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Reinstating endogenous antitumor immunity: The concept of therapeutic management of cancer

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Abstract: Strong evidence points to the role of cancer immunoediting and tumor immune infiltrates in regulating cancer progression. By understanding the immune tumor microenvironment, we can now target key pathways that suppress endogenous antitumor responses, thereby re-instating such immune responses and identifying novel targets for immune therapies. Therapies targeting oncogenic pathways and checkpoint blockades turn on a new paradigm shift in immune-therapy for cancer with remarkable clinical efficacy seen in various malignancies. However, a lot of cancer patients will fail to respond and therefore, it becomes crucial to identify biomarkers to predict who of the patients will most likely benefit from these therapies.

Keywords: *Cancer immunotherapy • Tumor infiltrating lymphocytes • Immunoscore • Cancer vaccines • Checkpoint inhibitors*

Introduction

There is now increasing evidence to suggest that adaptive immunity plays a major role in regulating the growth of tumour cells. T cells play a major role in coordinating the immune response against tumour-specific antigens during tumour progression. While T cell activation depends on the initial tumour antigen-specific signal provided to the T cell receptors via the antigen-loaded major histocompatibility complex (MHC) complex on dendritic cells (DCs), additional signals provided by costimulatory molecules fine-tune this response, determining its strength, nature and duration. To this end, the discovery of receptors regulating T cell activation against the autologous tumour was of paramount importance for understanding how cancer progresses under immunosurveillance. The CD28 co-receptor acts as a strong positive costimulatory receptor, and CTL antigen-4 (CTLA-4) as a potent co-inhibitory receptor. The programmed death 1 (PD-1) receptor: PD-Ligand (PD-L) pathway is another major receptor–ligand network that functions primarily to provide a co-inhibitory signal. PD1: PD-L interactions maintain peripheral tolerance and are exploited by tumours to evade immune eradication by memory T cells specifically recognizing tumour peptides [1]. As such, this pathway has emerged as a potential therapeutic target for enhancing the immune response.

A second important discovery, which also sheds light to our understanding of tumour evolution, is presented by the "immunoediting" theory. According to this theory, the immune system "edits" the tumour immunogenicity, resulting in the promotion or suppression of tumour growth, a phenomenon [2]. As a result of its capacity to shape tumour immunogenicity, an additional role for the immune system has emerged, namely that of prognostic indicator. Studies by Galon et al. [3], initiated almost a decade ago, have demonstrated that the quantity, quality and spatial distribution of immune cells within the tumour has a greater prognostic value than the standard tumour staging based on tumour burden, infiltration of draining and regional lymph nodes by tumour cells, and evidence of metastases. This so-called "immunoscore" came to complete the immunoediting theory by increasing the knowledge of the immune events inside the tumours, and by better understanding, the immune architecture of these tumours, as well as the functional programs of their constituents, all of which complete the idea of how tumours evade from immunosurveillance.

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The knowledge obtained from the above theories in the field of cancer immunology helped to identify new targets for the development of therapeutic strategies. In this review, we will discuss the basic concepts in cancer immunology, and their translation into the clinic by means of anticancer therapies aiming to stimulate the endogenous antitumour responses, thus providing the concept of the therapeutic management of cancer.

The pre-existent antitumour immunity as the basis for "immunoediting" and "immunoscore"

