divided patients into 3 treatment groups, ipilimumab plus glycoprotein 100 (gp100) peptide vaccine, ipilimumab alone, and gp100 alone. The result showed that ipilimumab significantly

improved patients overall survival compared to the gp100 alone or the combination (46). Ipilimumab has now also been approved for use in RCC in combination with nivolumab (47).

Tremilimumab, an IgG2 monoclonal antibody, is another anti-CTLA-4 that showed satisfactory result in phase I/II studies in advance melanoma (48). However, when test in a phase III trial compared with chemotherapy, tremilimumb induced toxicity and showed no survival benefit over chemotherapy (49). Therefore, tremilimumab was not approved, but further clinical trials are now ongoing to study this drug in combinations with current therapies and to assess potential biomarkers to predict treatment response.

2.3.4 Immune checkpoint inhibitors in colorectal cancer

Although checkpoint inhibitors have exhibited successful results in other tumours, in CRC, the results for immunotherapy are not as favorable. Initial studies showed some promising results in metastatic CRC patients with dMMR tumours, but not in MMR competent patients, which only represents a small proportion of metastatic patients. Therefore, pembrolizumab and nivolumab are only FDA-approved for this small group of patients with metastatic dMMR CRC, suggesting that other strategies are required for these inhibitors to be translated to a wider range of CRC tumours.

To address this, immune checkpoint inhibitors are now being trialed in combination with chemotherapy, radiotherapy, and other agents that might block factors that suppress the immune response or agents that directly stimulate the immune response, to prime for immunotherapy use (50). However, there is still a problem with resistance to checkpoint inhibitor due to various factors including signaling pathways which inhibit the anti-tumour activity of immune cells (51). At the moment, monotherapy or combination therapy to enhance the drug efficacy and reduce this resistance rate in CRC is being considered in parallel to examining new therapeutic targets.

Therefore, a combination of pembrolizumab with azacitidine chemotherapy was

performed in MMR competent metastatic CRC. The results observed an enhancement of pembrolizumab anti-tumour activity when combined with this chemotherapy in these patients. The trial is now in phase II with a cohort of 31 MMMR competent metastatic CRC patients receiving 200 mg pembrolizumab every 3 weeks and 100 mg azacitidine daily. However, the results showed a low response rate (3%) and the median overall survival was only 6.2 months. Ten patients did, however, developed rapid stabilization of tumour progression but treatment-related adverse events occurred in 63% of patients (52). Therefore, pembrolizumab plus azacitidine showed a low anti-tumour activity for MMR competent metastatic CRC, however, disease stabilization in some patients may suggest that biomarkers are needed to predict those patients that will achieve disease stabilization and those that will develop toxicity. Similarly, a combination of nivolumab and ipilimumab was preliminary studied and showed potential efficacy in the same cohort. The result from 27 patients showed a 41% objective response rate with 78% disease control rate. Tumour-related adverse events occurred in 37% of patients but there was no death due to this therapy (53). This study is still ongoing to further assess long-term efficacy and analysis of potential predictive biomarkers.

As the PD-L1 inhibitor, atezolizumab, has shown only a partial response in CRC Phase I studies, currently, studies of atezolizumab combined with the vascular endothelial growth factor (VEGF) inhibitor bevacizumab, or atezolizumab plus bevacizumab and FOLFOX in metastatic CRC are looking promising (54). Both studies showed significant anti-tumour effects. Many other anti-PD-L1 compounds are also in ongoing studies for combination therapy i.e. durvalumab and avelumab. Recently, the WNT/β-catenin signaling pathway has been reported to block anti-tumour activity of tumour-infiltrating lymphocytes and enhance resistance to anti-PD-1/PD-L1. In addition, immune evasion was further promoted by STAT3 signaling; BBI608 is an inhibitor that blocks STAT3 and down-regulate WNT/β-catenin signaling. Therefore, combination of pembrolizumab plus BBI608 is

currently undergoing assessment for efficacy and safety in a multicenter phase I/II trial (55).

The trial aims to block WNT/β-catenin and STAT3 signaling in 8 patients with MMMR competent metastatic CRC, to try to enhance the efficacy of pembrolizumab. In the phase I trial, patients were divided into 2 groups: for group 1, 5 patients received 240 mg BID everyday with 200 mg pembrolizumab; and for group 2, 3 patients received 480 mg BID everyday with 200 mg pembrolizumab. The result showed that one patient in group 1 developed dose-limiting toxicity and was discontinued, however, the rest of the patients in this group presented no toxicity related symptoms, with no toxicity seem in group 2. Interestingly, one patient in group 2 demonstrated tumour shrinkage over more than 12 weeks with a significant decline in carcinoembryonic antigen (CEA) levels in both lung and lymph node metastases. From these initial results, it suggests this combination might induce anti-tumour activity. This cohort will now continue into a phase II trial to confirm the efficacy and safety of this combination (56). These results suggest that for CRC the way forward for immunotherapy is combination with other drugs to prime the immune landscape. Developing predictive biomarkers for treatment response and toxicity may further enhance this.

3. Adoptive cell transfer to enhance T-cell functions

Passive immunotherapy or cell-based therapy is based on immune effector cells that are generated *ex vivo* and then transferred into the patient known as adoptive cell transfer (ACT). ACT is a type of cell-based therapy based on collecting tumour infiltrating or circulating lymphocytes from patients, processing them *ex vivo* to target a specific neoantigen, and then reinfusion of the cells back into patient (Figure 2). The predominant cell types used are T-lymphocytes and natural killer (NK) cells, which both have anti-tumour characteristics, cytolytic actions and produce cytokines to eliminate tumour cells (57).

