REVIEW

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Cancer immunotherapy comes of age

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Activating the immune system for therapeutic benefit in cancer has long been a goal in immunology and oncology. After decades of disappointment, the tide has finally changed due to the success of recent proof-of-concept clinical trials. Most notable has been the ability of the anti-CTLA4 antibody, ipilimumab, to achieve a significant increase in survival for patients with metastatic melanoma, for which conventional therapies have failed. In the context of advances in the understanding of how tolerance, immunity and immunosuppression regulate antitumour immune responses together with the advent of targeted therapies, these successes suggest that active immunotherapy represents a path to obtain a durable and long-lasting response in cancer patients.

The passive transfer of anticancer monoclonal antibodies and donor T cells in the context of allogeneic bone marrow transplantation are effective treatments for a variety of haematological and solid malignancies[1]. Although not always thought of as 'immunotherapy', the success of these biotherapeutics probably reflects the ability of the donor cells or antibodies to induce an immediate immune reaction against the cancer, bypassing the requirement to activate endogenous immunity. These immune treatments have been well-established in oncology for several decades, and continued advances in antibody and T-cell engineering should further enhance their clinical impact in the years to come (Box 1).

In contrast to these passive immunotherapy strategies, the active stimulation of specific and durable antitumour immunity has proved elusive. In 1891, William Coley, a young New York surgeon, began intratumoral injections of live or inactivated *Streptococcus pyogenes* and *Serratia marcescens* in an effort to reproduce the spontaneous remissions of sarcomas observed in rare-cancer patients who had developed erysipelas[2]. Given Elie Metchnikoff's contemporaneous work demonstrating the immune system's ability to cause inflammation and destroy invading bacteria, 'Coley's toxins' made sense by acting to stimulate antibacterial phagocytes that might kill bystander tumour cells. Some significant responses were recorded over the ensuing 40 years, but successes were sporadic, difficult to reproduce and not obtained in a scientifically rigorous fashion. Superficial bladder cancer was one notable exception, for which the intravesical injection of live bacillus Calmette-Guérin after surgical resection was shown to prolong patient survival[3]. Other than this particular clinical setting, the approach was never embraced by oncologists who continued to rely on surgery and, increasingly, on effective new methods, such as radiation therapy and ultimately chemotherapy. Coley's strategy was further discounted due to the very real risks associated with the administration of infectious, or at least pyrogenic, agents to already weakened cancer patients; this is ironic given the trauma associated with the treatments that did come into common use. Thus began the history of cancer immunotherapy. Before continuing, however, it is useful to summarize what must happen to elicit a protective immune response to cancer, and why overcoming these barriers has been so difficult.

Generating anticancer immunity is a multistep challenge

Based on our current understanding of the immune response, there are three distinct steps that must be achieved, either spontaneously or therapeutically, to mount effective antitumour immunity (Fig. 1). To

BOX 1
Established immune treatments

Nine monoclonal antibodies targeting six cancer-associated proteins (Her2/neu, EGFR, VEGF, CD20, CD52 and CD33) are approved for the treatment of solid and haematological malignancies. In addition to antagonizing oncogenic pathways, these biotherapeutics may act by opsonizing tumour cells and triggering their death or removal by antibody-dependent cellular cytotoxity or phagocytosis[94]. Ongoing investigations in murine models and patients raise the possibility that they may also stimulate adaptive immune responses in some settings[95]. Recently, the successful conjugation of toxins to antibodies has been achieved, and these have induced a clinical response in patients who are refractory to the naked antibody[96]. The concurrent administration of immunostimulatory cytokines such as IL-2 and GM-CSF may also enhance the efficacy of antibody therapy.

Allogeneic bone marrow transplantation and the infusion of donor lymphocytes can be a highly effective therapy for some leukaemias and lymphomas[24]. The graft-versus-leukaemia effects involve the direct killing of tumour cells by donor lymphocytes, together with the subsequent induction of broader innate and adaptive reactions. On the basis of these clinical benefits, many groups are exploring the use of adoptive T-cell therapy in the autologous setting. Promising strategies include the use of lymphodepletion before T-cell infusion, and the engineering of new T-cell specificities with CARs[97].

Other immune treatments that have received the FDA approval include recombinant cytokines, such as IL-2 (Proleukin), which is used for melanoma and renal cell cancer. Response rates are low (~15%) and the significant risk of serious systemic inflammation requires administration as an in-patient. Interferon-α is another agent that gained approval for 'immunological cancers' (that is, melanoma or renal cell cancer). Although also associated with low response rates and high-dose toxicity, a small subset of melanoma patients, who are also predisposed to autoimmunity, has been shown to exhibit an impressive survival response[98]. It has been, however, difficult to pre-identify these patients, which limits the use of the approach. Yet, when seen, responses are durable, suggesting they reflect active antitumour immunity.

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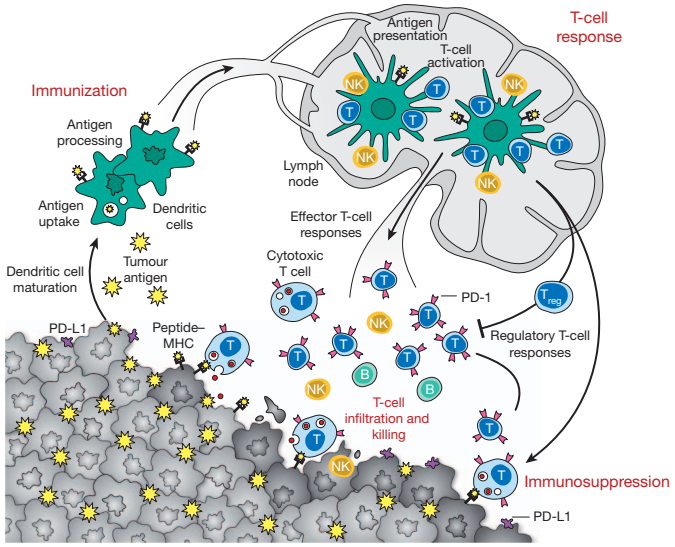