The pre-existent or endogenous antitumour immunity during cancer evolution constitutes the concept of cancer immunomodulation, as described in the "immunoediting" theory by Schreiber [4]. The immunoediting process is based on the knowledge obtained from progresses in our understanding of mechanisms regulating tumour cell immune recognition and immune evasion. During immunoediting, elements of the innate and adaptive immune system initially eliminate immunogenic tumour cells (elimination phase). Then comes a rather long period during which the immune system continuously interacts with the tumour, establishing a dynamic state of equilibrium which keeps the tumour cells in a dormant state. Thus, tumour immune surveillance is a major component of a long, or even permanently, lasting tumour dormancy, however, only when tumour cells are immunogenic. The equilibrium phase will progressively fade in the presence of epigenetic alterations, significantly affecting the biology of tumour cells, making them less immunogenic, highly suppressive, with a high angiogenic output. This may seriously impact the balance between the effector and regulatory cell compartments by favouring the infiltration and accumulation of regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSC) within tumours. In this way, the effector T cells that do infiltrate the tumour will be negatively controlled by these regulatory cellular subsets and inhibitory molecules. The outcome of this dysregulated balance between effector and regulatory cells is critical for the tumour to escape immune control. The immunoediting hypothesis provided an immune-mediated control of tumourigenesis by postulating opposing host-protective and tumour-promoting functions of the immune system. Based on this theory, studies conducted later on confirmed the role of endogenous adaptive antitumour immunity, both as prognostic and predictive biomarkers. To this end, it was demonstrated that the immune contexture (presence, location and density of T cells and cytokines within tumours) is related with a favourable prognosis, hence, emphasizing the ability of the immune response to maintain a sub-clinical tumour in equilibrium state [5–7]. Accordingly, it has been suggested that the predominance of immune infiltrates intratumourally, could be a reliable indicator for clinical outcome. This would suggest that once human cancer becomes clinically detectable, the adaptive immunity has a crucial role in preventing tumour progression. This is mainly due to the ability of effector memory T cells to recall previously encountered antigens which result in their expansion, so as to provide an effective and a protective immune response. Memory T cells have a long-lasting antitumour capacity, which could result in long-term immunity in human cancers. In addition to its prognostic significance, immunoscore could also play an important role as a biomarker for predicting clinical responses to conventional cancer treatments, as well as to novel immunotherapies targeting the immune checkpoints [8]. Thus, exploring and understanding the fundamental role of the endogenous intratumoural immune reaction could have important consequences in clinical cure of cancer.

Immune checkpoint inhibition – Basic aspects

It is well known that the process of T-cell activation consists of two major steps. In the first step, T-cell receptor (TCR) recognises the antigen in the context of MHC expressed on DCs; and in the second step, costimulation in the form of TCR interactions between T cell and DCs takes place [9–11]. This second step of costimulation is controlled by a number of "checkpoints" [10]. The two prominent checkpoints are CTLA4 and PD1 molecule. CTLA4 is expressed on the cell surface of T cells and interacts with costimulatory molecules B7-1 (CD80) and B7-2 (CD86), which are expressed by DCs. Functionally, CTLA4 competes with the T-cell costimulatory molecule CD28 [12], thus, negatively regulating T-cell activation. Ipilimumab is a fully humanised monoclonal antibody (mAb) that binds to and inhibits the function of CTLA4 [13]. Preclinical studies with CTLA4, examining its role as a Tregs costimulation, were followed by clinical development. As a result, ipilimumab was the first agent to be proven to prolong OS (Overall Survival) in patients with metastatic melanoma and achieved Food and Drug Administration (FDA) approval for this treatment indication.

The increased knowledge of immune checkpoint biology led to the development of antagonists of PD-1 and one of its ligands, PD-L1 with marked clinical efficacy. Following chronic T-cell activation, the inhibitory receptor PD-1 is induced on T cells and the expression of one of its ligands PD-L1 on macrophages and the tumour

cells can offer protection from immune destruction [14]. As a result, targeting either PD-1 or PD-L1 offers an opportunity to disable a major mechanism of tumour-mediated immune evasion. Nivolumab, the first anti-PD-1 fully human antibody to be developed, demonstrated impressive single-agent activity in heavily pre-treated patients, particularly in those with melanoma, kidney cancer and non-small cell lung cancer (NSCLC) [15].

Pembrolizumab, a humanised antibody, was the second anti-PD1 antibody to enter large-scale clinical trials [14]. Pembrolizumab demonstrated remarkable activity in patients with advanced-stage melanoma, both in patients previously treated with ipilimumab and in those who were not treated with ipilimumab [16]. Both nivolumab and pembrolizumab have received FDA approval for the treatment of advanced NSCLC and advanced melanoma.

Targeting PD-L1 is a promising approach as targeting PD-1. However, targeting PD-L1 may result in different biological effects than targeting PD-1. In addition to binding PD-1, PD-L1 is also believed to exert negative signals on T cells by interacting with B7 [17]. PD-L1–blocking antibodies prevent this interaction, but PD-1-blocking antibodies do not. Another slight difference is that PD-L1 antibodies do not prevent PD-1 from interacting with PD-L2, although the effect of this interaction remains unknown. BMS-956559 was the first PD-L1 antibody to show objective tumour responses in patients with a variety of solid tumours [18]. Atezolizumab (anti-PD-L1) has granted accelerated drug designation status by the FDA for advanced squamous NSCLC, while tremelimumab (MEDI4736) lies at a lesser advanced stage of its development.