3.1 Chimeric antigen receptor T-cell therapy

Current ACT strategy target T-lymphocytes that are genetically modified to expressed chimeric antigen receptor (CAR-T), which is specific to tumour cells. They recognise native tumour antigen on cell surface instead of epitopes presented by human leukocyte antigen (HLA) molecules. Engineered CAR-T cells are linked to the intracellular signalling domain of T-lymphocytes by using the antibody-derived single-chain variable fragment (scFv), resulting in T-lymphocytes being recognised via surface antigens independent of major histocompatibility complex (MHC) (58). After they bind to the tumour antigen, CARs then activate T-lymphocytes to kill the tumour. The most important CAR-T cells are generated to target specific tumour cells surface antigens; this avoids unexpected autoimmune diseases. As CARs do not rely on HLA, they can be employed in all patients regardless of HLA haplotype. Currently, CAR-T cell therapy is approved to treat hematologic malignancies, including chronic lymphocytic leukaemia (CLL) and acute lymphocytic leukaemia (ALL) patients and adults with certain types of large B-cell lymphoma (59, 60). Recently, a study reported the potential of CAR-T cells therapy in breast cancer however more evidence is needed before the approval of CAR-T cell therapy for this cancer type (61).

3.2 CAR-T cells therapy and colorectal cancer

In CRC, CAR-T cells are being investigated for metastatic disease. Animal model for CAR-T cells targeting CRC antigens, classic CEA and emerging guanylyl cyclase C (GUCY2C), show therapeutic potential that might translate to clinical use. When targeting CEA, animal experiments suggest that CAR-T cells may induce tumour regression; however, they also cause toxicity due to cytokine release syndrome. In human trials, the initial studies focussed on CRC with liver metastasis, and no patients were reported to developed severe adverse effects; however, disease progression was not suppressed, and cancer specific survival was not improved.

As for targeting GUCY2C, these have only been studies in animal models. Initial

results from pulmonary metastasis suggest that CAR-T cells targeting GUCY2C could reduce tumour burden and significantly prolong survival; however, this needs to be confirmed in human studies. Targeting GUCY2C showed no autoimmunity issues and had good safety and efficiency to treat metastatic CRC (62). From these initial results, CAR-T cells therapy has the potential to be a useful CRC metastasis treatment although further safety testing is required.

4. Enhancing the efficacy of current immunotherapies

Although immunotherapy has shown satisfactory results in multiple types of cancer, many patients show no response, for example, in MMR competent CRC. Therefore, strategies that enhance the efficacy of immunotherapy are now the focus of many studies. Some approaches to enhance the efficacy of immunotherapy include performing immune checkpoints combination therapy to increase treatment yield, evaluating biomarkers for treatment responses to observe treatment effectiveness, assessing biomarkers for treatment toxicities to reduce treatment failure, and investigating new potential targets.

4.1 Immune checkpoint inhibitor combination therapy

Currently, combinations of anti-PD-1/PD-L1 and CTLA-4 drugs are in clinical trials, focusing on ipilimumab with nivolumab or pembrolizumab. To date, combination trials in advanced melanoma have shown that a reduced dose of nivolumab combined with the standard dose of ipilimumab showed better response rates than ipilimumab alone, however, it caused more toxicity (63). Conversely, a Phase I study of standard-dose pembrolizumab combined with low-dose ipilimumab in advanced melanoma showed significant anti-tumour activity and controllable toxicity, therefore, a phase II trial is now underway (63, 64).

4.2 Predictive biomarkers for treatment response

Immunotherapy may have shown disappointing results in some tumours because of the genetic variability or differing strengths of the host immune response within each patient. Therefore, specific biomarkers or clinical features that could be used to predict response to treatment are likely to be key improving the effectiveness of immunotherapy in these patients. This is already suggested with MMR status being used as a predictive marker for pembrolizumab and nivolumab in a variety of cancers. Another potential biomarker candidate for immune checkpoint therapies includes immunological biomarkers, such as intratumoral PD-L1. However, a study from Aguiar et al. showed that PD-L1 negative tumours still respond to checkpoint inhibitor drugs (65). Suggesting that intratumoural PD-L1 might not be an effective marker for PD-1/PD-L1 inhibitor. Recently, PD-L2 expression was also found to be an independent prognostic factor that may also predict the effectiveness of anti-PD-1 therapy (66, 67).

Biomarkers related to clinical responses to anti-PD-1, anti-PD-L1, and anti-CTLA-4 checkpoint inhibitors therapies are currently being studied in both patient's tumour tissue and blood samples (66, 68) (Table 2). In tumour tissues, many biomarkers have been studied, for example, tumour-infiltrating lymphocytes (TILs), PD-L1 expression, and mutational load. TILs present in the intratumoral site have been shown to be associated with improved clinical benefit for anti-CTLA-4 therapy in advanced melanoma (69). Also, PD-L1 expression has been shown to associate with improved clinical benefit from anti-PD-1/anti-PD-L1 therapy in multiple cancer types including advanced melanoma and breast cancer (70, 71). Furthermore, mutational load has been performed in both anti-CTLA-4 and anti-PD-1 therapy. In melanoma, it was shown that high mutational load related to improved efficacy for anti-CTLA4 therapy (72); whereas in NSCLC high mutational load was associated with better efficacy for anti-PD-1 therapy (73). From these studies, it suggests that assessing treatment responses using various predictive biomarkers may benefit immunotherapy outcomes.

In blood samples, several biomarkers have been studied including circulating leukocytes (lymphocytes, neutrophils, eosinophils, and monocytes), myeloid-derived suppressor cells (MDSCs) level, and lactate dehydrogenase (LDH) level. For anti-CTLA-4, high lymphocytes level during treatment related to better overall survival on this therapy (74). Furthermore, the neutrophil-to-lymphocyte ratio (NLR) declined during on-going treatment and this showed association with a higher survival rate (75). In contrast, high serum LDH level prior to treatment with anti-CTLA-4 therapy was associated with resistance to treatment (76). Although wide varieties of biomarkers have been investigated in multiple cancers, validation is now required before translation into clinical setting can be achieved.

4.3 Predictive biomarkers for toxicity

One issue with checkpoint inhibitors is that the treatment can cause severe immune-related adverse events (irAEs), frequently affecting the skin, intestinal tract, liver, and endocrine system. These irAEs occur more frequently with anti-CTLA-4 antibody than anti-PD-1/anti-PD-L1 antibody [61]. As these can be life threatening they often lead to therapy being stopped. Therefore, predictive biomarkers that test for toxicity in patients are also needed to improve both the patient's quality of life and survival outcomes.