Figure 1 | Generation and regulation of antitumour immunity. Understanding the events in generating and regulating antitumour immunity suggests at least three sites for therapeutic intervention: promoting the antigen presentation functions of dendritic cells, promoting the production of protective T-cell responses and overcoming immunosuppression in the tumour bed. Antitumour immune responses must begin with the capture of tumour-associated antigens by dendritic cellls, either delivered exogenously or captured from dead or dying tumour cells. The dendritic cells process the captured antigen for presentation or cross-presentation on MHC class II and class I molecules, respectively, and migrate to draining lymph nodes. If capture and presentation occurred in the presence of an immunogenic maturation stimulus, dendritic cells will elicit anticancer effector T-cell responses in the lymph node; if no such stimulus was received, dendritic cells will instead induce tolerance leading to T-cell deletion, anergy or the production of T_{reg} cells. In the lymph node, antigen presentation to T cells will elicit a response depending on the type of dendritic cell maturation stimulus received and on the interaction of T-cell co-stimulatory molecules with their surface receptors on dendritic cells. Thus, interaction of CD28 or OX40 with CD80/86 or OX40L will promote potentially protective T-cell responses, while interaction of CTLA4 with CD80/86 or PD-1 with PD-L1/PD-L2 will suppress T-cell responses, and possibly promote T_{reg} formation. Antigen-educated T cells (along with B cells and NK cells) will exit the lymph node and enter the tumour bed, where a host of immunosuppressive defence mechanisms can be produced by tumours (or infiltrating myeloid cells) that oppose effector T-cell function. These include the upregulation of PD-L1/L2 on the cancer cell surface, release of PGE2, arginase and IDO (all T-cell suppressors), and the release of VEGF (triggered in part by intratumoral hypoxia), which inhibits T-cell diapedesis from the vasculature, and thus infiltration into the tumour bed.

initiate immunity, dendritic cells must sample antigens derived from the tumour, which can be ingested *in situ* or delivered exogenously as part of a therapeutic vaccine. These antigens might reflect one or more of the many mutated proteins that are typical of cancer, the products of non-mutated genes that are preferentially expressed by cancer cells (for example, cancer-testis antigens), or differentiation antigens associated with the cancer's tissue of origin, but against which thymic or peripheral tolerance has not been completely established (for example, melanosome-associated proteins in melanoma)[4,5]. On antigen encounter, the dendritic cells would also have to receive a suitable activation ('maturation') signal, allowing them to differentiate extensively to promote immunity as opposed to tolerance including enhanced processing and presentation of tumour-antigen-derived peptides[6,7]. Activation signals could be therapeutically supplied exogenously (for example, Toll-like receptor (TLR) ligands or agonist antibodies against activating receptors such as CD40) or endogenously: dying or necrotic tumour cells release factors (for example, high mobility group proteins or ATP) that are thought to result in the immunogenic maturation of dendritic cells. In addition, tumour cells seem to express resident endoplasmic reticulum proteins ectopically on the plasma membrane (for example, calreticulin) that promote their phagocytosis, possibly enabling the presentation of tumour antigens on major histocompatibility complex (MHC) class I and class II molecules. Some forms of chemotherapy or targeted therapy may promote a more immunogenic phenotype[8].

Next, in lymphoid organs, tumour-antigen-loaded dendritic cells must generate protective T-cell responses[9]. The precise type of T-cell response needed is unknown, but they certainly include the production of $CD8^+$ effector T cells with cytotoxic potential. Dendritic cells may also trigger antibody and natural killer (NK) or natural killer T (NKT)-cell responses, which may contribute to tumour immunity. The lymph node is a second potential site for therapeutic intervention, providing agents that may help guide the T-cell response. However, the dendritic cells are again key players because they must have been matured by a stimulatory adjuvant to have a chance at eliciting the desired T cells. Presentation of antigens by dendritic cells at the steady state (that is, dendritic cells that have not received an immunogenic maturation signal) promotes tolerance by regulatory T cell (T_{reg}) production[10–13], which would oppose an antitumour response.

BOX 2
Mechanisms of immune suppression

Tumours escape immune attack by a variety of complementary mechanisms of immunosuppression, many of which operate in parallel. Among paracrine mediators, adenosine, prostaglandin E2, TGF-β and VEGF-A exert multiple direct and indirect immunosuppressive activities. These mediators may function in the suppression of dendritic cells, indirectly inhibiting T-cell penetration into the tumour bed or directly suppressing effector T-cell activation while enhancing the function of T_{reg} cells. For example, adenosine, which is released by tumour cells under hypoxia, contributes to the suppression T-cell-activation and T_{reg} expansion. VEGF-A can also suppress proper T-celldevelopment and function: VEGF-A treatment of mouse splenocytes during T-cell stimulation was shown to induce IL-10 production from T cells while suppressing IFN-γ production[99]. Tumour cells can also directly escape T-cell recognition by downregulating MHC class I or by disabling other components of the antigen processing machinery. Shedding of soluble NKG2D ligands such as MIC-A or MIC-B can severely compromise the ability of effector T cells to function in the tumour microenvironment. In addition, tumour cells may upregulate surface ligands, which mediate T-cell anergy (or exhaustion), including PD-L1 and other ligands to inhibitory T-cell receptors. A variety of leukocyte subsets infiltrating tumours are also able to suppress T-cell function. In addition to T_{reg} cells (the accumulation of which in tumours correlates with poor prognostic outcome[14]), other suppressive lymphocyte subsets have been reported including IL-10 producing B cells and B regulatory cells, type II NKT cells, NK cells and γδ T cells. Myeloid lineage cells also promote immune suppression in tumours. Among these are mainly the poorly understood myeloid-derived suppressor cells (MDSC)[100]. Their characterization is ultimately based on their ability to suppress T and NK cell activation, probably through several mechanisms including nitric oxide, reactive oxidative species, arginase, IL-10 and TGF-β; there are also reports that MDSCs may specifically induce the expansion of T_{reg} cells. Tumour stroma cells have an important immune modulatory role. The so-called cancer-associated fibroblasts can promote the recruitment and function of immunosuppressive cells through the secretion of CCL2 and CXCL12. In addition, they can suppress effector T-cells through secretion of TGF-β. Finally, myeloid-derived mesenchymal stem cells exert important immunosuppressive functions by blocking proliferation and function of T effector cells. Further study is needed to determine which of these mechanisms are most important in general, and which determine the immune status in individual patients.

Finally, cancer-specific T cells must enter the tumour bed to perform their function at which point immune suppression becomes a challenge (Box 2). Tumours may (presumably by skewing dendritic cell maturation) prevent immunization, trigger the 'wrong' immune response or enable the local accumulation or expansion of T_{reg} cells that would oppose the activity of effector T cells. Indeed, infiltration of T_{reg} cells correlates with poor prognosis in a variety of epithelial tumour types[14,15]. Tumours may downregulate their expression of MHC class I molecules or their expression of target tumour antigens and can also produce a variety of surface molecules (for example, PD-L1 or PD-L2) that engage receptors on the surfaces of activated T cells (PD-1), causing T-cell anergy or exhaustion[16,17]. Expression of such suppressive ligands can be associated with oncogenic mutations seen in many cancers (for example, *PTEN* loss)[18]. Additionally, tumours can release immunosuppressive molecules, such as indoleamine 2,3-dioxygenase (IDO), which consumes tryptophan and limits T-cell function[19,20]. Myeloid-derived suppressor cells can also be recruited into the tumour, and release additional T-cell suppressors, such as arginase and nitrous oxide synthase[21]. Hypoxia in the tumour microenvironment may promote the generation of adenosine, which inhibits effector T-cell function in a similar way[22]. Hypoxia can also lead to the production of CCL28, which attracts immigration of T_{reg} cells[23]. Finally, tumour stroma cells can also suppress the function of effector lymphocytes. For example, mesenchymal stem cells block proliferation and function of effector T cells[24], whereas tumour vascular cells can suppress T-cell adhesion (to tumour endothelium) and prevent homing to tumours. This effect is, in part, mediated by vascular endothelial growth factor (VEGF)[25] as well as by the endothelin-B receptor (ETBR, also known as EDNRB)[26].

