Therapeutic vaccines for cancer: an overview of clinical trials

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Abstract | The therapeutic potential of host-specific and tumour-specific immune responses is well recognized and, after many years, active immunotherapies directed at inducing or augmenting these responses are entering clinical practice. Antitumour immunization is a complex, multi-component task, and the optimal combinations of antigens, adjuvants, delivery vehicles and routes of administration are not yet identified. Active immunotherapy must also address the immunosuppressive and tolerogenic mechanisms deployed by tumours. This Review provides an overview of new results from clinical studies of therapeutic cancer vaccines directed against tumour-associated antigens and discusses their implications for the use of active immunotherapy.

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Introduction

Immunotherapies against existing cancers include active, passive or immunomodulatory strategies. Whereas active immunotherapies increase the ability of the patient's own immune system to mount an immune response to recognize tumour-associated antigens and eliminate malignant cells, passive immunotherapy involves administration of exogenously produced components, such as lymphocytes or antibodies, to mediate an immune response. Immunomodulatory agents are not targeted at specific antigens, but enhance general immune responsiveness and are intended to amplify anticancer immune responses. Although there is some overlap between these categories, they provide a useful conceptual framework.

Active immunotherapy has been in clinical use for a long time (Figure 1 (Timeline)). The concept that the immune system can be harnessed by vaccination to specifically eradicate malignant cells has been repeatedly demonstrated in animal models, but was sometimes questioned by the results of trials in humans.[1,2] Malignant cells always harbour mutations and are often genetically unstable leading to numerous changes in the repertoire of epitopes (so-called neo-antigens) they present, suggesting that, in theory, tumours should be 'visible' to T lymphocytes.

The mechanisms required to mount effective antitumour responses have been reviewed by Mellman and colleagues (Figure 2).[3] In the first step, tumour-associated antigens (TAAs) must be directly presented by tumour cells or captured, processed and presented by dendritic cells. The second step requires presence of suitable activation and/or maturation signals that allow dendritic cells to differentiate, migrate to the lymph nodes, and present TAAs to naive T cells. The third step involves expansion of T cells in sufficient numbers so as to recognize and eliminate tumour cells. However, in the absence of suitable maturation signals, antigen presentation leads to T-cell anergy or production of regulatory T cells (T_{REG}) that suppress effector T cells. Finally, antigen-educated T cells must leave the lymph node, traffic to infiltrate the tumour and persist for long enough to kill the malignant cells. In this Review, we focus on immunotherapies that aim to induce or augment immune responses against TAA. The use of vaccines directed against oncogenic viruses for the prevention or treatment of cancer are not discussed.

Competing interests
I.M. has provided consultancy to and received honoraria and research funding from Bristol-Myers Squibb. G.G. is employed by Ultimovacs AS and has stocks in Ultimovacs AS and Targovax AS and he has provided consultancy to and received honoraria from KAEL-GemVax and Lytix AS. C.H. is an employee and has stocks in BioNTech and GANYMED Pharmaceuticals, has provided consultancy to Apceth, Bayer, Baxter, BioNTech, GANYMED, immatics, Merck KGaA, SuppreMol and TRON, and received honoraria from the Clinical Cancer Research (CCR, UK), Karolinska University, Stockholm (Sweden), Kurume Cluster (Japan), and Swiss National Science Foundation. G.P. has provided consultancy to CureVac and received honoraria from Recombio. S.S. and N.T. have provided consultancy and received honoraria from Merck KGaA and KAELGemVax, and has stocks in Kancera AB. I.F. has received a research grant from Merck KGaA. Views and opinions described do not necessarily reflect those of Merck KGaA. H.M. has received honoraria and research funding from Merck KGaA. W.G., J.W. and C.Z. declare no competing interests.

Principles of active immunotherapy

Antigens

Active immunotherapy encompasses a diverse range of strategies, some of which target multiple, undefined antigens whereas others specifically target a particular antigen or a group of antigens. Natural immune surveillance and artificial immunotherapy can lead to selective survival of tumour cells that lack immunogenic epitopes, a process called immunoediting.[4] Polyvalent vaccines (autologous or allogeneic) are derived from whole tumour cells or dendritic cells—fused with tumour cells,

Key points

- Development of vaccines for the treatment of cancer has posed many challenges, but results from some recent studies have confirmed the potential for clinical benefit
- Progress has been driven by advances in our understanding of cancer immunology and, in particular, the nature and dynamics of the tumour microenvironment
- Many clinical trials may have failed to adequately account for how vaccines differ from other cancer therapies, and for immunosuppressive mechanisms that operate in the tumour microenvironment
- Predictive biomarkers that can identify subpopulations of patients most likely to benefit from active immunotherapy are needed
- Evidence from clinical trials suggest that clinical benefit might be greatest in patients with less advanced-stage malignancies
- Future strategies should include steps to modify the tumour microenvironment to optimize tumour-specific immune responses

loaded with tumour lysates, or transfected with tumour-derived RNA or DNA—and should in theory be less susceptible to tumour antigen loss. Additionally, polyvalent vaccines are likely to carry mutations that drive the tumour's malignant phenotype. However, only a fraction of the targeted antigens will be specific to tumour cells, and the production of personalized polyvalent immunotherapies is often time and labour intensive.