Biomarkers associated with ipilimumab treatment toxicities in melanoma patients have been studied in colon tissue to investigate toxicity within the intestinal tract (77). The result showed that neutrophil infiltration within the lamina propria of colon biopsies and other markers of digestive dysregulation including histological observation, faecal calprotectin, and antibodies for enteric flora were associated with digestive toxicity. This suggests that ipilimumab induces colitis as confirmed by Marthey et al. (78), who then investigated these biomarkers during drug administration and showed they could be of benefit for predicting these adverse effects and improving the quality of life of the patients.

Similarly, another study using blood samples of melanoma patients treated with ipilimumab measured eosinophils level before and after treatment. The result showed that absolute eosinophil counts increased over treatment and were associated with the occurrence of irAEs (79). To address this further, gene expression profiles of peripheral bloods from advanced melanoma patients treated with ipilimumab, that had developed gastrointestinal tract irAEs, were investigated (80). The result showed increase expression of neutrophil markers, CD177 and carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1), during treatment supporting the potential role of neutrophil in irAEs of ipilimumab within the gastrointestinal tract. However, further studies from larger cohorts of patients are needed as well as other more specific predictive markers in order for early management of irAEs to occur.

4.4 Alternative targets for immunotherapy

At present, apart from targeting PD-1/PD-L1 and CTLA4 pathway, many other immune pathways are being targeted including macrophage targeting therapy, cytokine therapy, toll-like receptor (TLR) agonists, and other checkpoint inhibitors including T-cell membrane protein (TIM-3 or HAVcr2), lymphocyte activation gene (LAG-3 or CD223), B- and T-lymphocyte attenuator (BTLA or CD272), and V-domain Ig suppressor of T-cell activation (VISTA) (64). As a result, personalized medicine using these targeted immunotherapies is the main focus for future cancer treatments combined with actively seeking predictive biomarkers to increase responsiveness to these treatments.

Novel approaches to immunotherapy are also under investigation including utilizing a new-targeted drug delivery system for $CD8^+$ T-lymphocytes called nanoparticles that was proposed by Schmid et al. (81). The study in mice utilized antibody-targeted nanoparticles bound to $CD8^+$ T-lymphocytes in blood, lymphoid tissues, and the tumour. The result showed the nanoparticles successfully targeted $PD\text{-}1^+$ T-lymphocytes in the blood and tumour. Using

these nanoparticles, a TGFβ inhibitor was delivered to PD-1 expressing cells and showed extra survival benefit compared to delivering the inhibitor as a free drug at the same dose. Nanoparticles also modulated the ratio of tumour infiltrating $CD8^+$ T-lymphocytes and sensitized tumours to anti-PD-1 therapy (81). From the results, using nanoparticles targeting specific effector cell's function to kill tumour cells and inhibit suppressor cells at the same time could potentially change non-responder to responder for immunotherapy.

Other delivery methods are also under development including peptides and antibody-based systems. Furthermore, cellular mechanisms, such as cellular influx, are being investigated to deliver immune regulating compounds to the tumour area (82). Overall, it is promising time for the use of immunotherapy as a treatment for solid and hematological tumours. Furthermore, enhancing the efficacy of these treatments via combination therapies and utilizing biomarkers could expand the treatments prospects.

5. Conclusions

In conclusion, the immune system plays an essential role in cancer progression through mechanisms regulated by multiple immune cells types in tumour microenvironment. Of these, immune checkpoints, such as PD-1/PD-L1 and CTLA-4, are crucial proteins that inhibit the immune system anti-tumour effects and promote tumour progression. Therefore, immunotherapy targeting immune checkpoints to restore the killing function of immune cells is an alternative treatment strategy. Currently, there are many drugs targeting PD-1/PD-L1 and CTLA-4 that are approved to treat multiple tumour types, with nivolumab and pembrolizumab at the forefront of many clinical trials.

However, not all patients respond to these treatments and this might be due to differences at a genomic or immune level within each patient that is affecting their response to the drug's action. Therefore, biomarkers that predict response to treatment are also

essential for immunotherapies success. As a result, combination therapies of immunotherapy and conventional chemotherapy or radiotherapy are being investigated to see if these can prime the immune system to enhance the efficacy of the immunotherapy. Furthermore, targeting of other checkpoint inhibitors and immune pathways are being investigated to enhance the effectiveness of immunotherapy and move towards a more personalized therapy approach. Additionally, utilizing nanoparticles as a new-targeted drug delivery system to target $CD8^+$ T-lymphocytes to the tumour is a novel approach to immunotherapy that may enhance adoptive CAR-T cells therapy and is currently a major focus of many clinical trials. Overall, immunotherapy could be a powerful tool to fight multiple cancers; nevertheless, more investigation is needed to enhance efficacy and reduce toxicity within patients.

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Abbreviations- PD-1, programmed cell death-1; CTLA-4, cytotoxic T-lymphocyte antigen-4; PD-L1, programmed cell death ligand-1; PD-L2, programmed cell death ligand-2; APC, antigen presenting cell; MSI-H, microsatellite instability-high; dMMR, mismatch-repair deficient; VEGF, vascular endothelial growth factor; CEA, carcinoembryonic antigen; ACT, adoptive cell transfer; CAR, chimeric antigen receptor; HLA, human leukocyte antigen; MHC, major histocompatibility complex; scFv, single-chain variable fragment; GUCY2C, guanylyl cyclase C; TILs, tumour-infiltrating lymphocytes; MDSCs, myeloid-derived

suppressor cells; LDH, lactate dehydrogenase; NLR, neutrophil-to-lymphocyte ratio; irAEs, immune-related adverse events; CEACAM1, carcinoembryonic antigen-related cell adhesion molecule 1; TLR, toll-like receptor; TIM, T-cell membrane protein; LAG, lymphocyte activation gene; VISTA, V-domain, Ig suppressor of T-cell activation

References:

1. Urruticoechea A, Alemany R, Balart J, Villanueva A, Vinals F, Capella G. Recent Advances in Cancer Therapy: An Overview. Current Pharmaceutical Design. 2010;16(1):3-10.

2. Housman G, Byler S, Heerboth S, Lapinska K, Longacre M, Snyder N, et al. Drug Resistance in Cancer: An Overview. Cancers. 2014;6(3):1769-92.

3. Raguz S, Yague E. Resistance to chemotherapy: new treatments and novel insights into an old problem. British Journal of Cancer. 2008;99(3):387-91.

