Cancer immunotherapy – revisited

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Abstract | Our insight into antitumour immune responses has increased considerably during the past decades, yet the development of immunotherapy as a treatment modality for cancer has been hampered by several factors. These include difficulties in the selection of the optimal dose and schedule, the methods of evaluation, and financial support. Although durable clinical remissions have been observed with various immunotherapeutic strategies, the percentage of patients who benefited from these interventions has remained too small to justify the general use of such strategies. However, the recent positive results of clinical trials with novel immunoactive drugs as well as the unexpected finding of a positive interaction between immunotherapy and chemotherapy may herald a new era for the immunotherapy of cancer.

Cytotoxic T lymphocyte
Cytotoxic ($CD8^+$) lymphocytes can kill tumour cells following recognition of tumour-associated antigens that are presented by major histocompatibility complex class I.

Treatment with anticancer drugs is commonly categorized into four different classes: chemotherapy, which involves a large group of cytotoxic drugs that interfere with cell division and DNA synthesis; hormonal therapy, which involves drugs that interfere with growth signalling through hormone receptors on cancer cells; targeted therapy, which consists of a novel group of antibodies and small-molecule kinase inhibitors that specifically target proteins that are involved in growth signalling pathways in cancer cells; and immunotherapy, which targets the induction or augmentation of anticancer immune responses.

The concept of cancer immunotherapy goes back as far as the late nineteenth century, when William B. Coley observed tumour shrinkage and even disappearance following the injection of bacterial products in and around tumours[1]. Since then, many observations — such as the rare but well-documented occurrence of spontaneous remissions, the higher incidence of cancer in patients who are immunosuppressed, and the identification of tumour-specific antigens and lymphocytes — have stimulated research on strategies that aim to induce specific antitumour responses. Currently, allogeneic bone marrow transplantation and monoclonal antibodies that target tumour cells are two examples of broadly used and efficacious immunotherapies.

Over the past decades, considerable knowledge has been obtained on the components that are relevant in antitumour immune responses and immune escape mechanisms, and the cytotoxic T lymphocyte response has been identified as the most powerful and effective link in this vast network. This has led to a wealth of clinical studies in which these concepts have been tested. Given that most of the insight into specific immune responses was obtained in patients with melanoma, the majority of studies have been performed in this tumour type. Although clinical remissions have consistently been observed, for a long time the efficacy of most, if not all, immunotherapies in the treatment of solid tumours remained below a threshold that justified their use in the general patient population.

Several factors have hampered the development of cancer immunotherapy. First, compared to classic chemotherapy it is much more difficult to define the optimal dose and schedule for immunotherapies. This is due to an insufficient correlation between the maximal tolerated dose and the maximal effective dose, and the fact that many immunotherapies appear to have no maximal tolerated dose. If valid biomarkers for efficacy are also absent, as is almost invariably the case, the design of clinical trials becomes an educated guess. Given that there is a limit to the number of variations in dose and schedule that can be tested in clinical trials, potentially effective treatments may have been abandoned owing to negative outcomes of clinical trials that were not appropriate for testing the efficacy of the immunotherapeutic agent in question.

Second, the classical volumetric response criteria have been shown to be inadequate for evaluating the efficacy of immunotherapy. Immunotherapy is also considered to be less effective in patients with a large tumour burden, owing to the presumed correlation of immune suppression and tumour burden, and the delay in the time taken to translate immune responses into a survival benefit, which is not possible in most patients with advanced disease. This would favour the testing of these drugs in a low volume and/or microscopic disease setting, for which small sized Phase II studies are not appropriate and larger Phase III studies are required, which take more time and resources.

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Last, difficulties in the patentability of many immunotherapeutic strategies have resulted in a lack of interest and a subsequent lack of funding by the pharmaceutical industry, which has hampered their clinical development. In addition, the delicate collaborative links between academic and industrial partners have sometimes prevented access to adjuvants or key immunomodulators for developmental purposes. However, recent positive preclinical and clinical findings have given a great boost to immunotherapy. Here, we discuss some of the issues regarding the clinical development of cancer immunotherapies, the current status of the most commonly used strategies and promising new directions for the immunotherapy of solid tumours.

When is it ready for the ultimate test?

The selection of the optimal patient population and the optimal biological dose and schedule for immunotherapies are two major issues that still need to be resolved. With regard to the optimal patient population, patients with a minimal tumour burden are considered to have a better chance for response compared to patients with extensive and widespread disease, although this has never been formally investigated. Other clinical characteristics have not yet been shown to be of value. Promising results have been reported on immune signatures of tumours that may predict the efficacy of cancer vaccines[2]. However, finding the optimal biological dose and schedule is much more complicated for immunotherapies than for classic chemotherapy (BOX 1).

For example, the natural killer T cell (NK T-cell) activator α-galactosylceramide KRN7000, which showed antitumour activity in murine models in doses ranging from 0.01 μg per kg to 100 μg per kg, was tested in a Phase I study in patients with solid tumours, who were given weekly doses of KRN7000 starting at 50 μg per m^2. The trial was discontinued after the inclusion of 24 patients in whom the dose had been escalated to 4,800 μg per m^2 (a 96-fold increase from the starting dose); these patients did not show any signs of toxicity or an objective response, and some biological responses were observed that showed no correlation with dose[3].