Antigen-specific active immunotherapies are more suitable for reproducible, large-scale production than whole tumour or dendritic cell vaccines. Most antigen-specific immunotherapies have incorporated a single antigen, and narrow epitope specificity might have contributed to lack of efficacy in some trials.[5,6] Moreover, most tumour antigens are based on wild-type protein sequences that are overexpressed in many cancers of the same tissue origin. As tumour antigens are derived from self-antigens, it is likely that high-avidity T-cell receptors (TCR) will have been deleted from the repertoire. An immune response directed against a single antigen can induce immunity against other TAAs,[7,8] and this 'epitope spreading' might mitigate the potential weakness of narrow epitope specificity. Immunization with long peptides incorporating multiple epitopes[9,10] or a mixture of different peptides,[11,12] often incorporating both major histocompatibility complex (MHC) class I and class II epitopes, can improve immunogenicity.

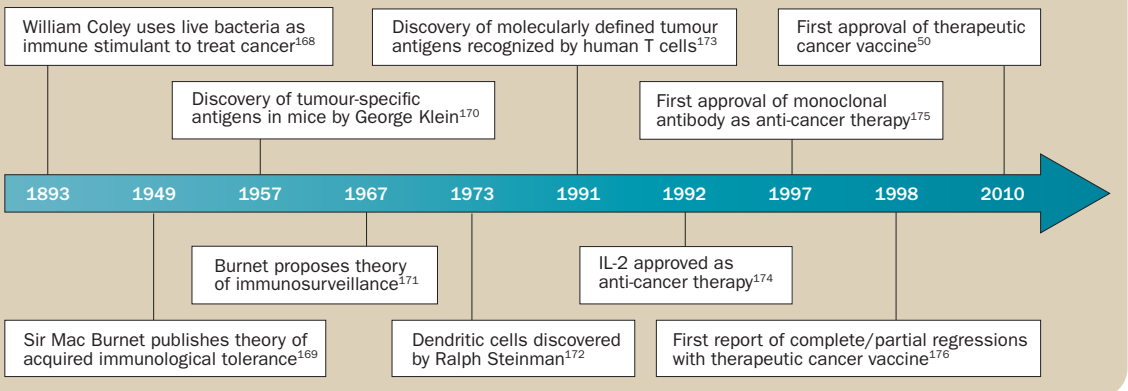
Many TAAs have been identified, some of which are shared with normal tissues, whereas others are unique to tumours (Box 1). Shared TAAs include cancer–testis and differentiation antigens that are either silent or expressed at low levels in normal tissues, but are transcriptionally activated in certain tumours. Individual TAAs are characteristic of single neoplasms, but the identification of these TAAs through mass screening is currently not feasible in routine practice. Immune peptidome analysis of HLA-bound peptides,[13] and whole-exome sequencing of tumour cells[14] coupled with epitope prediction by bioinformatics are being used to identify new TAAs and disease biomarkers and might bring truly personalized immunotherapy a step closer to becoming an affordable reality in clinical practice.

Adjuvants and delivery vehicles

Adjuvants are substances or interventions that, when combined with an antigen, enhance antigen immunogenicity and elicit the desired immune response. In cancer therapy, the desired response involves predominantly the activation of IFNγ-producing type 1 T helper cells (T_H1) and cytotoxic T lymphocytes (CTLs). Classical adjuvants—such as alum, used in prophylactic vaccines—promote type 2 T helper cells (T_H2)-dependent humoral immunity, but rarely induce strong T_H1-dependent responses.[15] This shortcoming has driven the development of a range of new adjuvants (Box 1).

Water-in-oil emulsions, such as Freund's adjuvants, have been used widely in cancer vaccines, but failed to prove efficacy in clinical trials. Water-in-oil emulsion adjuvants were originally designed for slow release of antigen from a depot at the vaccination site. This strategy might work well with vaccines aimed to elicit potent antibody responses, but, as Hailemichael and colleagues[16] demonstrated, this slow-release mechanism might be detrimental if the purpose is to generate tumour-specific CTL responses, as activated T cells become trapped and accumulate at the vaccination site rather than in the tumour. This discrepancy might explain the puzzling lack of an association between immune responses and clinical outcomes observed in many trials. For example, a glycoprotein 100 (gp100) peptide vaccine with water-in-oil adjuvant has failed to improve survival when