4. Ahmad M, Rees RC, Ali SA. Escape from immunotherapy: possible mechanisms that influence tumor regression/progression. Cancer Immunology Immunotherapy. 2004;53(10):844-54.

5. Seager RJ, Hajal C, Spill F, Kamm RD, Zaman MH. Dynamic interplay between tumour, stroma and immune system can drive or prevent tumour progression. Converg Sci Phys Oncol. 2017;3.

6. Hanahan D, Weinberg RA. Hallmarks of Cancer: The Next Generation. Cell. 2011;144(5):646-74.

7. Shanker A, Marincola F. Cooperativity of adaptive and innate immunity: implications for cancer therapy. Cancer Immunology Immunotherapy. 2011;60(8):1061-74.

8. Blair GE, Cook GP. Cancer and the immune system: an overview - Introduction. Oncogene. 2008;27(45):5868-.

9. Stanley J. Oiseth MSA. Cancer immunotherapy: a brief review of the history, possibilities, and challenges ahead. J Cancer Metastasis Treat. 2017;3:12.

10. Farkona S DE, Blasutig IM. Cancer immunotherapy: the beginning of the end of cancer? Bmc Medicine. 2016;14.

11. Galluzzi L, Vacchelli E, Bravo-San Pedro JM, Buque A, Senovilla L, Baracco EE, et al. Classification of current anticancer immunotherapies. Oncotarget. 2014;5(24):12472-508.

12. Passardi A, Canale M, Valgiusti M, Ulivi P. Immune Checkpoints as a Target for Colorectal Cancer Treatment. Int J Mol Sci. 2017;18(6).

13. Dine J, Gordon R, Shames Y, Kasler MK, Barton-Burke M. Immune Checkpoint Inhibitors: An Innovation in Immunotherapy for the Treatment and Management of Patients with Cancer. Asia-Pac J Oncol Nu. 2017;4(2):127-35.

14. Singh PP, Sharma PK, Krishnan G, Lockhart AC. Immune checkpoints and immunotherapy for colorectal cancer. Gastroenterology Report. 2015;3(4):289-97.

15. Robert C, Schachter J, Long GV, Arance A, Grob JJ, Mortier L, et al. Pembrolizumab versus Ipilimumab in Advanced Melanoma. New England Journal of Medicine. 2015;372(26):2521-32.

16. Buchbinder EI, Desai A. CTLA-4 and PD-1 Pathways Similarities, Differences, and Implications of Their Inhibition. American Journal of Clinical Oncology-Cancer Clinical Trials. 2016;39(1):98-106.

17. Leach D KM, Allison J. Enhancement of anti- tumor immunity by CTLA-4 blockade. Science. 1996;271:3.

18. Schachter J, Ribas A, Long GV, Arance A, Grob JJ, Mortier L, et al. Pembrolizumab versus ipilimumab for advanced melanoma: final overall survival results of a multicentre, randomised, open-label phase 3 study (KEYNOTE-006). Lancet. 2017;390(10105):1853-62.

19. Garon E RN, Hui R, Leighl N, Balmanoukian A, Eder J. Pembrolizumab for the Treatment of Non–Small-Cell Lung Cancer. N Engl J Med. 2015;372(21):11.

20. Seiwert TY, Haddad RI, Gupta S, Mehra R, Tahara M, Berger R, et al. Antitumor activity and safety of pembrolizumab in patients (pts) with advanced squamous cell

carcinoma of the head and neck (SCCHN): Preliminary results from KEYNOTE-012 expansion cohort. Journal of Clinical Oncology. 2015;33(18).

21. Cohen EEW, Machiels JPH, Harrington KJ, Burtness B, Shin SW, Gause CK, et al. KEYNOTE-040: A phase III randomized trial of pembrolizumab (MK-3475) versus standard treatment in patients with recurrent or metastatic head and neck cancer. Journal of Clinical Oncology. 2015;33(15).

22. Moskowitz C, Zinzani PL, Fanale MA, Armand P, Johnson N, Ribrag V, et al. Pembrolizumab for Relapsed/Refractory Classical Hodgkin Lymphoma: Multicohort, Phase 2 Keynote-087 Study. Haematologica. 2016;101:44-.

23. Bajorin DF, Plimack ER, Siefker-Radtke AO, Choueiri TK, De Wit R, Sonpavde G, et al. KEYNOTE-052: Phase 2 study of pembrolizumab (MK-3475) as first-line therapy for patients (pts) with unresectable or metastatic urothelial cancer ineligible for cisplatin-based therapy. Journal of Clinical Oncology. 2015;33(15).

24. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. N Engl J Med. 2015;372(26):2509-20.

25. Le DT, Yoshino T, Jager D, Andre T, Bendell JC, Wang RX, et al. KEYNOTE-164: Phase II study of pembrolizumab (MK-3475) for patients with previously treated, microsatellite instability-high advanced colorectal carcinoma. Journal of Clinical Oncology. 2016;34(4).

26. O'Neil BH WJ, Lorente D, Elez, E RJ, Gomez-Roca C. Safety and antitumor activity of the anti–PD-1 antibody pembrolizumab in patients with advanced colorectal carcinoma. Plos ONE. 2017;12(12):e0189848.

27. Diaz L, Marabelle A, Kim TW, Geva R, Van Cutsem E, Andre T, et al. Efficacy of pembrolizumab in phase 2 KEYNOTE-164 and KEYNOTE-158 studies of microsatellite instability high cancers. Annals of Oncology. 2017;28.

28. Fuchs CS, Doi T, Jang RW, Muro K, Satoh T, Machado M, et al. Safety and Efficacy of Pembrolizumab Monotherapy in Patients With Previously Treated Advanced Gastric and Gastroesophageal Junction Cancer Phase 2 Clinical KEYNOTE-059 Trial. Jama Oncology. 2018;4(5).

29. Chung HC, Schellens JHM, Delord JP, Perets R, Italiano A, Shapira-Frommer R. Pembrolizumab treatment of advanced cervical cancer: Updated results from the phase 2 KEYNOTE-158 study. Journal of Clinical Oncology. 2018;36(15).