Consequently, for most immunotherapies there is a lack of tools to select the best dose and schedule, and the decision to take a regimen to the ultimate test (usually a Phase III trial) is extremely difficult. Two examples of this dilemma are discussed in BOX 2. For interleukin-2 (IL-2), Phase III trials were initiated too late; for dendritic cell vaccines, Phase III trials were initiated too early.

Successes and promises

The clinical development of immunotherapy has received a new impulse resulting from several breakthroughs that have led to the approval of drugs and treatments for cancer (TIMELINE). Several other drugs that are currently in clinical trials hold great promise, either as single agents or in combination with other treatments.

Vaccine-based strategies. In contrast to the many accepted childhood vaccines that provide protection from dangerous infectious diseases, few vaccines exist that provide protection from cancer. Two vaccines that target the high-risk human papilloma virus (HPV) serotypes HPV-16 and HPV-18 have been approved for administration in young girls who are not yet sexually active[4,5]. These vaccines induce a humoral immune response against these serotypes of HPV, but they mostly fail once chronic HPV infection with genomic integration of viral genes encoding oncoproteins E6 and E7 has occurred. Unfortunately, there are usually several aetiological agents for most human cancers and therefore instead of being prophylactic, vaccine strategies need to be therapeutic.

Overlapping synthetic long peptides from HPV-16 E6 and E7 oncoproteins in incomplete Freund's adjuvant have been used to vaccinate women who are suffering from HPV-associated vulvar intraepithelial neoplasia Grade III (VINIII), which is a premalignant disease. At a follow-up after 1 year, nearly 50% of women had a complete response and HPV-16 had been eradicated[6]. Although these early results clearly show the potential of this approach, further clinical development is required. HPV-associated cancers and other virally induced cancers such as Hepatitis B and Hepatitis C virus-induced hepatocellular carcinomas and Epstein–Barr virus (EBV)-associated nasopharyngeal carcinomas may be most susceptible for vaccine-based immunotherapy strategies because the vaccine can be directed against viral proteins. It is estimated that approximately 15% of all cancers are virally induced[7]. In all other cases, cancer antigens are often nonmutated self proteins to which the rules of self tolerance can be applied. The T cell repertoire is mostly devoid of high-avidity clones as a result of thymic negative selection, and T cells that have escaped negative selection are subjected to highly regulated peripheral tolerance mechanisms. Immunotherapists face several challenges in overcoming these major obstacles and finding ways to break this tolerogenic milieu in order to attack the cancer.

Several viral vaccines have shown promising results in Phase II trials. One of these is the attenuated herpes simplex virus type 1 that is genetically modified to produce granulocyte–macrophage colony-stimulating factor (GM-CSF) (OncoVEX; BioVex). This so-called oncolytic immunotherapeutic agent is injected into the tumour and is being developed for the treatment of melanoma and head and neck squamous cell carcinoma[8]. The herpes simplex virus infects both healthy and tumour cells, but selectively multiplies in cancer cells. After causing destruction of the cancer cell, which results in the release of many tumour antigens that can be taken up by professional antigen-presenting cells, the virus may also induce a systemic antitumour immune response acting at distant sites. In addition, the virus can undergo another replication cycle in neighbouring cancer cells. In both diseases, a Phase III study is underway.

The first therapeutic cancer vaccine that was approved by the US Food and Drug Administration (FDA) for targeting a self protein is sipuleucel-T (Provenge; Dendreon), which is used for the treatment of metastatic castration-resistant prostate cancer. This

Box 1 | **Problems in designing immunotherapy trials**

Clinical end points for drug development, such as the maximal tolerated dose and tumour response rate, have been proven to be invalid for the development of immunotherapeutic agents. Compared to chemotherapeutic agents, for these agents there is much less, if any, correlation between drug exposure and efficacy and/or toxicity. Insufficient understanding of the mechanism of action of immunotherapies has further hampered the development of clinically useful methods to assess and monitor the desired immune responses as an alternative way to select the optimal dose, schedule and route of administration. In general, compared to classic chemotherapy it usually takes a prolonged period of time for immunotherapy to achieve clinical efficacy, with even initial transient tumour progressions having been observed. This has implications for the selection of patients, the monitoring of the clinical efficacy and the design of clinical trials. The biology of disease should allow a relevant immune response to develop, which may exclude patients in whom a quick tumour shrinkage is indicated. Established criteria for the evaluation of objective response and progression-free survival have been modified to suit the efficacy assessments of immunological drugs more effectively. Although some progress has been made in the development of immune monitoring assays, these assays have not shown consistent results among trials and they have not been validated. Consequently, an international collaboration has been initiated to standardize commonly used assays.

cell-based vaccine consists of autologous peripheral blood mononuclear cells, which include professional antigen-presenting cells that have been activated with a fusion protein (PA2024) of the prostate antigen prostatic acid phosphatase and the immunostimulant GM-CSF. Sipuleucel-T was approved based on results from a placebo-controlled Phase III randomized trial. Despite showing a lack of benefit in progression-free survival and the fact that tumour regressions were rare, an overall survival benefit of 4.1 months was demonstrated compared to placebo[9]. This is the first cell-based vaccine that has been brought to the market by a private company. However, the firm does face the challenge of producing sufficient vaccine for the target population: thousands of patients who are diagnosed with metastatic prostate cancer every year. In addition, the costs of this treatment — US$93,000 for three infusions — will not make it widely available[10].