Predictive biomarkers in immune checkpoint immunotherapy

PD-L1 expression

PD-L1 expression can be induced by tumour antigen-specific T cells [19]. Thus, the expression of PD-L1 can be considered as a dynamic process during the effective activation of T-cells during immunotherapies. PD-L1 is expressed on tumour cells and/or tumour infiltrating macrophages (TIM). Studies have demonstrated that PD-L1 expression on these types of cells can predict the response to PD-1/PD-L1 checkpoint inhibition [20–24]. However, not all tumors express simultaneously PD-L1 both on tumor cells and TIM. So, it is logical to ask which of these is more important in predicting the response? PD-L1 expression by tumour cells was correlated significantly with clinical benefit to anti-PD-1 therapy, while the correlation of PD-L1 expression by TIM with objective response rates (ORRs) did not reach statistical significance in multiple cancer types [24]. However, in metastatic bladder cancer, PD-L1 in TIM proved to be the most predictive for the response to an anti-PD-L1 antibody [22]. In addition, in colon cancers with microsatellite instability (MSI), PD-L1 was found to be expressed predominantly in TIM, rather than tumour cells [21]. The association of PD-L1 expression on TIM with treatment response to the anti-PD-L1 antibody atezolizumab reached the statistical significance, while the association with PD-L1 expression on tumour cells was not observed in several solid cancers [20]. In contrast, in NSCLC, PD-L1 expression on both tumour cells and TIM was clearly shown to identify patients with improved OS and PFS (Progression Free Survival), and from atezolizumab treatments [25]. The expression of PD-L1 can be induced by activated tumour antigen-specific T cells [19].

PD-L1 can also be expressed during the tyrosine kinase inhibitors (TKI)-targeted therapy [26]. In pre- and post-TKI biopsies in the epidermal growth factor receptor-mutant and anaplastic lymphoma kinase-positive metastatic NSCLC, the expression levels of PD-L1 were increased due to TKI therapy [26]. However, taking into consideration this dynamic expression of PD-L1, its evaluation at a single time point may not accurately reflect an evolving immune response, or predict the response to PD-1/PD-L1 pathway blockades.

It is also difficult to decide the cut-off values to define the positive rate of PD-L1 staining. Different cut-off values may lead to a difference in predicting function. As outlined above, the expression of PD-L1 relates to the activation status of tumour infiltrating T cells, which depends on their interaction with the surrounding tumour cells. Therefore, it is difficult to decide the cut-off value according to different cancers because there are so many kinds of cancers and various biological properties in the same cancer, as seen in the case of NSCLC consisting of a variety of mutations.

Mutational landscape

The presentation of self-antigens in the thymus may result in the elimination of high-avidity T cells, and therefore, mutant neoantigens, which are recognised as “foreign” should be more immunogenic. The combined application of mass spectrometry with whole exome sequencing has enabled the comprehensive characterisation of somatic mutations in tumour samples coding for such neoantigens [27]. Melanomas and lung cancers display high non-synonymous mutations per tumour [28], and ongoing efforts are being made to employ mutational landscape to identify candidate patients who will benefit

from checkpoint blockade immunotherapy. In melanoma and NSCLC, a high mutational burden is correlated with sustained clinical benefits [23, 29]. The possible explanation for association between mutation burden and efficacy of checkpoint blockade is that tumour antigens as a consequence of somatic mutations, functions as the target of T cells activated by checkpoint blockade immunotherapy [23, 30]. In a mouse model, it was confirmed that pre-existent immunity against tumour-specific mutation antigens was reinvigorated by anti-PD-1 and anti-CTLA-4 therapies [30]. The mutation landscape has an important impact on the understanding of response to PD-1/PD-L1 blockades. However, there are limitations for using mutation landscape to identify potential patients. First, there were tumours with higher non-synonymous mutations that did not respond to checkpoint blockades [23, 31, 32]. Second, the mutation frequency varies in diverse cancers [33], and even in one cancer type it is influenced by the degree of exposure to the environment mutagens [27]. The large variability of somatic mutation makes it difficult to set a particular cut-off point for mutation burden to predict the response to immunotherapy based on checkpoint blockade. Third, the whole-exome sequencing analysis yields too many candidate mutant peptides, which makes the process of immunogenicity testing laborious.