30. Michot JM, Armand P, Ding W, Ribrag V, Christian B, Marinello P, et al. KEYNOTE-170: Phase 2 study of pembrolizumab in patients with relapsed/refractory primary mediastinal large B-cell lymphoma (rrPMBCL) or relapsed or refractory Richter syndrome (rrRS). Annals of Oncology. 2016;27.

31. Raedler LA. Opdivo (Nivolumab): Second PD-1 Inhibitor Receives FDA Approval for Unresectable or Metastatic Melanoma. Am Health Drug Benefits. 2015;8(Spec Feature):180-3.

32. Long GV, Atkinson V, Ascierto PA, Robert C, Hassel JC, Rutkowski P, et al. CHECKMATE 066: effect of nivolumab (NIVO) on quality of life (QoL) in patients (pts) with treatment-naive advanced melanoma (MEL) Asia-Pacific Journal of Clinical Oncology. 2015;11:58-.

33. Kazandjian D, Suzman DL, Blumenthal G, Mushti S, He K, Libeg M, et al. FDA Approval Summary: Nivolumab for the Treatment of Metastatic Non-Small Cell Lung Cancer With Progression On or After Platinum-Based Chemotherapy. Oncologist. 2016;21(5):634-42.

34. Motzer RJ, Escudier B, Mcdermott DF, George S, Hammers HJ, Srinivas S, et al. CheckMate 025: a randomized, open-label, phase Ill study of nivolumab versus everolimus in advanced renal cell carcinoma (RCC). Bju International. 2015;116:17-.

35. Ferris RL, Blumenschein G, Jr., Fayette J, Guigay J, Colevas AD, Licitra L, et al. Nivolumab for Recurrent Squamous-Cell Carcinoma of the Head and Neck. N Engl J Med. 2016;375(19):1856-67.

36. Papadopoulos KP, Owonikoko TK, Johnson M, Brana I, Martin MG, Perez RP, et al. Cemiplimab (REGN2810): A fully human anti-PD-1 monoclonal antibody for patients with unresectable locally advanced or metastatic cutaneous squamous cell carcinoma (CSCC)-Initial safety and efficacy from expansion cohorts (ECs) of phase I study. J Clin Oncol. 2018;36(5).

37. Alsaab HO, Sau S, Alzhrani R, Tatiparti K, Bhise K, Kashaw SK, et al. PD-1 and PD-L1 Checkpoint Signaling Inhibition for Cancer Immunotherapy: Mechanism, Combinations, and Clinical Outcome. Frontiers in Pharmacology. 2017;8.

38. Hoffman-Censits JH, Grivas P, Van der Heijden MS, Dreicer R, Loriot Y, Retz M, et al. IMvigor 210, a phase II trial of atezolizumab (MPDL3280A) in platinum-treated locally advanced or metastatic urothelial carcinoma (mUC). J Clin Oncol. 2016;34(2).

39. Rittmeyer A, Barlesi F, Waterkamp D. Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial (vol 389, pg 255, 2016). Lancet. 2017;389(10077):E5-E.

40. Fehrenbacher L, Spira A, Ballinger M, Kowanetz M, Vansteenkiste J, Mazieres J, et al. Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): a multicentre, open-label, phase 2 randomised controlled trial. Lancet. 2016;387(10030):1837-46.

41. Kaufman HL, Russell J, Hamid O, Bhatia S, Terheyden P, D'Angelo SP, et al. Avelumab in patients with chemotherapy-refractory metastatic Merkel cell carcinoma: a multicentre, single-group, open-label, phase 2 trial. Lancet Oncology. 2016;17(10):1374-85.

42. Patel MR, Ellerton J, Agrawal M, Gordon M, Dirix L, Lee KW, et al. Avelumab (MSB0010718C; anti-PD-L1) in patients with metastatic urothelial carcinoma progressed after platinum-based therapy or platinum ineligible. Annals of Oncology. 2016;27.

43. Powles T, O'Donnell PH, Massard C, Arkenau HT, Friedlander TW, Hoimes CJ, et al. Efficacy and Safety of Durvalumab in Locally Advanced or Metastatic Urothelial Carcinoma: Updated Results From a Phase 1/2 Open-label Study. JAMA Oncol. 2017;3(9):e172411.

44. Antonia SJ, Ozguroglu M. Durvalumab in Stage III Non-Small-Cell Lung Cancer. N Engl J Med. 2018;378(9):869-70.

45. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nature Reviews Cancer. 2012;12(4):252-64.

46. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved Survival with Ipilimumab in Patients with Metastatic Melanoma. New England Journal of Medicine. 2010;363(8):711-23.

47. Hammers HJ, Plimack ER, Sternberg C, McDermott DF, Larkin JMG, Ravaud A, et al. CheckMate 214: A phase III, randomized, open-label study of nivolumab combined with ipilimumab versus sunitinib monotherapy in patients with previously untreated metastatic renal cell carcinoma. Journal of Clinical Oncology. 2015;33(15).

48. Comin-Anduix B, Escuin-Ordinas H, Ibarrondo FJ. Tremelimumab: research and clinical development. Oncotargets and Therapy. 2016;9.

49. Ribas A, Kefford R, Marshall MA, Punt CJA, Haanen JB, Marmol M, et al. Phase III Randomized Clinical Trial Comparing Tremelimumab With Standard-of-Care Chemotherapy in Patients With Advanced Melanoma. Journal of Clinical Oncology. 2013;31(5):616-22.

50. Boland PM, Ma WW. Immunotherapy for Colorectal Cancer. Cancers. 2017;9(5).

51. Jenkins RW, Barbie DA, Flaherty KT. Mechanisms of resistance to immune checkpoint inhibitors. British Journal of Cancer. 2018;118(1):9-16.

52. Lee JJ, Sun WJ, Bahary N, Ohr J, Rhee JC, Stoller RG, et al. Phase 2 study of pembrolizumab in combination with azacitidine in subjects with metastatic colorectal cancer. Journal of Clinical Oncology. 2017;35.

53. Lonardi S, Andre T, Wong KYM, Morse M, McDermott R, Hill A, et al. Combination of nivolumab (nivo) 1 ipilimumab (ipi) in the treatment of patients (pts) with deficient DNA mismatch repair (dMMR)/high microsatellite instability (MSI-H) metastatic colorectal cancer (mCRC): CheckMate 142 Study. Annals of Oncology. 2017;28:3-.