Recently, the results of a randomized placebo-controlled Phase III study of idiotype-based vaccination plus GM-CSF in follicular lymphoma were presented, and they showed a prolonged duration of remission in patients who were treated with the vaccine[11]. In addition, a positive Phase III vaccination study was presented in metastatic melanoma, in which a melanocyte protein PMEL (also known as gp100) vaccine that was combined with high-dose IL-2 was compared with high-dose IL-2 alone; a statistically significant benefit in progression-free survival and a borderline significant benefit in overall survival was observed for the combination therapy[12]. Other examples of vaccination strategies that have now entered Phase III trials include a prostate-specific antigen-targeted poxviral vaccine in prostate cancer[13] and a melanoma-associated antigen 3 protein vaccine in non-small cell lung cancer[14].

Although these are examples of promising vaccine-based approaches, numerous other vaccine-based strategies have so far failed. Many of these approaches, including tumour cell vaccines, peptide vaccines, DNA vaccines and dendritic cell vaccines (reviewed in REF. 15), can induce immune responses in patients and sometimes they also induce clinical responses, but they have been unsuccessful at achieving clinical responses in a reproducible manner in a substantial number of patients with cancer. Interestingly however, in cases where clinical responses were induced, these were often long-lasting[16]. More knowledge on the schedules, routes, doses and adjuvants is required to optimally use these strategies. Also, in order to compare between and within different strategies, consistent methods for readouts, like validated T cell assays, need to be established (see below).

Cytokines and immune-targeted agents. In addition to IL-2, which has been discussed earlier, interferon-α is the only other cytokine to produce consistent and comparable response rates with perhaps a small survival benefit in renal cell carcinoma[17]. Given the need for its prolonged use and its associated toxicity, interferon-α treatment is not common, except in some countries in which it is used in the adjuvant setting in patients with high-risk melanoma in whom it may prolong disease-free survival but not overall survival[18].

A novel class of immune-targeted agents have demonstrated efficacy and durable responses in patients with metastatic melanoma. Cytotoxic T lymphocyte-associated antigen 4 (CTLA4) is part of a large family of molecules that are involved in the activation or inhibition of T cell immune responses. CTLA4 is expressed on both CD8 and CD4 T cells, including the FOXP3$^+$ regulatory T cells[19]. The role of CTLA4 as an attenuator of an immune response was demonstrated in mice that were deficient in CTLA4. These animals succumb to a lymphoproliferative disease within weeks after birth. Blockade of CTLA4 using monoclonal antibodies in tumour-bearing animals resulted in the rejection of established tumours. In mouse models of melanoma, this was accompanied by the development of skin depigmentation or vitiligo — an autoimmune destruction of skin-resident melanocytes[20].

These studies led to the development of monoclonal antibodies targeting human CTLA4. Two fully human antibodies have been developed and tested in metastatic melanoma. Both tremelimumab and ipilimumab (Yervoy; Bristol-Myers Squibb) showed objective response rates of approximately 10%[21,22] in early clinical trials. Interestingly, these responses were durable, sometimes lasting for years. Infusion of these antibodies was associated with the development of immune-related adverse reactions, such as colitis, dermatitis, hepatitis and endocrinopathies (such as thyreoiditis and hypophysitis), thus illustrating the role of CTLA4 in maintaining peripheral tolerance.

For ipilimumab, induction of these immune-related adverse events appeared to correlate with response, although these were not a prerequisite for a response. Ipilimumab was studied in a large placebo-controlled randomized trial in pretreated patients with metastatic melanoma, and it was the first treatment in 30 years to show a survival benefit in this disease[23]. In this study, the overall survival of patients in ipilimumab treatment groups after 2 and 3 years was above 20% compared to approximately 10% in the control vaccine group.

Cytotoxic T lymphocyte-associated antigen 4 (CTLA4). A co-inhibitory molecule that is expressed by T cells. Binding of its ligands B7.1 or B7.2 on antigen-presenting cells results in negative regulation of T cell activity.

Based on the outcome of this pivotal trial, ipilimumab was recently approved by the FDA for the treatment of patients with advanced-stage melanoma.

Interestingly, tremelimumab failed to show a survival benefit in previously untreated patients with metastatic melanoma[24]. Possible explanations for the discrepant results with these two antibodies may be the unplanned use of salvage treatment with ipilimumab in the control group, or the difference in the schedule of administration (monthly for ipilimumab versus every 3 months for tremelimumab) given the dynamics of lymphocyte rebound, which favours a monthly schedule. It could also be postulated that the positive results of the second-line ipilimumab trial were related to the positive immunological effects of prior exposure of patients to cytotoxic chemotherapy, which is discussed later. However, the first results of a randomized controlled trial comparing dacarbazine plus placebo with dacarbazine plus ipilimumab in previously untreated patients with unresectable stage III or stage IV melanoma are now available. These results show an improved overall survival for the ipilimumab treatment group[25], thus illustrating the potency of this drug in patients with melanoma.