Clinical implications of immune checkpoint blockade: Developments beyond melanoma and NSCLC

Immune checkpoint inhibitors became clinically relevant in 2010, when ipilimumab, an anti-CTLA4 mAb, was found to offer survival benefit in metastatic melanoma patients. This paved the way for an unprecedented rate of development, not only for CTLA-4 blockade, but for PD-1 axis as well, given that these agents have been tested in the recent years in almost all human solid malignancies.

Colorectal cancer

There is a growing body of evidence that colorectal cancer (CRC) can be amenable to immune modulation. CRC is associated with a relatively high mutation burden, similar to gastric, head and neck cancer. Furthermore, as in other immune-responsive cancers, it seems that there is a correlation between increased lymphocytic infiltration by Th1 cytotoxic T cell and better prognosis [3].

According to the proposed molecular taxonomy, 14% of CRCs exhibiting MSI are related to BRAF mutations and worse survival at relapse. Tumours with deficient mismatch repair proteins (dMMR) are rich in mutations that are recognised as neoepitopes when presented to the immune system. It seems logical that this subtype of CRC would exhibit a higher stimulating capacity for T cell activation and should be more prone to immune therapeutic modalities [34–36].

In a recently published phase II trial with pembrolizumab, 40% of CRC patients with dMMR (deficient mismatch repair) had a partial response (PR) (4 out of 10), while at the same trial, none of the patients with MMR proficient cancer showed response. Disease control rate reached 90% in dMMR patients versus only 11% in patients with MMR-p disease, and ORR was 71% for the patients with MSI-H non-CRC versus 0% for the MSS CRC. Following these results, a phase III trial has been conducted, which is still ongoing, testing pembrolizumab versus chemotherapy in MSI-H or dMMR stage IV CRC (Keynote – 177) as a first-line therapy (EudraCT no.: 2015-002024-89) [37].

Gastric cancer

This type of cancer can be divided into four distinct molecular subtypes, with two of them, the MSI-related (20%) and the Epstein–Barr virus-related (10%) types, considered as more immunogenic. In the phase I trial of pembrolizumab for gastric cancer (Keynote-012), 40% of the screened patients (65 of 162) were eligible for enrolment based on PD-L1 positivity. Among the 39 patients who received the drug, 22.2% showed PR. A correlation was also observed between PD-L1 expression and overall response. Various phase II trials and a phase III trial versus paclitaxel chemotherapy are ongoing [38, 39].

Anal cancer

During the 2015 European CanCer Organisation/ European Society for Medical Oncology symposium, the efficacy results from another trial of pembrolizumab in PD-L1 positive squamous anal cancer patients were reported. ORR reached 20% in a heavily pre-treated patient population, while disease control rate was 64%. The more promising result of this trial was the duration of the response, which has not been reached yet, with three out of five responses ongoing at the time of analysis [40].

Pancreatic cancer

Cancers of the pancreas are highly suppressive for the immune system. TILs (Tumor-Infiltrating Lymphocytes) are rarely found within pancreatic tumours, thus posing an impetus for immunotherapeutic approaches. Given

these facts, it seems rather pointless to investigate immunotherapeutic approaches outside the frame of a combinatorial strategy. Vaccine therapy offers a sensitisation of immune cells, and in pancreatic cancer, at least two vaccines have reached a phase II trial development: (i) GVAX, an irradiated whole-cell modified vaccine composed of two irradiated pancreatic cell lines engineered to express granulocyte/macrophage-colony stimulating factor (GM-CSF) and (ii) CRS-207, a live-attenuated *Listeria monocytogenes* vaccine. After the initial encouraging results, these vaccines are now being tested in combination (GVAX+CRS-207) with or without nivolumab, in previously treated metastatic pancreatic cancer patients (https://clinicaltrials.gov/ct2/show/NCT02243371)) [41,42].

Over expression of PD-L1 is related to poor prognosis for hepatocellular cancer patients. Results from a phase I/II trial testing nivolumab in patients with advanced HCC that failed or were intolerant to sorafenib were quite encouraging. With clinical response evaluation in 39 patients, there were 2 complete responses and 7 partial remissions. The ORR (20%) was comparable to that of sorafenib. Complete response duration exceeded 17 months. Dose limiting toxicities within the expected range from what is known with previous nivolumab experience were reported [43].