54. Bendell JC, Powderly JD, Lieu CH, Eckhardt SG, Hurwitz H, Hochster HS, et al. Safety and efficacy of MPDL3280A (anti-PDL1) in combination with bevacizumab (bev) and/or FOLFOX in patients (pts) with metastatic colorectal cancer (mCRC). Journal of Clinical Oncology. 2015;33(3).

55. Shinozaki E, Kawazoe A, Kuboki Y, Komatsu Y, Nishina T, Hara H. Multicenter phase I/II trial of BBI608 and pembrolizumab combination in patients with metastatic colorectal cancer (SCOOP Study): EPOC1503. Journal of Clinical Oncology. 2018;36(15).

56. Overman MJ, McDermott R, Leach JL, Lonardi S, Lenz HJ, Morse MA, et al. Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study. Lancet Oncology. 2017;18(9):1182-91.

57. Androulla MN, Lefkothea PC. CAR T-cell Therapy: A New Era in Cancer Immunotherapy. Current Pharmaceutical Biotechnology. 2018;19(1):5-18.

58. Houot R, Schultz LM, Marabelle A, Kohrt H. T-cell-based Immunotherapy: Adoptive Cell Transfer and Checkpoint Inhibition. Cancer Immunology Research. 2015;3(10):1115-22.

59. KYMRIAH (tisagenlecleucel) 2018 [Available from: https://www.fda.gov/BiologicsBloodVaccines/CellularGeneTherapyProducts/ApprovedProducts/ucm573706.htm.

60. Marco L. Davila RJB. CD19-targeted CAR T cells as novel cancer immunotherapy for relapsed or refractory B-cell acute lymphoblastic leukemia. Clin Adv Hematol Oncol. 2016;14(10):7.

61. Bajgain P, Tawinwung S, D'Elia L, Sukumaran S, Watanabe N, Hoyos V, et al. CAR T cell therapy for breast cancer: harnessing the tumor milieu to drive T cell activation. Journal for Immunotherapy of Cancer. 2018;6.

62. Baybutt TR, Aka AA, Snook AE. Immunotherapy in Colorectal Cancer: Where Are We Now? Current Colorectal Cancer Reports. 2017;13(5):353-61.

63. Long GV, Atkinson V, Cebon JS, Jameson MB, Fitzharris BM, McNeil CM, et al. Standard-dose pembrolizumab in combination with reduced-dose ipilimumab for patients with advanced melanoma (KEYNOTE-029): an open-label, phase 1b trial. Lancet Oncology. 2017;18(9):1202-10.

64. Kirchberger MC, Hauschild A, Schuler G, Heinzerling L. Combined low-dose ipilimumab and pembrolizumab after sequential ipilimumab and pembrolizumab failure in advanced melanoma. European Journal of Cancer. 2016;65:182-4.

65. Aguiar PN, Santoro IL, Tadokoro H, Lopes GD, Filardi BA, Oliveira P, et al. The role of PD-L1 expression as a predictive biomarker in advanced non-small-cell lung cancer: a network meta-analysis. Immunotherapy. 2016;8(4):479-88.

66. Hopkins AM, Rowland A, Kichenadasse G, Wiese MD, Gurney H, McKinnon RA, et al. Predicting response and toxicity to immune checkpoint inhibitors using routinely available blood and clinical markers. British Journal of Cancer. 2017;117(7):913-20.

67. Yearley JH, Gibson C, Yu N, Moon C, Murphy E, Juco J, et al. PD-L2 Expression in Human Tumors: Relevance to Anti-PD-1 Therapy in Cancer. Clinical Cancer Research. 2017;23(12):3158-67.

68. Manson G, Norwood J, Marabelle A, Kohrt H, Houot R. Biomarkers associated with checkpoint inhibitors. Annals of Oncology. 2016;27(7):1199-206.

69. Hamid O, Schmidt H, Nissan A, Ridolfi L, Aamdal S, Hansson J, et al. A prospective phase II trial exploring the association between tumor microenvironment biomarkers and clinical activity of ipilimumab in advanced melanoma. Journal of Translational Medicine. 2011;9.

70. Kefford R, Ribas A, Hamid O, Robert C, Daud A, Wolchok JD, et al. Clinical efficacy and correlation with tumor PD-L1 expression in patients (pts) with melanoma (MEL) treated with the anti-PD-1 monoclonal antibody MK-3475. Journal of Clinical Oncology. 2014;32(15).

71. Baptista MZ, Sarian LO, Derchain SFM, Pinto GA, Vassal J. Prognostic significance of PD-L1 and PD-L2 in breast cancer. Human Pathology. 2016;47(1):78-84.

72. Van Allen EM, Miao D, Schilling B, Shukla SA, Blank C, Zimmer L, et al. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. Science. 2015;350(6257):207-11.

73. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science. 2015;348(6230):124-8.

74. Delyon J, Mateus C, Lefeuvre D, Lanoy E, Zitvogel L, Chaput N, et al. Experience in daily practice with ipilimumab for the treatment of patients with metastatic melanoma: an early increase in lymphocyte and eosinophil counts is associated with improved survival. Annals of Oncology. 2013;24(6):1697-703.

75. Di Giacomo AM, Calabro L, Danielli R, Fonsatti E, Bertocci E, Pesce I, et al. Long-term survival and immunological parameters in metastatic melanoma patients who responded to ipilimumab 10 mg/kg within an expanded access programme. Cancer Immunology Immunotherapy. 2013;62(6):1021-8.

76. Kelderman S, Heemskerk B, van Tinteren H, van den Brom RRH, Hospers GAP, van den Eertwegh AJM, et al. Lactate dehydrogenase as a selection criterion for ipilimumab treatment in metastatic melanoma. Cancer Immunology Immunotherapy. 2014;63(5):449-58.

77. Berman D1 PS, Siegel J, Chasalow SD, Weber J, Galbraith S, Targan SR, Wang HL. Blockade of cytotoxic T-lymphocyte antigen-4 by ipilimumab results in dysregulation of gastrointestinal immunity in patients with advanced melanoma. Cancer Immun. 2010;10:10.