CTLA4-specific antibodies are a novel treatment paradigm for the blockade of several other immune checkpoint molecules from the CD28:B7 family, such as programmed cell death protein 1 (PD1; also known as PDCD1), programmed cell death ligand 1 (PDL1; also known as CD274), B and T lymphocyte attenuator, CD276 antigen (also known as B7H3) and V-set domain-containing T cell activation inhibitor 1 (also known as B7x)[26]. For several cancers, it was shown that the expression of PDL1 is correlated with poor clinical outcome[27]. Ligation of PD1, which is expressed by tumour-specific T cells infiltrating the tumour, renders these cells inactive. Blockade of this interaction by PD1-specific monoclonal antibodies revitalizes these tumour-specific T cells in some patients, which results in objective tumour responses. In addition, autoimmune phenomena have been reported with PD1-specific antibodies, albeit less frequently than with CTLA4-specific antibodies.

It has been demonstrated that regulatory T (T_{Reg}) cells suppress immune responses in the tumour microenvironment of patients with cancer[28]. Initially, it was observed that the intratumoural presence of T_{Reg} cells was correlated with a poor prognosis in several cancer types[28,29]; further studies in colorectal cancer showed that the presence of T_{Reg} cells was in fact associated with an improved clinical outcome[30], whereas in follicular lymphoma it was the architectural distribution of T_{Reg} cells in the tumour that determined prognosis[31].

Studies that target the depletion of T_{Reg} cells in patients with cancer are hampered by the fact that T_{Reg} cells do not have an exclusive surface molecule that can be targeted. Two agents have been used that target the IL-2 receptor, which is upregulated on T_{Reg} cells: daclizumab (Zenapax; PDL BioPharma), a monoclonal antibody that targets the α-chain of the IL-2 receptor, and denileukin diftitox (Ontak; Eisai), which is a fusion protein of IL-2 and diphtheria toxin. Small clinical studies in melanoma and prostate cancer that have combined these agents with vaccines have shown conflicting results[32–35], which could be explained by the fact that the IL-2 receptor is upregulated by not only T_{Reg} cells but also by activated T effector cells, and the timing of the two treatment strategies

Box 2 | **When to put immunotherapy to the final test? Two examples**

Interleukin-2

Interleukin-2 (IL-2) is a T cell growth factor that has been shown to induce objective tumour regressions in some patients with metastatic renal cell cancer and melanoma[75,76]. In both diseases there was a high unmet need for active drugs, and high-dose IL-2 was subsequently approved by the US Food and Drug Administration for use in these tumour types. High-dose IL-2 has consistently produced response rates of approximately 15% in selected groups of patients, with approximately half of these responses being durable and complete[115]. With IL-2 being the first drug to show clinically relevant efficacy in a minority of patients with these tumour types, the predominant view in the 1980s was that only a few adjustments in dose or testing in combination with other drugs were required to achieve a breakthrough in cancer treatment.

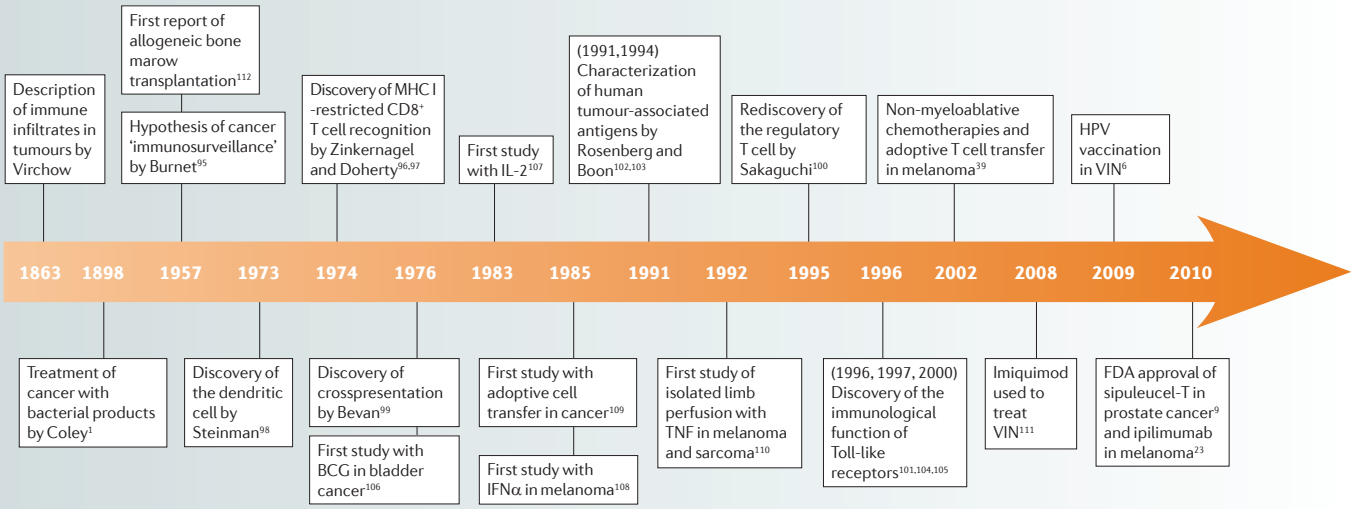
Numerous Phase I–II studies were subsequently carried out, which took many years and involved thousands of patients who received IL-2 in many different doses and schedules, at different routes of administration, and in combination with a variety of other cytokines and/or cytotoxic drugs. However, the initial efficacy results were not improved and toxicity had increased, especially in combination with chemotherapy[77]. The few randomized Phase III trials that were performed were either underpowered or they did not have a control group that reflected standard treatment in general practice. A Phase III trial comparing IL-2 with observation was never performed, as IL-2 was considered by many to be too promising for such a simple trial and thus a waste of time. To date, despite the fact that durable complete remissions have consistently been observed — albeit in a minority of patients — the use of high-dose IL-2 is largely limited to patients in the United States and has generally not been accepted as standard treatment, owing to the high costs and acute toxicity that accompany its use, and the fact that a benefit in overall survival has never clearly been demonstrated.