Thus, the success of immunotherapy seems unlikely. Any approach would have to overcome several significant barriers: that tumour-associated antigens are typically closely related or identical to self antigens, that it will be very difficult to separate therapeutic responses from pathological autoimmune responses, that both central and peripheral tolerance would have conspired to deplete or inactivate the relevant T-cell repertoire, and that the tumour microenvironment is inherently immunosuppressive. However, there now seems to be a path to clinical success.

Cancer vaccines are finally showing early signals of activity

Vaccines come in two formats: prophylactic and therapeutic[9]. Prophylactic (or preventative) vaccines have been used with considerable success for the prevention of cancers of viral origin, such as hepatitis B virus and human papillomavirus (HPV), where the aetiological agent is known. In contrast, the development of therapeutic vaccines to treat existing disease has proved problematic. The long history of failure has tainted the entire strategy of immunotherapy in the eyes of many oncologists.

The idea of a therapeutic cancer vaccine originated with the discovery that patients can harbour $CD8^+$ and $CD4^+$ T cells specific for cancer-testis or differentiation antigens expressed in their tumours[4]. Vaccination might reasonably be expected to amplify the frequency and strength of these pre-existing responses or perhaps induce some *de novo* reactions. Additionally, clinicopathological studies have demonstrated a strong association between prolonged patient survival and the presence of intratumoral $CD3^+$ or $CD8^+$ cytotoxic T cells and an interferon-γ (IFN-γ) gene signature[27,28]. Thus, if vaccination could trigger these types of T-cell responses, then a clinical benefit might be expected.

Unfortunately, the many initial attempts were compromised by a poor understanding of the mechanism of immunization, specifically the role of dendritic cells. Mostly conducted by academic groups, thousands of patients were treated with vaccines consisting of short peptides, often without an effective dendritic-cell-activating adjuvant[29]. Free peptides are likely to have poor pharmacokinetic properties and may be rapidly cleared before being loaded onto dendritic cells, where their half-life may also be short. Without an adjuvant, the dendritic cells might remain in the steady state and promote tolerance, as much as immunity. As a result, there was typically poor immunization, infrequent response to the selected tumour antigens (assuming they were even the correct ones) and minimal therapeutic benefits. Recently, however, the co-administration of interleukin (IL)-2 as an immune stimulant with a short peptide derived from glycoprotein 100 (gp100), a melanocyte differentiation antigen, was shown, under some conditions, to augment tumour responses and prolong progression-free survival compared with IL-2 alone in advanced melanoma patients[30]. These findings indicate that increasing immune activation with peptide vaccines is a critical step for improving therapeutic efficacy.

Because the importance and function of dendritic cells in stimulating T-cell responses is now well known, current vaccine trials are designed more rationally. One potentially promising approach involves the use of peptides (~20-mer) that are somewhat longer than those that bind to MHC class I molecules (10–12-mer). In the presence of a suitable dendritic-cell-activating adjuvant, these peptides are thought to be more efficient at generating effector T cells, perhaps because some processing may be required. A recent study of peptides derived from the HPV-16 E6 and E7 viral oncoproteins administered in incomplete Freund's adjuvant showed clinical responses in 15 of 19 women with vulvar intraepithelial neoplasia[31]. Tumour regressions were associated with the generation of HPV-specific, IFN-γ-producing $CD4^+$ and $CD8^+$ T cells. These favourable results might reflect in part the selection of viral gene products for immunization, because these proteins might be more readily recognized as foreign by the host. Indeed, a small trial of long peptides derived from TP53 (previously known as p53), a tumour suppressor often mutated in cancer, delivered in Montanide (an emulsion-adjuvant) induced a weaker IFN-γ-producing T-cell-response and no tumour regressions in advanced ovarian cancer patients, indicating the need to optimize these formulations further, or that p53 is simply not sufficiently immunogenic in this setting; in contrast to viral antigens, p53 might also have to break pre-existing tolerance to self[32].

Full-length proteins are also being explored as targets for cancer vaccinations, as they contain a broader profile of epitopes that might be presented by dendritic cells. Among these approaches, GlaxoSmithKline is currently conducting a large (>2,500 patients) randomized phase III trial using a recombinant fusion protein encoding a single cancer-testis antigen (MAGE-A3) in HLA-A2-positive non-small cell lung cancer patients, together with their ASO2B adjuvant consisting of a saponin/lipid-A emulsion combined with TLR4 and TLR9 agonists. Initial read-outs from the phase II trial (180 patients) showed some survival response (27%), but this did not reach statistical significance[33]. Objective T-cell responses were not reported, nor were data on the level or homogeneity of MAGE-A3 expression in the study group; this latter parameter might be especially important, as immune attack on sub-populations of lung cancer cells that lack MAGE-A3 would require diversification of the T-cell response to additional cancer antigens.

Another target that has attracted some attention is the antigen receptor on B-cell lymphomas (idiotype), which is an example of a clonally expressed tumour-specific antigen. Three randomized phase III trials testing recombinant idiotypes (prepared individually for each patient) administered with granulocyte-macrophage colony-stimulating factor (GM-CSF) to attract dendritic cells were undertaken. One trial, in which eligibility was restricted to subjects who first achieved a complete response to cytotoxic chemotherapy, indicated that vaccination might prolong progression-free survival. However, the two other studies failed to reveal a clinical benefit, which might reflect differences in patient populations, vaccine manufacturing methods or that the approach is not sufficiently robust[34].

Viral vectors encoding tumour antigens are another vaccine platform undergoing evaluation. These strategies exploit the strong immune response directed against viral components to enhance reactivity against the cancer antigen. One such phase II trial (125 patients), conducted by Bavarian-Nordic and the United States National Cancer Institute, involved an initial inoculation of a recombinant vaccinia virus encoding prostate-specific antigen and the adhesion molecules B7-1, ICAM-1 and LFA-3; a similarly configured fowlpox vector was administered subsequently in a prime-boost strategy, and GM-CSF was administered with the vectors for further immune stimulation (the entire vaccine

product is termed PROSTVAC). In addition to reactivity against the pox viral gene products, the introduction of adhesion molecules into the infected cells was intended to have them serve as surrogate dendritic cells, although the specializations of dendritic cells for T-cell stimulation extend well beyond these three adhesion molecules. Whereas vaccination had no impact on progression-free survival, there was an overall survival benefit: 25.1 months versus 16.6 months in the control group (patients treated with empty vector plus saline)[35]. A larger phase III trial is planned using vector alone, vector plus GM-CSF (as a dendritic cell adjuvant or attractant) or empty vector plus GM-CSF.