78. Marthey L, Mateus C, Mussini C, Nachury M, Nancey S, Grange F, et al. Cancer Immunotherapy with Anti-CTLA-4 Monoclonal Antibodies Induces an Inflammatory Bowel Disease. Journal of Crohns & Colitis. 2016;10(4):395-401.

79. Schindler K, Harmankaya K, Kuk D, Mangana J, Michielin O, Hoeller C, et al. Correlation of absolute and relative eosinophil counts with immune-related adverse events in melanoma patients treated with ipilimumab. Journal of Clinical Oncology. 2014;32(15).

80. Shahabi V, Berman D, Chasalow SD, Wang LS, Tsuchihashi Z, Hu BH, et al. Gene expression profiling of whole blood in ipilimumab-treated patients for identification of potential biomarkers of immune-related gastrointestinal adverse events. Journal of Translational Medicine. 2013;11.

81. Schmid D, Park CG, Hartl CA, Subedi N, Cartwright AN, Puerto RB, et al. T cell-targeting nanoparticles focus delivery of immunotherapy to improve antitumor immunity. Nature Communications. 2017;8.

82. Dougan M, Dougan SK. Targeting Immunotherapy to the Tumor Microenvironment. Journal of Cellular Biochemistry. 2017;118(10):3049-54.

Figure legends:

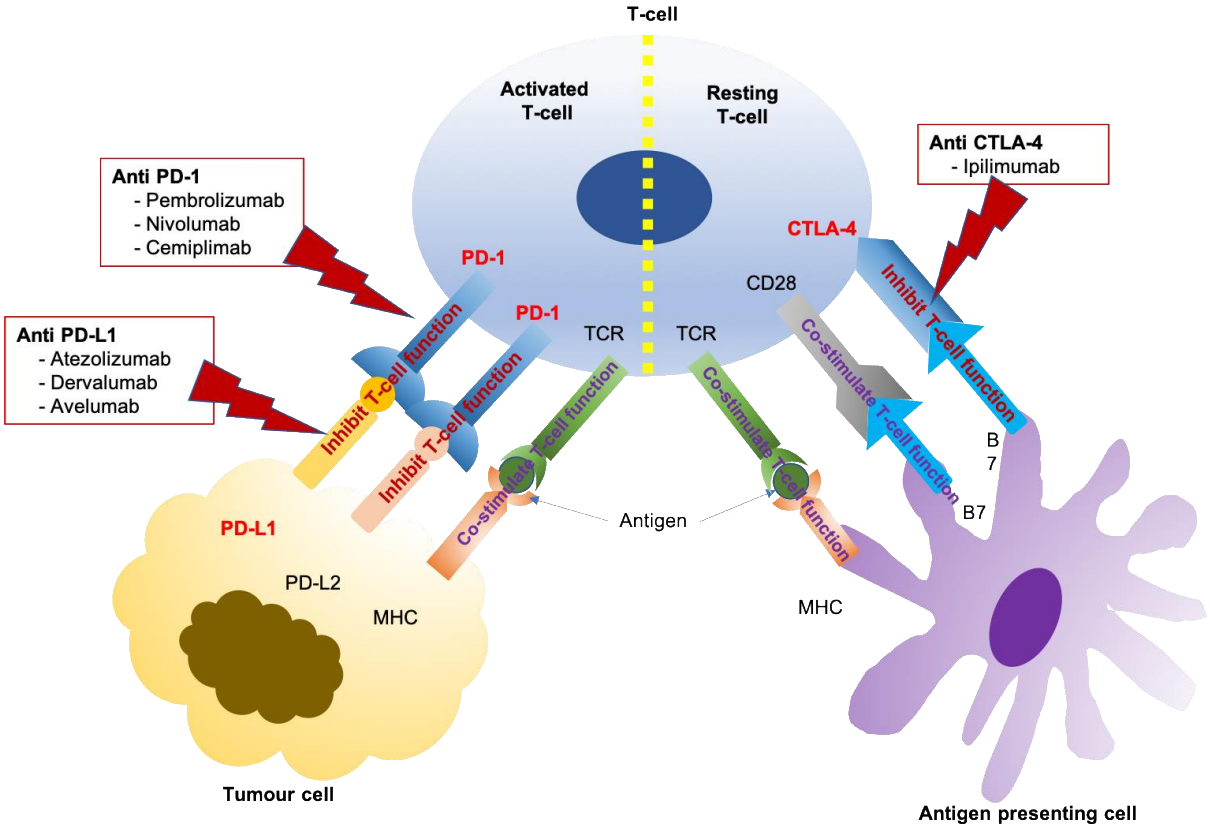

Figure 1. Immune checkpoint interactions that inhibit T-cells function and their inhibitors. Immune checkpoint, PD-1 on activated T-cell binds to specific receptors, PD-L1 and PD-L2, on tumour cells, whereas CTLA-4 on resting T-cell binds to B7 on antigen-presenting cells, both to inhibit T-cells function. Immune checkpoint inhibitors, anti PD-1, anti-PD-L1, and anti CTLA-4 can block these complexes to restore T-cells function.