Remarkably, there is still limited knowledge on the exact mode of action of IL-2, as the majority of clinical trials did not involve a translational research programme. The role of IL-2 in the homeostasis of regulatory T cells has been identified as a factor that may negatively influence its antitumour response[78].

Dendritic cells

Dendritic cells are professional antigen-presenting cells that are capable of inducing antigen-specific T cell and B cell responses[79]. As such, they have been applied in clinical studies in patients with cancer. Given that most of the immunogenic antigens have been identified in melanoma, the majority of studies have been performed in this tumour type[80]. Although from a clinical point of view the limited use of one or two antigens may be inferior to the use of whole tumour preparations when they are pulsed on dendritic cells, the advantage of this strategy is that, at least in theory, it allows the induced immune response to be monitored more effectively. As is the case for immunotherapy in general, many practical questions also arose for dendritic cell therapy, concerning for instance the optimal method of culture, the number of dendritic cells to administer, the schedule and the route of administration. Although progress was certainly made by small-scale studies using extensive monitoring and translational research[48–50,81], and objective and durable clinical remissions have been observed — albeit in a minority of patients[80] — the best way to administer dendritic cells has not yet been established. However, less than 2 years after one of the first publications on dendritic cell therapy was published[82], a prospective Phase III trial was initiated in 2000 that compared standard dacarbazine chemotherapy with a dendritic cell vaccine as first-line treatment of patients with metastatic melanoma[83]. The trial was prematurely discontinued at the first interim analysis after the inclusion of 103 patients owing to lack of efficacy. The authors identified several possible negative contributing factors, including a variable quality of the dendritic cell vaccine among participating centres, the lack of a T helper epitope, and a suboptimal maturation state, dose, and route of administration of the dendritic cells. In retrospect, this trial was carried out too soon and was performed at a time when dendritic cell vaccination was too early in its development. Obviously, although dendritic cell vaccination is still under investigation in many centres worldwide, one should not underestimate the negative impact of such a trial result.

Timeline | **The history of cancer immunotherapy**



Important basic immunological discoveries and key clinical trials are shown. BCG, bacille Calmette–Guérin; IFNα, interferon-α; IL-2, interleukin-2; MHC, major histocompatibilty complex; TNF, tumour necrosis factor; VIN, vulvar intraepithelial neoplasia.

appears to be crucial[34,36]. Novel strategies for specifically targeting T_{Reg} cells and abolishing their suppressive function are therefore required.

Although its exact mechanism of action is not completely understood, instillations of bacille Calmette–Guérin (BCG) for the treatment of intermediate or high-risk Stage Ta and T1 urothelial carcinoma of the bladder have become standard of care. BCG instillation was compared to epirubicin in a large randomized Phase III trial that was carried out by the European Organization for Research and Treatment of Cancer (EORTC). The study showed unequivocally that immunotherapy reduced local recurrences and the development of distant metastases, and improved overall and disease-specific survival[37]. It is assumed that the inflammatory response that is induced by the mycobacteria results in tumour cell death and the spread of tumour antigens to the immune system, which leads to immune control of bladder cancer.

Adoptive T cell therapies. Whereas vaccine-based strategies and CTLA4-specific antibody treatment aim to induce or augment endogenous antitumour T cell responses, adoptive T cell therapies are based on the infusion of large numbers of tumour-specific T cells. These tumour-specific T cells can be derived from the tumour environment (such as tumour-infiltrating lymphocytes (TILs)), from peripheral blood or they can be genetically modified to express a high affinity antitumour T cell receptor (TCR). The feasibility of this strategy was first shown in post-transplant lymphoproliferative disease, which is an EBV-associated B cell lymphoma that develops under conditions of severe T cell immunosuppression. Infusion of EBV-specific T cells led to the rejection of lymphomas and restored the severely hampered EBV-targeted immunity[38].

In 2002, the National Cancer Institute (NCI) reported the outcome of a Phase II study of adoptive transfer of tumour-reactive TILs in pretreated patients with metastatic melanoma[39]. TILs that were obtained from a melanoma metastasis were grown *in vitro* with high-dose IL-2 to overcome their anergic state, which had been induced by the tumour environment. Large quantities of cells were cultured and re-infused into patients. Preconditioning of patients with cyclophosphamide and fludarabin, agents that induce severe lymphocytopaenia, was shown to be necessary for the survival and expansion of the infused TILs. Single-arm Phase II studies from separate institutions have shown objective response rates of 50% in patients with metastatic melanoma who are receiving this treatment[40,41]. Approximately 10% of patients obtain a complete response, which may be durable. Side effects resulting from the infusion of TILs were mostly mild, consisting of vitiligo or uveitis in some patients, but both the non-myeloablative chemotherapy and the high-dose IL-2 resulted in significant well-known toxicities.