A variety of earlier stage trials are also in progress, using defined antigens that are delivered to dendritic cells by coupling to dendritic-cell-targeted monoclonal antibodies (for example, DEC-205) or further peptide and viral vector trials, in conjunction with various adjuvants. The ability of these approaches to facilitate durable anticancer immune responses, however, remains to be demonstrated, particularly on their own.

Another strategy for vaccine therapy involves the use of cell-based approaches. One of the ideas underlying this strategy is that an actual cancer cell would already harbour a wide range of tumour-associated antigens (including mutant proteins), so that if used as a vaccine the problem of antigen selection would be reduced. A meta-analysis of 173 published peer-reviewed immunotherapy trials in various solid tumour types revealed that patients immunized with whole-tumour antigen had low, but significantly higher rates of objective clinical response (8.1%) than patients immunized with molecularly-defined tumour antigens (3.6%)[36]. Although autologous tumour cells are the best choice of immunogen for this approach, the complexities of vaccine manufacture for individual patients has led to the application of allogeneic tumour cell lines. Among these strategies, the one that progressed furthest into clinical development was GVAX for prostate cancer, advanced by Cell Genesys (no longer exists). The vaccine product was comprised of two prostate cancer cell lines that were stably engineered to secrete GM-CSF. After early promising results, GVAX failed in phase III trials due to lack of clinical efficacy[37]. This failure might reflect a lack of sufficient immunogenicity of the approach, alterations in preparation of the vaccine product necessitated by commercial scale-up or the inability of allogeneic tumour cell lines to represent adequately the spectrum of antigens characteristic of individual prostate cancers in patients.

There has also been considerable interest, particularly among academic groups, in developing dendritic-cell-based vaccines. In this approach, dendritic cells are isolated from a cancer patient, loaded with antigens (peptides or even tumour cell lysates) *ex vivo*, activated and then re-infused back into the patient[9,38]. Although there have been some promising hints of efficacy and response, as yet no clearly positive studies have been reported and the approach has not gained broad support from the biotech-pharmaceutical industry, given the complexities of cell isolation, *ex vivo* manipulation and re-infusion. There is, however, one notable exception, which will be discussed shortly.

In general, there are many barriers to success, or at least to quantifying the success, of cancer vaccines administered as 'single agents'. First, the criteria for defining optimal tumour antigens remain to be fully defined[39,40]. Mere expression in the target tumour population may be inadequate for predicting the ability to generate protective T-cell response. Identification of peptides bound to MHC class I on the tumour by mass spectroscopy can identify those antigens that yield potential targets[41], but these peptide–MHCI complexes might not be sufficiently immunogenic rejection antigens (that is, able to generate effective T-cell responses). Moreover, antigen expression within a tumour bed can be heterogeneous. Even the allogeneic tumour cell line based approaches are limited by the fact that a given patient's tumour will probably harbour mutations that are not found in the vaccine product. Second, the optimal adjuvant for producing antitumour $CD8^+$ T-cell responses that can be used safely and effectively in humans is not yet clear. The desired adjuvant (or adjuvant combination) will be one capable of triggering the maturation of dendritic cells to a state where they can facilitate the generation of tumour-reactive, $CD8^+$ cytotoxic T cells. Finally, although it is likely that conditions for immunization will eventually be optimized, the effectiveness of a tumour-specific T-cell population may still be limited by the multiple mechanisms of immunosuppression used by tumours to guard against T-cell killing. These are not reasons to eliminate a vaccine arm from consideration as part of immunotherapy, but rather to highlight some of the difficulties in assessing success in the absence of other immunomodulatory agents. In the same way as other forms of targeted therapy for cancer, the discovery and application of predictive biomarkers or diagnostics, which could identify those patients most likely to benefit from a given vaccine, will be an important challenge for future development.

An efficacious cell-based vaccine for prostate cancer?

A fact of drug development is that performance in the clinic is the final arbiter of success. The first validation of active immunotherapy as a viable approach to cancer treatment was the Food and Drug Administration (FDA) approval in April 2010 of Provenge (sipuleucel-T) for advanced prostate cancer. Provenge was originally assumed to be an autologous dendritic-cell-based vaccine[42] (it was developed by the eponymously named company, Dendreon); however, it actually comprises an incompletely characterized, complex mixture of peripheral blood mononuclear cells supplemented with a cytokine and tumour-derived differentiation antigen. In the pivotal phase III trial[43], total peripheral blood mononuclear cells were collected from patients by leukopheresis at 0, 2 and 4 weeks and then cultured for 36–44 h at 37 °C in medium containing a fusion protein composed of prostatic acid phosphatase (PAP, a tumour-associated differentiation antigen) and GM-CSF before reinfusion. This was compared with a placebo that was prepared by incubating one-third of the leukopheresis product for 36–44 h at 2–8 °C without exposure to the fusion protein (the remaining two-thirds were cryopreserved to allow for subsequent vaccine manufacture in a salvage protocol). Thus, the control not only lacked the presumptive immunizing antigen, but was also processed differently.

The clinical results showed little evidence of tumour shrinkage or delay in disease progression. By standard response evaluation criteria in solid tumours (RECIST) criteria, only 1 out of the 341 patients in the active arm showed a partial response. At least a 50% reduction in prostate-specific antigen levels on at least two visits was shown in 2.6% of the patients versus 1.3% in the placebo group. Nevertheless, a 4.1 month improvement in median survival was achieved (25.8 months versus 21.7 months), which was deemed significant by the FDA in a patient population that has few, if any other, effective therapeutic options.

While Provenge is clearly a cell-based therapy, there may be other mechanisms involved. Although the majority (66%) of survivors showed an antibody response to the fusion protein, the fraction of patients producing antibodies that recognized endogenous PAP was much lower (28.5%). Moreover, T-cell responses to either the fusion protein or PAP were not associated with survival. These discrepancies might reflect a limitation of monitoring antitumour immune responses in the peripheral blood compared with the tumour microenvironment. However, they also raise the possibility that other undefined factors in the cellular product may have an important role. Further studies are required to understand the therapeutic mechanism of Provenge, and to define the impact of the different cell-processing procedures on the placebo product.

The lack of tumour shrinkage, the criterion typically used to gauge the efficacy of cancer treatments, in the face of a survival benefit is surprising, but perhaps not unexpected for immunotherapy. As seen pre-clinically, an effect on pre-existing tumours due to immune manipulations can be delayed while an immune response develops[44,45]. Furthermore, biopsies of metastases after vaccination in some clinical trials revealed the presence of immune infiltrates that mediate tumour destruction in association with extensive oedema, which may be followed by fibrosis[46]. These histopathological findings suggest that monitoring tumour size alone may be inadequate for assessing the overall therapeutic effects of vaccination. As discussed later, these considerations apply to the evaluation of CTLA-4 antibody blockade, highlighting the need to modify

tumour response criteria in light of new insights into the biology of immunotherapy[44].