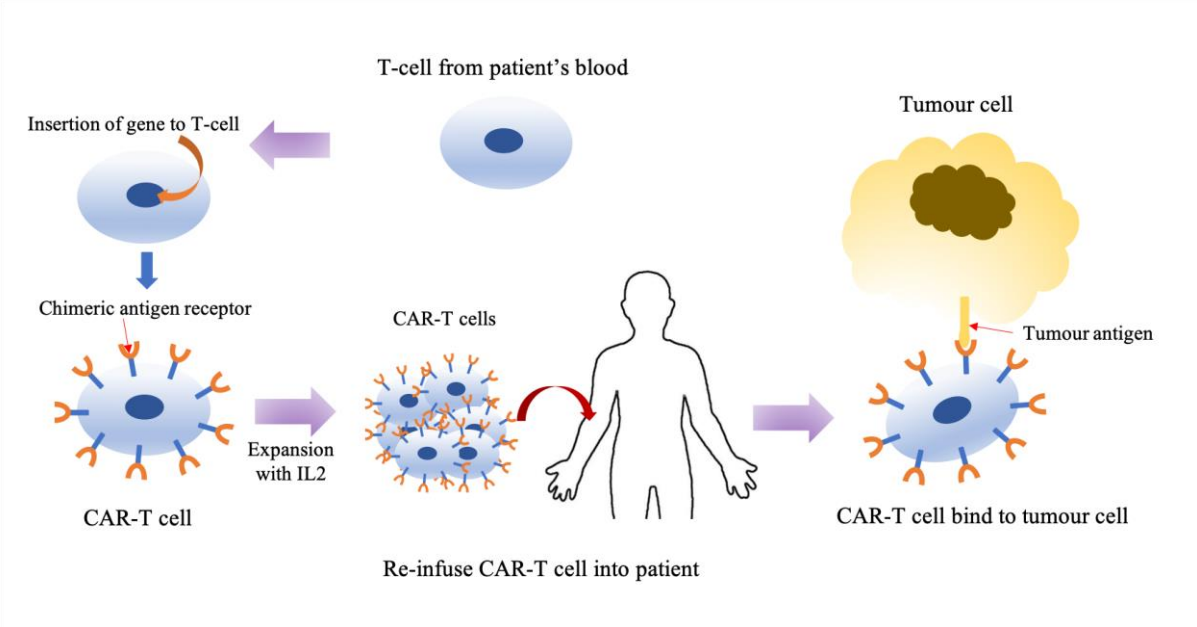

Figure 2. General principle of CAR-T cell therapy T-lymphocytes from patient's blood are engineered *in vitro* to express chimeric antigen receptor (CAR) to enhance T-cells binding to tumour antigens, after expansion with IL2 they are re-infused into patient to bind to tumour receptors and enhance killing of tumour cells.

Table 1. Summary of immune checkpoint inhibitors approved for multiple cancers.

Antibody	Drug name	Year	Approved for	Reference studies	Phase	Response rate	References
Anti PD-1	Pembrolizumab	2014	Advanced melanoma	KEYNOTE-006 (NCT01866319)	Phase III	33.7%	(18)
		2015	Advanced non-small-cell lung cancer (NSCLC)	KEYNOTE-001 (NCT01295827)	Phase I	19.4%	(19)
		2016	Recurrent or metastatic head and neck squamous cell carcinoma (HNSCC)	KEYNOTE-012 (NCT01848834)	Phase II	16.0%	(20)
		2017	Classical Hodgkin lymphoma (cHL)	KEYNOTE-087 (NCT02453594)	Phase II	69.0%	(22)
		2017	Metastatic urothelial carcinoma (mUCC)	KEYNOTE-052 (NCT02335424)	Phase II	38.0%	(23)
		2017	Non-resectable or metastatic MSI-H/dMMR	KEYNOTE-016 (NCT01876511)	Phase II		(24)
				KEYNOTE-164 (NCT02460198)	Phase II		(25)
				KEYNOTE-012 (NCT01848834)	Phase II	39.6% (Pooled)	(20)
				KEYNOTE-028 (NCT02054806)	Phase Ib		(26)
				KEYNOTE-158 (NCT02628067)	Phase II		(27)
		2017	Gastroesophageal junction (GEJ) carcinoma	KEYNOTE-059 (NCT02335411)	Phase II	13.3% (MSI-H 57%)	(28)
		2018	Recurrent or metastatic cervical cancer (RCC)	KEYNOTE-158 (NCT02628067)	Phase II	13.3%	(29)
		2018	Large B-cell lymphoma (PMBCL)	KEYNOTE-170 (NCT02576990)	Phase II	45.0%	(30)
	Nivolumab	2014	Metastatic melanoma	CHECKMATE-037 (NCT01721746)	Phase III	32.0%	(31)
				CHECKMATE-066 (NCT01721772)	Phase III	40.0%,	(32)
		2015	Metastatic non-small-cell lung cancer (NSCLC)	CHECKMATE-057 (NCT01673867)	Phase III	19.2%	(33)
		2015	Advanced metastatic renal cell carcinoma (RCC)	CHECKMATE-025 (NCT01668784)	Phase III	25.0%	(34)
		2016	Recurrent or metastatic head and neck squamous cell carcinoma (HNSCC)	CHECKMATE-141 (NCT02105636)	Phase III	13.3%	(35)
	Cemiplimab	2018	Metastatic cutaneous squamous cell carcinoma (CSCC)	NCT02383212	Phase I	52.0%	(36)
Anti PD-L1	Atezolizumab	2016	Advanced urothelial carcinoma	IMVigor210 (NCT02108652)	Phase II	23.5%	(38)
		2016	Metastatic non-small cell lung cancer (NSCLC)	OAK (NCT02008227)	Phase III	14%	(39)
				POPLAR (NCT01903993)	Phase II	14.3 months	(40)
	Avelumab	2017	Merkel cell cancer	NCT02155647	Phase II	31.0%	(41)
		2017	Urothelial carcinoma	NCT01772004	Phase Ib	16.5%	(42)
	Durvalumab	2017	Metastatic urothelial carcinoma (mUCC)	NCT02516241	Phase III	17.8%	(43)
		2018	Metastatic non-small cell lung cancer (NSCLC)	NCT02125461	Random	28.4%	(44)
Anti CTLA-4	Ipilimumab	2014	Metastatic melanoma	NCT00094653	Phase III	10.9%	(46)
		2018	Renal cell carcinoma	CHECKMATE-214 (NCT02231749)	Phase III	42%	(47)

Table 2. Potential biomarkers associated with immune checkpoint inhibitors treatment response and toxicity.

Samples	Biomarkers	Antibody	References
Potential biomarkers associated with treatment response			
Tumour tissues	Tumour infiltrating lymphocytes (TILs)	Anti CTLA-4	(60)
	PD-L1 expression	Anti PD-1	(61,62)
	Mutational load	Anti CTLA-4	(6)
		Anti PD-1	(64)
Blood	Circulating leukocytes	Anti CTLA-4	(65)
	Neutrophil-to-lymphocyte ratio (NLR)	Anti CTLA-4	(66)
	Lactate dehydrogenase (LDH) level	Anti CTLA-4	(67)
Potential biomarkers associated with treatment toxicity			
Tumour tissues	Neutrophil infiltration	Anti CTLA-4	(68,69)
Blood	Eosinophil level	Anti CTLA-4	(70)