As well as the costs and labour intensity of the treatment, a major drawback of this approach is that TILs cannot be cultured from all patients. In addition, the specificity of T cells within the TIL graft that are responsible for the clinical effects is unknown. This strategy could be refined by determining the tumour antigen specificity. It could also be exploited to clone high-affinity tumour-specific TCRs from these cells that can serve as donor TCRs to genetically modify unselected peripheral blood T lymphocytes for adoptive transfer[42]. This so-called *TCR* gene transfer is currently being tested in several clinical trials. The first studies performed by the NCI using melanoma antigen recognized by T cells 1 (MART1) and/or gp100-specific TCRs led to objective

responses in 20–30% of patients, but they also led to more severe toxicity as was observed using TILs. Some patients developed extensive vitiligo, acute uveitis and hearing loss owing to destruction of melanocytes in the epidermis, the eye and stria vascularis of the inner ear, respectively[43].

In order to use adoptive transfer of genetically modified T lymphocytes with tumour-specific TCRs, the choice of target antigen is crucial. Lethal toxicity can be induced by infusion of T lymphocytes that are genetically engineered to express a trastuzumab (Herceptin; Roche/Genentech)-based chimeric antigen receptor targeting ERBB2 (also known as HER2) (REF. 44), which illustrates that T cells that are activated *in vitro* can detect low-level ERBB2 expression by the lung parenchyma. Moreover, in patients with metastatic renal cell carcinoma, the adoptive transfer of genetically modified T cells expressing a chimeric antigen receptor that is specific for carbonic anhydrase 9 — which is highly expressed in renal carcinoma cells — led to acute liver toxicity owing to low-level expression of the target protein on bile ducts[45].

By contrast, antigens that have their expression restricted to tumour cells, such as cancer/testis antigen 1 (also known as NY-ESO-1), can be targeted by TCR-redirected T cells without the risk of inducing systemic toxicity[46]. Another new therapeutic target is CD19, which is expressed by mature B cells and most non-Hodgkin's lymphoma B cells. In patients with follicular lymphoma who were treated with T cells that were transduced with the CD19-specific chimeric antigen receptor, early results have been promising and have shown acceptable toxicity[47].

Monitoring the immune response

The ability to reliably measure the expected immune response of a given immunotherapy would greatly facilitate its development, and assays that could correlate clinical responses with the induced immune response would provide a strong support from a mechanistic point of view. However, at present there is a lack of robust assays to monitor the antitumour immune response. Although there is an abundance of different assays that are being used to measure tumour antigen-specific T cell responses, with some of these having indeed been shown to correlate with clinical outcome[48–50], these assays have not shown consistent results among trials, and none has been validated in prospective clinical trials.

Researchers from Europe and the United States have started a project called MIATA (Minimal Information About T cell Assays) to standardize commonly used assays such as the enzyme-linked immunosorbent spot assay (ELIspot), major histocompatibility complex tetramer assays and intracellular cytokine assays[51]. The establishment of universally accepted guidelines for performing and presenting immunological assays for and in scientific publications will create a framework that will allow the comparison of immune responses across clinical trials. However, the use of immune responses as a surrogate end point in clinical trials remains limited so far. In addition, more emphasis should be put on monitoring immune responses at the effector site, given that T cell responses in peripheral blood and the tumour microenvironment can show markedly different patterns[52].

Monitoring the clinical response

The classic World Health Organization (WHO)[53] and Response Evaluation Criteria In Solid Tumours (RECIST)[54,55] criteria that are applied to measure the efficacy of cytotoxic chemotherapy depend on tumour shrinkage, and any increase in tumour size beyond a certain level as well as the appearance of new lesions is considered as a treatment failure. However, there is now ample evidence that these criteria do not apply to immunotherapy. Immunotherapy-induced tumour regressions have been well documented after initial progression and even after the appearance of new lesions, which are presumably caused by the infiltration of lymphocytes into tumours. For example, in some patients receiving ipilimumab, metastases may grow or new lesions may even develop before there is a decline in total tumour burden[56].

These observations have led to the proposal of novel immune-related response criteria, as response evaluation according conventional response criteria (such as WHO and RECIST) can lead to unwanted early cessation of treatment owing to initial tumour growth[57]. These observations reflect the different dynamics of the immune response compared with the direct effects of cytotoxic drugs on cancer cells[58]. This also has important implications for the design and conduct of clinical trials, such as the planning of interim analyses.

Immunotherapy with chemotherapy

For a long time, the dogma has been that the myelosuppressive effects of chemotherapy would prevent its combined use with immunotherapy. However, in recent years this has been challenged by a large amount of experimental data (FIG. 1; reviewed in REF. 59).