The potential of directly harnessing lymphocytes

Adoptive transfer of lymphocytes with tumoricidal properties can, in theory, bypass the daunting task of breaking tolerance to tumour antigens and generating a high frequency of high avidity effector T cells. The discovery that host lymphodepletion facilitates engraftment of adoptively transferred T cells has enabled the successful transfer of *ex-vivo*-expanded tumour-infiltrating lymphocytes (TILs) from patients with melanoma, with marked clinical responses, some of which are complete and durable[47,48].

Advances in T-cell culturing methods and T-cell engineering, through retroviral vectors carrying cloned T-cell receptors or chimaeric antigen receptors (CARs) enriched by co-stimulatory signalling domains, has expanded the opportunities for adoptive T-cell therapy beyond patients with resectable tumours harbouring reactive T cells to a larger population with solid tumours expressing the cognate target(s)[49]. The advent of CARs bypasses the need for tumour cells to possess functional antigen-processing machinery and express antigen through MHC class I or II molecules; transduced T cells can recognize the intact surface protein through the affinity domain (usually a scFv antibody) of the artificial CAR. Early clinical results appear very promising[50,51]. However, safety issues surrounding the selection of the target, the paucity of such targets, manufacturing complexities and costs, and the lack of durable response in many patients indicates that additional interventions are required to properly direct and activate T cells in the tumour microenvironment.

A far more convenient approach may be the therapeutic use of bispecific antibodies that engage both the TCR and an antigen on the tumour cell surface. In B-cell lymphoma and leukaemia, MicroMet[52] has applied this approach with impressive clinical success. However, the platform has a number of liabilities including neurotoxicity and the necessity for continuous pump-mediated infusion due to the very rapid clearance of the antibody fragment. Nevertheless, such bispecific antibodies further emphasize the possibility of repurposing T-cell specificity for therapeutic benefit.

Ipilimumab's emergence as an effective therapy

The most important development for cancer immunotherapy, and hopefully for the benefit of many cancer patients, was the recent readout of the ipilimumab phase III trials in late-stage metastatic melanoma. Not only was a clear survival advantage observed for a patient group with no other therapeutic options, but it was achieved with an agent whose mechanism of action is virtually certain to involve the modulation of endogenous T-cell responses. The results were deemed significant enough that in March 2011 the FDA granted broad approval for use in patients with metastatic melanoma, either as initial therapy or after relapse.

Ipilimumab is a monoclonal antibody to CTLA4, whose role in regulating T-cell function has been studied for many years by a number of groups, notably by Allison and colleagues[53]. CTLA4 is a key negative regulator that is recruited to the plasma membrane on T-cell activation where it binds to members of the B7 family of accessory molecules expressed by dendritic cells and other antigen-presenting cells (Fig. 2). CTLA4 ligation effectively inhibits further activation and expansion, thereby controlling the progress of an immune response and attenuating the chances for chronic autoimmune inflammation. The negative regulation is overcome by use of a blocking antibody. The fundamental importance of CTLA4 in controlling T-cell function is well-illustrated by the phenotype of *Ctla4*$^{-/-}$ mice, which die of an aggressive lymphoproliferative disorder at a young age[54,55]. Interestingly, CTLA4 ligation is also important for the immune suppressive function of T_{reg} cells, by further assisting to dampen T-cell responses[56]. T_{reg} function is also thought to be blocked by anti-CTLA4.

The rationale for using anti-CTLA4 in cancer therapy was to unrestrain pre-existing anticancer T-cell responses and possibly trigger

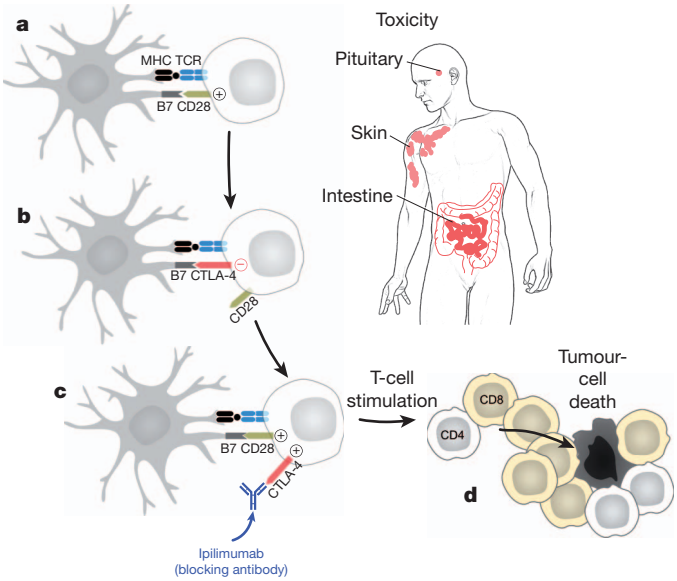

Figure 2 | Biological activities of CTLA-4 antibody blockade. **a**, On encountering a dendritic cell presenting a cognate tumour-antigen-derived peptide epitope and expressing B7 co-stimulatory molecules (CD80, CD86), specific antitumour T cells become activated through TCR and CD28 signalling. **b**, CTLA-4 is subsequently upregulated and preferentially engages B7 to attenuate T-cell response. **c**, Ipilimumab blocks CTLA-4 function, thereby allowing enhanced T-cell stimulation and a more potent antitumour reaction. Ipilimumab may also antagonize CTLA-4 on regulatory T cells to limit their ability to suppress the antitumour T-cell effector response (not shown). **d**, CTLA-4 antibody blockade compromises tolerance to some normal tissue antigens, provoking inflammatory toxicities that can have an impact on the skin, pituitary gland and intestine in human patients.

new responses. It is well-known for melanoma (and other diseases) that TILs exist, and they can bear specificity for tumour antigens[4,57–59]. Pre-clinical studies using mouse models were promising, which led two companies (Pfizer and BMS/Medarex) to put two different anti-CTLA4 antibodies into the clinic. Phase II trials failed to reach their endpoints of tumour regression, but BMS/Medarex felt there was sufficient potential for long-term benefit, and so a lengthy randomized phase III trial was initiated in relapsed-refractory metastatic melanoma patients to determine overall survival. Their antibody, ipilimumab, was given to one arm, antibody plus a short peptide (gp100) for a melanoma differentiation antigen was given to a second arm and the control arm received the short peptide alone. Although the Kaplan–Meier plots of survival were inseparable for the first few months, a twofold survival benefit was detected at 12–15 months in both antibody arms, which was still durable after 2.5 years and included a complete response in some patients[60].