Ovarian cancer

Ovarian cancer harbours a low burden of mutagens in relation to other solid tumours, but the increased levels of TILs are associated with improved prognosis. Emerging clinical data indicates that targeting the PD-1/PD-L1 axis may be a promising strategy in ovarian cancer. In phase I trial with pembrolizumab, 22% of the 17 treated patients had evidence of an objective response or stable disease lasting at least 24 weeks. In another study with nivolumab treatment, total disease control rate was 45% and larger studies using these agents are currently underway. Administration of avelumab, an anti PD-L1 antibody in the setting of a phase Ib trial, resulted in similar results (17% PR and 48% stable disease). Therapies aiming at enhancing T cell activation, such as adoptive cell therapies and vaccination, have also been used, albeit with limited clinical efficacy. A small phase II study, using DCs presenting mucin 1, showed a modest clinical activity that was measured as a response to Ca-125 tumour marker values [15, 44–48].

Renal cancer

Renal cancer therapy was for many years exclusively dedicated to immunotherapy, with some infrequent but durable responses observed after administration of high doses of IL-2, an approved therapy since 1992. During the last decade, several targeted therapies including the five vascular endothelial growth factor (VEGF) inhibitors (bevacizumab, sunitinib, sorafenib, axitinib and pazopanib) and two mTOR inhibitors (everolimus and temsirolimus) have been tested in phase III trials with positive results, and were granted approval. In a recently published trial, nivolumab was tested against everolimus in patients with previously treated advanced renal cell cancer. Nivolumab treatment resulted in longer overall survival (OS) and was related to fewer serious adverse events. The median OS was 25 months in the nivolumab group, versus 19.6 months in the everolimus group (HR (Hazard Ratio) 0.73, p = 0.002). The benefit with nivolumab was observed irrespective of PD-L1 expression. Grade 3 or 4 adverse events occurred in 19% of patients treated with nivolumab and in 37% of patients treated with everolimus. Following these results, the FDA granted approval for use of nivolumab in patients with advanced renal cell cancer, who have received prior therapy [49].

Urothelial cancer

Bacillus Calmette-Guerin, derived from attenuated mycobacterium bovis, has been for long the mainstay of local (intravesical) treatment for superficial bladder cancer, as it induces a robust inflammatory response that eventually results in tumour regression. Recently, the PD-L1 inhibitor atezolizumab was tested in a phase II trial. In a platinum pre-treated patient population, 12 patients achieved complete response (CR) and 35 PRs. Ninety-two per cent of the responding patients maintained response at a minimum follow-up of 24 weeks, with a median PFS of 2.1 months across all groups. Preliminary data suggested higher PD-L1 expression on immune cells associated with higher overall response rates and better survival. Updated results confirmed an ORR of 26% in the cohort with more intense expression compared to 9.5% of participants who were classified as “negative” for PD-L1 expression. After a median follow-up of 11.7 months, ongoing responses for 84% of responders were reported. Following this encouraging data, atezolizumab was granted FDA approval for the treatment of advanced/metastatic urothelial cancer (FDA immediate release press announcement 18 May 2016, www.fda.gov).

In the urothelial cohort of Keynote – 012 trial of pembrolizumab in PD-L1+ urothelial cancer patients, the overall response rate was 28%, with 3 out of 29 patients achieving CR (10%). Half of the patients were alive at 12 months, and the response duration ranged from 8 to more than 64 weeks. Based on these results, a phase III trial of pembrolizumab versus chemotherapy, and a

phase II trial for pembrolizumab as first line for patients ineligible to cisplatin are ongoing [50–52].

Incorporating vaccines into clinical practice

DC-based therapies

The major function of DCs is to process and effectively present cancer antigens in the context of major histocompatibility complex molecules to effector T cells, bearing appropriate receptors. The advantage of using DCs cell-based vaccines is that DCs are patients' autologous cells that are primed ex vivo, implying that the vaccine would be immunologically compatible with any patient who undergoes the process [53]. The only currently approved DC vaccine is sipuleucel-T, for the treatment of advanced castrate-resistant prostate cancer. It is an autologous DC targeting prostatic acid phosphatase (PAP). The procedure in sort includes patient's blood leukapheresis, and then ex vivo exposure to PAP fused with GM-CSF and re-infusion. The clinical efficacy of the vaccine versus placebo was tested in 341 asympromatic men with CRPC (castration-resistant prostate cancer) and at 36.5 months median follow-up showed a 4.1 months median survival benefit (HR for death 0.759, p = 0.017). Following these results, sipuleucel-T gained approval from European Medicines Agency and FDA in 2010. The immunogenic effect of vaccination based on T-cell proliferation and interferon gamma (IFNγ) was evident in 80% responders, versus 13% for the placebo arm. There was also evidence of cross priming with other tumour antigens, which can act as possible candidates for further testing. The administration was safe and well tolerated with only mild side effects [54 –56]. This is also the method for the HER2-based breast cancer vaccine lapuleucel-T (Neuvenge, Dendreon Corp), which has been used in early clinical trials [57]. Another DC vaccine approach being explored in breast cancer uses leukapheresed APCs (antigen presenting cells) transfected with a replication of defective adenoviral vector loaded with wild-type p53 transcript [58]. The viral infection triggers the activation of the DCs, and because p53 is made in the DC cells, the p53 is processed and presented on the surface. These DCs can trigger p53-specific effector T-cell responses against cancer cells with abnormally accumulated mutant p53.