Early studies in animal models in the 1970s demonstrated that local intratumoural administration of cytotoxic compounds such as doxorubicin and actinomycin D could induce a systemic immune response. Treatment with these agents resulted not only in clearance of the injected tumour and loco-regional metastases but also in subsequent protective immunity when the immune system was re-challenged with tumour cells[60]. At the same time, systemic treatment with cyclophosphamide was found to enhance immune responses[61], and this is now known to be mediated through the depletion of T_{Reg} cells[62].

The notion of a possible synergistic effect of chemo- and immunotherapy has been revived through studies by Nowak and colleagues[63], who showed that the treatment of tumour-bearing mice with gemcitabine results in enhanced crosspresentation and T cell activation. Subsequent studies by Zitvogel, Kroemer and colleagues[59] have demonstrated that chemotherapy-induced cell death can indeed invoke an immune response, depending on the biochemical cell death cascade that is induced by the drug. For example, calreticulin is translocated to the surface of tumour cells following treatment with anthracyclins,

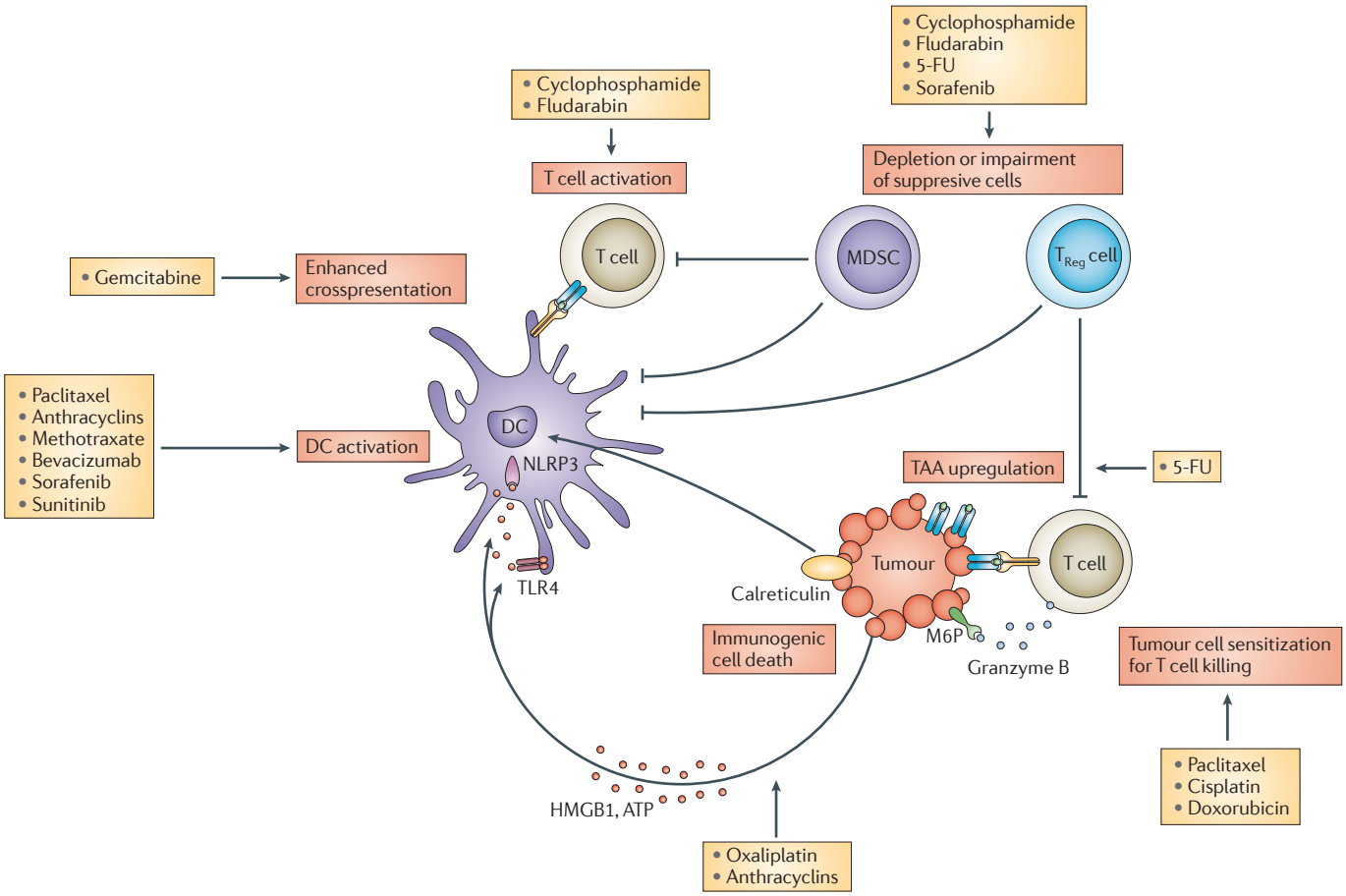


Figure 1 | **Examples of positive immunological effects of cytotoxic chemotherapeutics and targeted therapies.** Some of the positive immunological effects of cytotoxic chemotherapeutics and targeted therapies include: the induction of immunogenic cell death through the exposure of calreticulin and release of ATP and HMGB1 (high mobility group protein box 1) and subsequent activation of dendritic cells through NLRP3 (NOD-, LRR and pyrin domain-containing 3) and TLR4 (Toll-like receptor 4) respectively[64–66]; direct activation of dendritic cells (DCs)[84,85]; enhanced crosspresentation[63,86]; T cell activation[87,88]; depletion or impairment of suppressive immune cells[89,62,90–92]; tumour-associated antigen (TAA) upregulation[93] and enhancement of tumour cell susceptibility to be killed by T cells[94]. 5-FU, 5-fluorouracil; M6P, mannose 6-phosphate; MDSC, myeloid-derived suppressor cell.