In a second BMS/Medarex randomized trial involving 502 patients with previously untreated metastatic melanoma, the addition of ipilimumab to standard dacarbazine therapy was shown to improve overall survival compared with dacarbazine alone (11.2 months versus 9.1 months). Furthermore, the combination treatment significantly increased the proportion of surviving patients with at least 3 years of follow-up (20.8% versus 12.2%)[61]. Although only a relatively small fraction of patients derived clinical benefit, these studies clearly establish ipilimumab as an active agent, which offers patients, who would normally be at the terminal stage of this disease, clinically meaningful benefits and the possibility of long-term survival. Furthermore, the results validate the idea that activating the T-cell compartment can, on its own, provide a significant therapeutic effect.

The use of ipilimumab does present some clinical and scientific challenges. First is the significant rate of on-target toxicities observed. Up to 23% of the ipilimumab-treated patients developed serious (grade 3–4) adverse events including colitis and hypophysitis due to induced inflammation (possibly autoimmune in nature), and in conjunction with

dacarbazine, approximately 20% showed significant elevations in liver function tests. However, toxicity does not accurately predict positive therapeutic outcome, indicating that many patients will experience inflammatory pathology without benefiting from an antitumour effect. These toxicities might be expected given that the removal of *CTLA4* from mice leads to virulent inflammatory disease, as mentioned earlier. The different spectrum of toxicities with ipilimumab compared with standard cancer treatments means that practising oncologists will need to acquire additional expertise in the management of inflammatory disorders.

A second clinical challenge with ipilimumab relates to the kinetics of the antitumour response. In contrast to conventional cytotoxic therapies that may trigger rapid tumour shrinkage due to direct killing of cancer cells, the stimulation of T-cell response with ipilimumab may take several months to occur. Tumours may increase in size during this period, and some component of this growth may be a result of the evolving inflammatory reaction. Indeed, as many as 10% of patients treated with ipilimumab, who were scored with progressive disease using the modified WHO (World Health Organization) criteria for tumour size, were shown to achieve disease stabilization and prolonged survival[44,60]. This unusual pattern of treatment response has led to the proposal of new immune-related criteria that may aid clinical decision making regarding continuation of therapy[62] (Box 3).

The rationale for ipilimumab monotherapy is that its use so far assumes that tumour-protective T cells exist in the patient before therapy, and that these cells will exert antitumour activity if CTLA4 is blocked. The previously mentioned clinical studies were carried out without concomitant effective immunization. An uncoupled peptide to the melanoma differentiation antigen epitope was included in some arms, but dosed without adjuvant or a dendritic cell maturation agent. Although it would be expected for CTLA4 to be induced in tumour-reactive T cells only after immunization, the clinical response observed in the absence of prior exogenous vaccination indicates that tumour-reactive TILs expressing CTLA4 are responsive to checkpoint blockade and acquire effective tumour-rejecting functions. The notion of exogenous versus endogenous vaccination is discussed later.

Despite these limitations, ipilimumab provides realistic hope for melanoma patients, particularly those with late stage disease who otherwise had little chance of survival. More broadly, it provides clear clinical validation for cancer immunotherapy in general. The results will also intensify the search for predictive biomarkers for positive responders. Other applications of ipilimumab are already being vigorously pursued, and the door has been opened for the development and investigation of a host of other potential immunotherapeutic strategies, some of which may prove safer and more effective than targeting CTLA4. At long last, there is the prospect of combining this treatment with other immunotherapeutic regimens, such as effective vaccination, which, arguably, should have been considered much earlier in the clinical study history of anti-CTLA4.

The next generation T-cell immunomodulators

The success of anti-CTLA4 in melanoma should create interest in evaluating other antibodies that can be used to activate T-cell responses. There are a number of known receptors that could serve as targets for agonist antibodies, including 4-1BB, OX40, GITR, CD27 and CD28 (Fig. 3). The latter, however, introduces a cautionary note owing to an early clinical trial of an agonist anti-CD28 (TGN1412) in which severe toxicities and even death resulted from unexpected cytokine release[63]. These serious events emphasize the power of the immune system and the need for extreme care and a conservative trial design when using any immune activator. The use of agents that clear more rapidly from the circulation than intact IgGs may help mitigate the potential for such toxicities, or at least enable the more rapid removal of the inducing drug. The same consideration may apply to anti-CTLA4 therapy, where alternative dosing strategies may serve to increase its therapeutic index.

LAG-3 is another T-cell receptor that, like CTLA4, is largely suppressive. Not as well studied as CTLA4, LAG-3 appears similar in that it acts to limit the activity of $CD4^+$ and $CD8^+$ T cells, and augment the activity of T_{reg} cells[64,65]. In the same way as CTLA4, there is also a significant intracellular pool of LAG-3 (ref. 66). However, the functional consequences of its deletion are far less dramatic because it may work alongside other regulatory molecules (for example, PD-L1)[67]. This situation suggests, however, that antagonizing LAG-3 may provide an alternative to antagonizing CTLA4, and perhaps have a better safety profile.

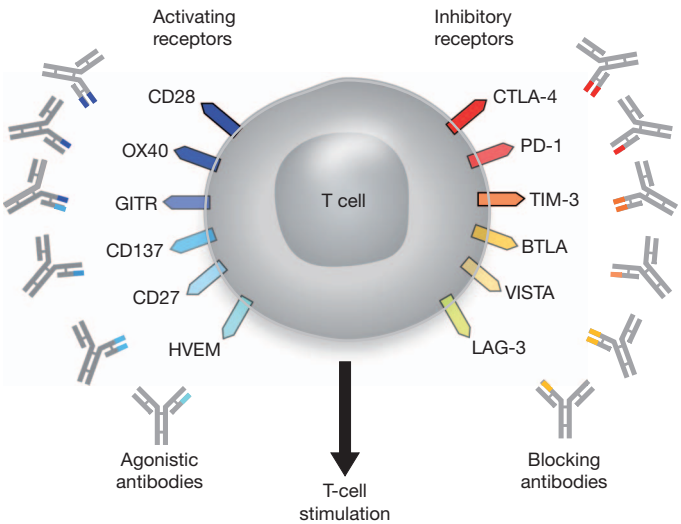

Figure 3 | T cell targets for immunoregulatory antibody therapy. In addition to specific antigen recognition through the TCR, T-cell activation is regulated through a balance of positive and negative signals provided by co-stimulatory receptors. These surface proteins are typically members of either the TNF receptor or B7 superfamilies. Agonistic antibodies directed against activating co-stimulatory molecules and blocking antibodies against negative co-stimulatory molecules may enhance T-cell stimulation to promote tumour destruction.