Recombinant virus vaccines

Viral-based vaccination vectors represent an active field of investigation in cancer immunotherapy. The selection of viral vectors makes use of their natural ability to trigger immune responses and carry genetic material into cells for production of the target antigen. The antigens are processed intracellularly by APCs, and are presented on major histocompatibility complexes to receptive effector T cells. A vaccine that has shown a survival advantage in metastatic castrate-resistant prostate cancer is prostate-specific antigen (PSA)-TRICOM. This vector (vaccinia and fowl pox viruses)-based vaccine expresses transgenes for PSA and three T-cell costimulatory molecules (B7.1, ICAM-1, LFA-3) collectively designated as TRICOM. The PSA-TRICOM vaccine showed an 8.5 month improvement in overall survival relative to placebo (p = 0.006) in a multicenter randomised phase II trial [59]. In a similar but smaller trial at the NCI, PSA-TRICOM was shown to generate an antigen-specific immune response, which was associated with favourable survival outcomes [60]. A multi-centre phase III trial of PSA-TRICOM in metastatic castrate-resistant prostate cancer is underway (http://www.bavariannordic.com/pipeline/prostvac.aspx). A similar study in metastatic breast cancer utilised the same viral vectors loaded with a carcinoembryonic antigen (CEA) peptide and TRICOM. In a completed phase I trial, 40% of patients had stable disease and one patient developed a pathologic CR [61, 62].

Oncolytic virus vaccination

Oncolytic viruses can be divided into two major classes: one with an intrinsic property to preferentially replicate inside tumour cells, while leaving normal cell uninfected, and the genetically engineered viruses that encompass genes promoting tumour-tropism and replication of the viruses within the tumour cells [63]. Each oncolytic virus normally leads to a specific cell death pathway. The most effective delivery of oncolytic viruses is a direct injection inside the tumour [64].

The major clinical development of these agents is represented by talimogenelaherparepvec (T-VEC), which utilises a weakened herpes simplex virus type 1, to over express GM-CSF that promotes DCs-mediated antigen presentation and enhances the systemic antitumour immune responses. The virus is injected intratumouraly and is designed to selectively replicate within tumours, having the ICP34.5 and ICP47 genes deleted in order to enhance tumour-selective replication and immunostimulation [65]. In a randomised phase III trial, T-VEC was compared with GM-CSF alone in patients with unresected stage IIIB to IV melanoma. The primary end point was durable (≥6 months) response rate (DRR), with OS and ORR as key secondary end points. Intratumoural injections of T-VEC produced an

improved DRR (16.3%, odds ratio 8.9; $p < 0.001$) and a longer median OS of 23.3 months versus 18.9 months with only GM-CSF ($p = 0.051$), with a 3-year survival rate of 41% versus 28% for the GM-CSF arm. These encouraging results eventually led to the first of this kind of therapy, for which it was granted FDA approval on October 2015 [66].

The success of T-VEC has widened the range of clinical applications. There are already ongoing clinical trials combining HSV (herpes simplex virus) with ipilimumab and pembrolizumab. HSV is also being tested against other tumour types, like glioblastoma, where a phase Ib clinical trial is under way. Numerous other virus backbones are under clinical or preclinical development with viruses like adenovirus, reovirus, vesicular stomatitis virus and Newcastle disease virus [67, 68].