Toll-like receptor 4
(TLR4). A member of the Toll-like receptor family of innate immune receptors that recognize molecular patterns of microbes or danger signals derived from tissue damage.

NLRP3
NOD-, LRR and pyrin domain-containing 3. This is a pyrin-like protein that is involved in inflammation and immune responses.

Crosspresentation
The mechanism by which certain APCs take up, process and present extracellular antigens on MHC class I molecules to stimulate cytotoxic T cells. This property is atypical, as most cells exclusively present peptides from endogenous proteins on MHC class I molecules.

Immunogenic cell death
The process of immunogenic cell death takes place when dying tumour cells provide an alerting or activating signal to the immune system.

which results in enhanced phagocytosis of tumour cells by dendritic cells[64]. In addition, anthracyclin- or oxaliplatin-treated tumour cells release the alarmin protein high mobility group protein B1 (HMGB1) and ATP, which results in Toll-like receptor 4 (TLR4) and NLRP3 inflammasome stimulation, respectively[65,66].

These data clearly show that chemotherapeutic agents can have a beneficial effect on the antitumour immune response, and may even imply that at least part of the clinical effect of chemotherapy depends on its immunological effects. This is supported by the observation that patients with colorectal cancer carrying a loss-of-function *TLR4* allele that results in a low binding affinity for HMGB1 exhibited reduced progression-free and overall survival in response to oxaliplatin compared to patients carrying the normal *TLR4* allele[67].

In addition, recent data from clinical studies in patients with cancer have shown that T cell induction is not hampered by chemotherapy treatment[68,69]. Therefore, the goal for the near future is to enforce this partial immunotherapeutic effect of classic cancer cytotoxic chemotherapy by combining it with immune stimuli. Obvious candidates for combination treatments with chemotherapeutics include cancer vaccines, TLR agonists and antibodies that target immune checkpoints, such as CTLA4, PD1, PDL1 or PDL2.

The optimal sequence of immunotherapy–chemotherapy combination treatments remains to be established. For example, it has been shown that for effective crosspresentation it is crucial for TLR ligands and antigens to be in the same place at the same time[70,71]. Theoretically, this would favour a more or less simultaneous scheduling of TLR ligands and chemotherapeutics, as the latter provide tumour antigen release. There is currently a lack of studies to draw any conclusion on a possible optimal treatment schedule for the combination of chemotherapeutics and antibodies targeting immune checkpoints. Furthermore, it should be stressed that as different cytotoxic drugs have different immunological effects, it is conceivable that

optimal treatment strategies will differ depending on both the cytotoxic compound and the immunotherapeutic approach. Consequently, additional studies in animal models and small clinical studies are urgently needed to help us to design the most optimal treatment schedules.

Similarly to some classical chemotherapeutic agents, the therapeutic effect of some novel targeted agents appears to depend on the immune system; for example, it has been shown that the presence of $CD4^+$ T cells is required to achieve the full therapeutic efficacy of imatinib (Gleevec; Novartis) against *Myc* oncogene-addicted tumour cells[72]. These data imply that immunocompetent models should be used during the preclinical developmental phase of targeted anticancer agents.

Given the accumulating evidence for the positive immunological effects of novel targeted tyrosine kinase inhibitors and antibodies (FIG. 1), there is also a rationale to test the combination of these targeted drugs with immunotherapy. One interesting combination is the BRAF kinase inhibitor vemurafenib (previously named PLX4032) and ipilimumab. Vemurafenib has shown a high response rate in a recent Phase I–II trial and a subsequent Phase III study[113] in patients with *BRAF*-mutated metastatic melanoma[73]. However, these responses were short-lived in many patients. The progression-free survival interval, however, forms a potential time frame for the expansion of tumour-specific T cells by CTLA4-specific antibodies in the absence of tumour-induced immune suppression. Lastly, local therapies such as radiation[65,74] and radiofrequency ablation[114] have also been shown to induce immune effects that may be further exploited in combination with immunotherapies.

Conclusions

For many years, clinical progress in the field of immunotherapy has been slow. However, the recent proof of clinical efficacy of several immunotherapeutic drugs in patients with cancer has boosted the development of this treatment modality. An improved understanding of immunotherapy-induced responses has resulted in novel ways of evaluating responses to treatments. The development of valid assays to monitor the relevant immune responses currently remains one of the greatest hurdles to overcome. A completely new and promising field of clinical research has been uncovered by recent data that show a previously unexpected but beneficial effect of the combination of immunotherapy with both new and more classic cancer treatment modalities such as targeted agents and chemotherapy. Although many questions remain to be answered — such as the selection of the dose, sequence and patients — the greatest challenge is in the design of clinical trials in which these effects can be maximally exploited.

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