BOX 3

Clinical assessment of immunotherapy

Oncologists traditionally evaluate the activity of cancer therapies through measurements of tumour area or volume. These standard metrics include the RECIST and modified WHO criteria. Clinical responses to cytotoxic treatments, such as chemotherapy, radiation therapy and some targeted agents usually occur quickly (within a few weeks to months) because their presumed mechanism of action involves a direct effect on tumour cells. Moreover, these treatments generally result in a reduction in tumour size because cancer cells undergo apoptosis or other modes of programmed cell death. Although tumour regression indicates the therapy is beneficial , this may not always translate into improvements in survival due to the potential emergence of lethal drug-resistant cells. Immunotherapy-induced tumour destruction, in contrast, may be delayed or even preceded by a period of apparent tumour growth. In clinical trials of ipilimumab, 10–20% of patients showed an increase in tumour size when evaluated 3 months after starting treatment, but subsequently achieved prolonged tumour control or regression without any additional intervention. These patients demonstrated long-term survival comparable with patients who had more rapid tumour regression. The mechanisms underlying the delayed response are not yet well understood, but might include the effects of immune infiltrates in tumours or just the long period of time required to generate sufficient T cells to accomplish tumour killing. This distinctive biology has led to the proposal of immune-related response criteria[40], which allow for greater flexibility in following the increase in tumour size during immunotherapy before declaring treatment failure.

Another attractive approach, which is beginning to receive some clinical validation, is targeting immunosuppression in the tumour bed. Even if a vaccine or T-cell modulation therapy is successful, the ability of tumours to counteract immune effectors may limit the clinical benefit. Of current clinical interest is the PD-1/PD-L1(-L2) axis. PD-1 is expressed by T cells, particularly activated T cells, and binds to its ligands PD-L1/L2 that can be expressed by potential target cells, thereby rendering the T cell unresponsive or 'exhausted'[68]. This axis is well-characterized as limiting the T-cell response to chronic virus infection[69], but increasingly it is thought to have a role in limiting the immune response in cancer as well[70]. A variety of tumours, including melanoma, ovarian, renal, hepatocellular and glioblastoma, have been found to express PD-L1 (and occasionally PD-L2). PD-L1 expression has been found to correlate with poor prognosis[71,72], and to increase on activation of the oncogenic PI3K pathway (for example, by *PTEN* deletion)[18]. Antibodies to PD-1 have now reached the clinic. In early phase I trials they have shown good activity in a variety of cancer types and, so far, have a toxicity profile that seems safer than ipilimumab[73]. The reduced toxicity is consistent with the generally milder autoimmune phenotype seen in *PD-1*$^{-/-}$ mice[68] compared with *Ctla4*$^{-/-}$ mice. However, lung inflammation and cardiomyopathy observed in mice[74,75] may prove to be a concern in the clinic. An important biological and clinical question is whether the effector function of tumour-reactive TILs co-expressing multiple inhibitory receptors can be fully recovered by targeting a single receptor, or whether combinatorial checkpoint blockade is required for sustained tumour protection.

Other potential approaches to T-cell immunosuppression include targeting T_{reg} cells for inactivation or depletion because, in many tumour beds, the infiltration of T_{reg} cells may act to oppose effector T-cell function as much as the presence of negative regulatory molecules on the surface of effector cells (for example, PD-1). Whereas no specific surface marker of T_{reg} cells has yet been identified, some proteins, such as GITR and OX40, may be transiently expressed, possibly enabling them as targets (although they are also expressed by activated effector T cells in humans)[76,77]. Anti-CD25 antibodies may preferentially deplete T_{reg}, at least following short-term therapy, and may help increase the efficacy of active immunization[78]. Finally, low-dose cyclophosphamide may preferentially target T_{reg} and allow for attenuation of T_{reg} in the context of immunization protocols[79,80].

As mentioned earlier, tumour beds can produce a number of soluble mediators that counteract T-cell function, such as IDO (indoleamine 2,3-dioxygenase), arginase and prostaglandin E2 (PGE2)[81]; to the extent that these agents can be diagnostically demonstrated as predictive biomarkers. Efforts to inhibit their activities with either small molecule or antibody antagonists might also be useful additions to a portfolio of cancer immunotherapeutics. Furthermore, as knowledge on the immunoregulatory function of tumour vasculature increases, therapeutic manoeuvres to 'normalize' the tumour vessels in combination with immunotherapy seem to be an interesting development. Indeed, VEGF blockade has resulted in increased T-cell homing to tumours[82] and has enhanced the efficacy of immunotherapy in the mouse[83]. Lastly, the importance of tumour-promoting inflammatory pathways, such as STAT3 and NF-κB signalling, and cytokines including IL-6, IL-17, IL-23 and tumour-necrosis factor-α (TNF-α) has been demonstrated[84]. Inhibition of these circuits might not only antagonize tumour progression, but also enhance the activity of immunotherapy.

Combination immunotherapy

The use of combinations of chemotherapeutic drugs has traditionally been a mainstay of oncology. Even in the relatively recent age of molecularly targeted therapies, the development of combinations is proving to be of benefit to broaden the response and to treat resistance. The difference is, in theory, combinations of targeted agents can be combined rationally, in a scientifically guided fashion. There is no reason to believe that the same will not hold true for immunotherapy.

In melanoma, there has been another recent success in the small molecule realm. For patients bearing the V600E activating mutation of *BRAF*, the Roche inhibitor vemurafenib has been shown to yield a marked response in more than 50% of patients[85]. Yet, resistance develops rapidly (<1 year), creating the necessity for additional therapy. Although one probable effective approach is to add a second small molecule inhibitor to *MEK*, to prevent the tumour from activating a compensatory pathway, one might predict the tumour will also circumvent this strategy. Therefore, the prospect of generating long-lasting protective immunity during the remission period is intensely attractive and trials combining ipilimumab with vemurafinib are in the planning stages. Since vemurafinib-induced death of tumours can be expected to release endogenous tumour antigens, it is possible that the small molecule and immunotherapeutic approaches may synergize, with *BRAF* inhibition acting to help prime *de novo* T-cell responses that can then be facilitated by the anti-CTLA4. Success, however, makes the assumption that the *BRAF* inhibitor is not suppressive of immune responses under the conditions that the two agents would be combined in the clinic.

In this and all other instances where immunotherapeutics are to be combined with targeted or chemotherapeutic agents, it will be critical to assess the potential interactions of the combination(s). Small molecule inhibitors, and cytotoxic chemotherapy alike, may act on cells of the immune system to block dendritic cells or T-cell function, or modify the tumour or tumour microenvironment in a way that will antagonize the development of immunity. Alternatively, conditions may be found where such inhibitors or cytotoxic drugs may be immunostimulatory to T cells, as recently suggested for an IAP antagonist[86] or, surprisingly, for the mTORC1 inhibitor, rapamycin, which is normally used as an immunosuppressive agent in the transplant setting[87]. Similarly, although conventional wisdom viewed the effects of chemotherapy as obligatorily deleterious to immune mechanisms, such effects are drug-, dose- and/or schedule-dependent. As the same agent may prove inhibitory, benign or even stimulatory depending on the stage of immune response being targeted and the dose/schedule being used[88,89], great care must be exercised when designing clinical protocols in case an efficacious drug is dismissed due to negative interactions or suboptimal dosing schedules.