The AE37 therapeutic cancer vaccine paradigm

Vaccines targeting HER-2/neu have been tested in preclinical/mouse/tumour models as well as in human clinical trials, and have shown that significant levels of T-cell HER-2/neu immunity can be generated with active immunisation [69]. Holmes et al. [70] conducted a phase I study of the AE37 hybrid peptide in HER-2/neu+ breast cancer patients and showed strong immunologic responses to the vaccine. In the phase I trial, 29 prostate cancer patients were immunizsed with AE37 plus GM-CSF as adjuvant. For 6 months, the vaccine was given monthly and was well-tolerated with minimal toxicity. AE37 induced strong immunological responses in vivo (delayed-type hypersensitivity) and in vitro (IFNγ production) and it could be measured in majority of the patients. Long-term immunity to AE37 was still detectable 6 months post-vaccinations, and it could be considerably prolonged for an additional period of 36 months after one single booster AE37 injection [71–73]. AE37 has also been utilised to vaccinate breast cancer patients in a randomised phase II trial. In the trial, 301 patients with node positive or high risk node negative and with any level of HER2 expression were enrolled to receive AE37+ GM-CSF or only GM-CSF vaccine in a 1:1 randomisation order, following the standard care therapy. The vaccine was found to be safe and well tolerated, and demonstrated benefits in patients with HER2 non-over expressing tumours, especially those with triple negative disease, where the 5-year relative risk reduction for recurrence reached 35%. In a separate sub study conducted in our laboratory, we found that pre-existing immunity either as in vitro (levels of IFNγ) or in vivo (dermal reaction) parameter, might serve as a predictive biomarker for clinical response in patients vaccinated with AE37 [74–76].

Table 1 summarises the most important phase II and phase III vaccine trials.

Table 1. Clinical trials using various therapeutic vaccines.

STUDY NAME	TARGET ANTIGEN	VACCINE NAME/TYPE	CANCER
IMPACT	PAP	Mo/DCs PAP-GM-CSF	mCRPC
PROSPECT	PSA	PSA-TRICOM	mCRPC
Stimuvax	MUC1	BLPD25	NSCLC
PANVAC	MUC1/CEA	DCs PSA-TRICOM	Colorectal
GVAX	multiple	T47D&SKBR3 lines	Breast
DERMA	MAGE-A3	Rec Protein	Melanoma
MAGRIT	MAGE-A3	Rec Protein	NSCLC
IMPRINT	11 tumour peptides	soluble	Renal
TG4010	MUC1	MVA MUC-1-IL-2	Prostate NSCLC
CDX110	EGFRvIII	Soluble 13-mer	Glioblastoma

PAP: prostatic acid phosphatase, PSA: prostatic specific antigen, mCRPC: metastatic castrate-resistant prostate cancer, NSCLC: non-small cell lung cancer, Mo/Ds: monocyte/dendritic cells, TRICOM: triad costimulatory molecules, MUC1: mucin 1, MVA: modified vaccinia Ankara virus

Combination of checkpoint blockade with other therapeutic modalities

It may be speculated that the efficacy of checkpoint blockade may be limited in two circumstances. On one hand, the high tumour burden can exert a suppressive load on the immune system that cannot be overcome by checkpoint blockade alone. On the other hand, there are many malignancies that are not intrinsically immunogenic, and thus, are not considered to be good candidates for immunotherapy. In these scenarios, conventional cancer therapies can reduce tumour burden and cause a release of tumour antigens after tumour cell death that may favour the conversion of a tumour from a nonimmunogenic to an immunogenic state (vaccine effect) after relieving the suppressive pressure. In melanoma patients, both radiotherapy and treatment with BRAF inhibitors have shown to be good candidates for combination with ipilimumab [77, 78]. A large number of clinical trials are currently evaluating the combination of radiotherapy not only with checkpoint blockade but also with a broad range of immunotherapeutic approaches against immunogenic and nonimmunogenic malignancies [reviewed in Ref. 79]. In tumours that have been considered refractory to

checkpoint blockade, such as gastrointestinal stromal tumours, promising results have been observed when the TKI imatinib is combined with CTLA-4 blockade in mouse models [80]. In this case, the mechanisms underlying the synergistic effects of the combination not only included a possible vaccine effect after imatinib treatment, but also the activation of cytotoxic T cells and depletion of Tregs within the tumour.