The effects of conventional chemotherapeutics on the immune system may be more nuanced than previously believed. Evidence is emerging that tumour cells can die in multiple ways, with some forms of (apoptotic) death actually leading to the enhancement of an immune response to the tumour[90]. So-called immunogenic cell death is characterized in part by the release of ATP and high mobility group protein B1, which could activate local infiltrating myeloid cells and dendritic cells by a purinergic receptor or TLR4, respectively. Cytotoxic agents that elicit this death fingerprint may have the ability to help induce antitumour immune responses and, therefore, be better candidates for combination therapy with immunologically active agents. Again, great care must be exercised to ensure such agents are used at doses and schedules that do not suppress effector CTLs.

Finally, the prospect of combining immunotherapeutic agents themselves must be considered. To do this rationally, one must return again to the various steps of the immune response that need to be addressed to generate anticancer immunity (Fig. 1), consider the step(s) under the control of each agent, and assess the potential for overlapping or synergistic toxicity that would decrease rather than increase therapeutic index. For example, combining anti-CTLA4 with anti-PD-1 makes sense biologically, as the two agents remove the brakes from T-cell activation at two distinct stages: proliferation (CTLA4) and effector function (PD-1). Yet, both might be expected to show similar adverse events, underscoring the need to define carefully the potential for serious toxicity.

Revisiting vaccine use

Agents that act at the effector stage (for example, anti-PD-1 or inhibitors of immunosuppression) can only act by re-energizing the pre-existing T cells. Agents that act at the proliferation or activation stage (for example,

ipilimumab) can probably enhance not only pre-existing responses but also *de novo* responses. Thus, either could work well in conjunction with a vaccine approach, which is the place where immunotherapy in cancer got its start. However, it is debateable whether endogenous or exogenous vaccines would be preferable for this purpose.

Exogenous vaccines involve introducing preselected antigens on antibody delivery vehicles targeted to dendritic cells, encoded in viral vectors, or administered as peptides or proteins in a suitable adjuvant and carrier. The approach has been highly effective in generating prophylaxis and protective immunity against infectious agents[9]. In fact, such vaccines represent some of medicine's greatest successes. Vaccines have been far less impressive in cancer, which could be a reflection of the use of poor platforms that fail to elicit optimal antigen processing or presentation by dendritic cells, suboptimal adjuvants, or the absence of co-administered agents that facilitate T-cell responses or overcome immunosuppression in the tumour bed. In addition, the antigens selected may have been poorly chosen, unable to generate protective T cells with high affinity T-cell receptors because the tolerance barrier was too high or unable to generate a sufficient quantity of T-cell receptor ligands (peptide–MHC class I complexes) at the tumour cell surface.

Endogenous vaccines involve mobilizing antigens from a patient's own tumour, *in situ*. This would have to be accomplished by inducing tumour cell death under conditions that favour the ability of endogenous dendritic cells to capture, process and present tumour-derived antigens. Although less controllable than the exogenous approach, endogenous vaccines have the distinct advantage of potentially allowing the presentation of the dozens, or hundreds, of mutations harboured by cancer cells[5]. If even only a fraction of these can form MHC-binding peptides that serve as T-cell epitopes, then as neo-antigens they would certainly not have been subject to suppression by central tolerance. Whether chemotherapy alone is sufficient to induce such *in situ* vaccination, however, is unknown. Because of the powerful immunosuppressive circuitries operating in the tumour microenvironment, additional signals are probably required to correctly polarize the tumour-infiltrating-antigen presenting cells, which can be now achieved by pathogen-derived signals or other dentritic cell maturation agents.

A recent attempt at endogenous vaccination involves the intratumoral injection of replication-conditional herpes simplex viruses engineered to secrete GM-CSF. These oncolytic viruses (OncoVex), which have entered phase III testing in melanoma, cause lysis of tumour cells on infection, when GM-CSF release may enhance dentritic cell function in the tumour microenvironment to stimulate responses against autologous cancer antigens[91]. A second attractive approach involves the combination of cytotoxic therapies in conjunction with agonistic antibodies to CD40. Whereas this strategy might be predicted to augment dendritic cell priming of antitumour T cells, an important role for macrophage-mediated killing was implicated instead[92]. Lastly, combinations of chemotherapy with TLR agonists could provide the right ingredients of tumour antigen release and tumour antigen presenting cell activation.

Perspectives

Despite its long, if not always distinguished, history, these are early days for the new science and clinical practice of cancer immunotherapy. Nevertheless, as an exciting therapeutic strategy that merits serious consideration by the biopharmaceutical industry and clinical oncology community alike, recent results have allowed cancer immunotherapy to finally come of age. This transition coincides with another development of the cancer immunology field. For much of the twentieth century, the focus was on cancer 'immunosurveillance' given the prominent view that the immune system has a homeostatic role in controlling cancer. When oncogenic or other mutations occurred, the immune system was, in theory, thought to respond, thereby preventing the development of a tumour, at least when the system was operating normally. This concept was refined by 'immunoediting', which recognizes the complex and dynamic cross-talk between the tumour and the host throughout all stages of disease development, including the possibility of tolerance[93]. In the twenty-first century, however, we believe emerging pre-clinical and clinical data has turned attention directly towards the primary role of 'tolerance and immune suppression'. This leaves aside the issue of whether the immune response has a homeostatic function in prevention. The cancer develops and escapes control either because the tumour cells are too similar to their normal counterparts and, therefore, do not elicit sufficient immunity, or because they are adept at inducing peripheral tolerance. Whereas ipilimumab might work either to induce a *de novo* response or enhance a pre-existing one, the clinical activity of anti-PD-1 antibodies seems to be more closely associated with overcoming tolerance than with generating an anticancer T-cell repertoire.

Cancer immunotherapy has come of age at just the right time. The advent of a cohort of inhibitors that target oncogenic pathways with ever greater specificity is starting to reveal significant and sometimes spectacular responses in several indications. Yet, even in diagnostically defined populations, these responses can be transient or require continued dosing. If such drug regimens can be matched to appropriate immunotherapies, activating a patient's immune system during a time of tumour reduction and remission may be the best way to ensure that responses are converted to a long-term and durable benefit. As well as the development of additional agents to prime and guide the immune response, appropriate pharmacodynamic biomarkers will have to be implemented to determine if a given immunotherapy is having the desired effect and appropriate diagnostics used to identify which strategy to apply to which patient. Finally, scientists, drug developers and oncologists will have to work together to implement new metrics for evaluating the effectiveness of immunotherapies. Their mechanism of action is so distinct, both mechanistically and temporally, compared with conventional cytotoxic drugs, that they cannot be expected to perform according to standards developed a generation ago, even though the result may ultimately be curative.

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