The VEGF has been recognised as a critical mediator of immune suppression, suggesting that VEGF blockade, which has proven effective for the treatment of several cancers, may have a favourable impact on the antitumour immune response in addition to its direct effects on the tumour vasculature. Studies on tumour samples obtained from advanced melanoma patients after treatment with ipilimumab revealed immune-mediated vasculopathy associated with tumour necrosis and heavy infiltration with mononuclear cells [81]. These findings indicate that CTLA-4 inhibition may directly modulate tumour vessels in addition to its effect on the activation of T cells [81]. Moreover, high serum levels of VEGF are associated with decreased overall survival in advanced melanoma patients treated with ipilimumab, suggesting that sVEGF levels may predict outcomes after immune checkpoint inhibition [82]. Combined therapy with ipilimumab and bevacizumab in melanoma patients resulted in encouraging antitumour activity and had beneficial effects on the host's antitumour immune response, including increase of memory cells in the peripheral blood, increased effector cell trafficking and enhanced antibody responses to galectins. Many trials are underway exploring the concept of checkpoint inhibition in combination with angiogenesis inhibition, including VEGF blockade and inhibition of novel angiogenesis targets, such as angiopoietin 2. These include bevacizumab combined with (i) ipilimumab in melanoma (NCT00790010, NCT01950390), (ii) MPDL-3280A in various solid tumours (NCT01633970), (iii) nivolumab in RCC (renal cell carcinoma) and NSCLC (NCT02210117, NCT01454102) and (iv) pembrolizumab in RCC, NSCLC, Glioma and melanoma (NCT02348008, NCT02039674, NCT02313272, NCT02141542)

The idea that chemotherapy can be used in combination with immunotherapy is very attractive, and multiple studies have demonstrated that in addition to direct cytotoxic effects on cancer cells, some chemotherapeutic agents may actually promote immunogenic cell death and activation of antitumour immune response [83]. The pro-immunogenic effects of chemotherapy have been demonstrated to be mediated by a variety of mechanisms, which may differ depending on the agent used. Just as radiation, conventional chemotherapies have also demonstrated the release of antigens and DAMPs (damage associated molecular pattern molecules), thus triggering immunogenic cell death [84]. In addition to the effect on cancer cells, chemotherapeutic agents exert various effects on the host immune system. Some agents such as gemcitabine, 5-FU and taxanes have been shown to reduce the number of tumour-infiltrating and splenic MDSCs, while selective depletion of Tregs has been demonstrated with cyclophosphamide in several studies [84, 85]. These studies provide a rationale for the exploration of chemotherapy in combination with antibodies targeting costimulatory and co-inhibitory receptors. Indeed, ipilimumab and nivolumab have been explored in combination with chemotherapy in several trials [86–89]. Although in some studies, the combination appears to be well-tolerated [86], in others, the combination has been associated with increased toxicity, particularly noted in the combination of nivolumab with platinum-based chemotherapies in NSCLC, where grades 3–4 treatment-related adverse events were reported in 45% of patients [89].

Multiple preclinical studies have demonstrated synergistic efficacy of autologous modified tumour cellular vaccines with immunomodulatory antibodies [90]. This strategy has been explored in clinical trials, where allogeneic cancer cells transfected with GM-CSF (GVAX) have been evaluated in patients with metastatic pancreatic cancer and hormone-refractory prostate cancer in combination with ipilimumab with clinical benefits [91, 92]. Other studies explored virus-vectored vaccines as a means to augment the immune response to a specific antigen within the context of immunomodulatory antibody therapy. The combination of CTLA-4 blockade and modified vaccinia Ankara poxvirus encoding mutated p53 protein vaccine resulted in an improved therapeutic efficacy over either of the approaches [93]. Combination of the recombinant vaccinia vector carrying the genes for CEA, B7.1, ICAM-1 and LFA-3 (rV-CEA-TRICOM), and recombinant fowl pox-boosted vaccines with systemic CTLA-4 blockade led to an enhanced antitumour immunity [94]. This strategy was explored in a phase I trial of combination of poxviral-based PSA-TRICOM vaccine with ipilimumab in patients with metastatic castration-resistant prostate cancer. In this study, the use of the vaccine was not associated with increased rate of adverse events and had some evidence of activity with PSA declines in 58% of the chemotherapy-naïve patients [95].

Conclusions

As our knowledge regarding the immense potential of immune system to fight cancer widens, there is an

accelerated pace of drug development entering in the field of immunooncology. The complex interrelations between the tumour, its microenvironment and the elements of immunity point towards the need to reinstate our therapeutic strategy against cancer, on the basis of developing combinations between the various forms of immuno-oncology and molecular targeted therapies. After a long period, marked mostly by failures and drawbacks, cancer immunotherapy has reach to a point, where it is finally gaining momentum inside our collective effort to make faster strides against cancer.

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