Timeline | Milestones in the development of active immunotherapy



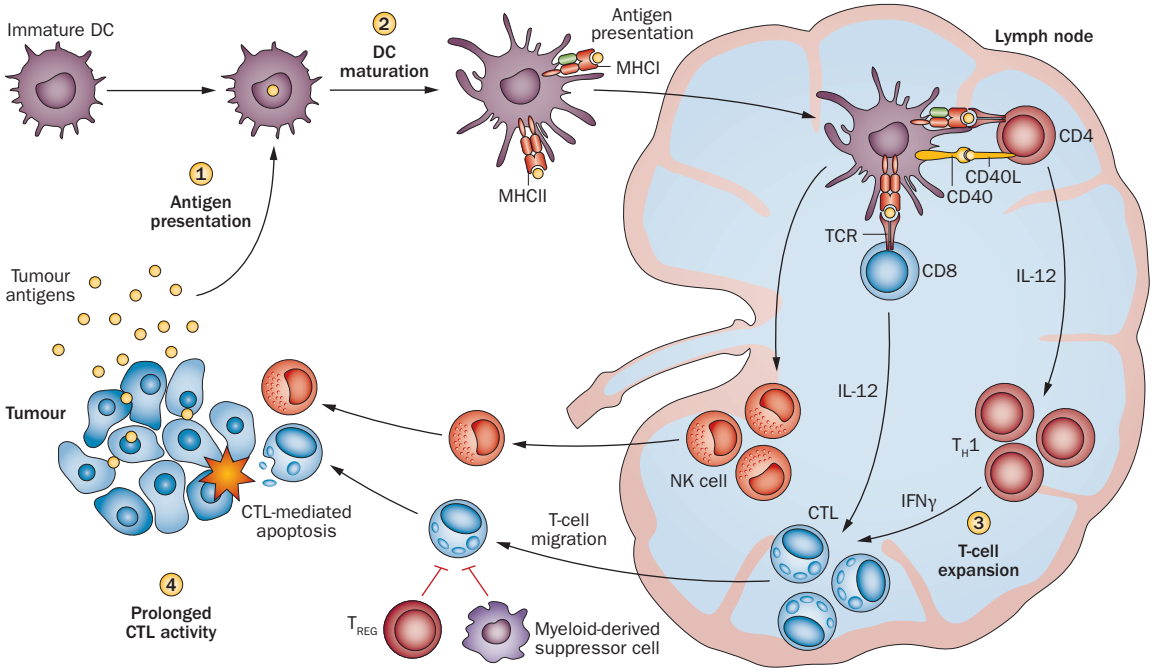


Figure 2 | Steps in the development of a cellular immune response against tumour-associated antigen. Multiple steps and processes are involved in the generation of an immune response directed against tumour antigens, offering multiple opportunities for therapeutic enhancement. For example, immunization can be used to present tumour-associated antigens to DCs (1). Tumours can deploy a number of immunosuppressive factors, including TGF-β and activators of STAT3 phosphorylation, which inhibit DC maturation (2). Small-molecule inhibitors of these factors can be used to promote DC maturation and enhance antitumour activity.[177–179] T-cell expansion (3) can be supplemented by adoptive transfer of activated antitumour T cells, expanded or genetically modified in culture to recognize tumour antigens.[105] Immunostimulatory monoclonal antibodies (such as agonists of CD40, CD137 or OX40)[180,181] and cytokines (such as IL-12, IL-15, IL-21)[182] can also enhance the performance of active immunotherapies or the action of adoptively transferred T cells. Finally, new studies have demonstrated the clinical potential of checkpoint modifiers that interfere with key immunosuppressive mechanisms (such as CTLA-4 and PD-1) and prolong CTL activity (4).[17,46] Abbreviations: CD40L, CD40 ligand; CTL, cytotoxic T cell; DC, dendritic cell; IFN-γ, interferon-γ; MDSC, myeloid-derived suppressor cell; MHC, major histocompatibility complex; MHCI, MHC class I; MHCII, MHC class II; NK, natural killer cell; PD-1, programmed cell death protein 1; TCR, T-cell receptor; TGF-β, transforming growth factor β; T_H1, type 1 T helper cells; T_{REG}, regulatory T cells.

added to ipilimumab (a monoclonal antibody directed against cytotoxic T-lymphocyte protein 4 [CTLA-4]) in patients with metastatic melanoma.[17] These findings should encourage the use of other forms of adjuvants in future trials.

A single adjuvant is unlikely to result in clinically-relevant antitumour activity and consequently, many active immunotherapies incorporate multiple adjuvants. Vaccines could also incorporate antigens and adjuvants in a delivery vehicle that is immunogenic, such as a recombinant viral vector or liposomal microspheres. Cultured mature dendritic cells are a type of delivery vehicle with natural adjuvant properties. However, the best source of dendritic cells, the most suitable way to load tumour antigens, the most efficacious regimen to activate and mature the dendritic cells, the best route of administration and the optimal dosing scheme are debated. In an orthotopic mouse model of head and neck or lung cancer, intranasal (but not intramuscular) immunization with dendritic cells from the lung parenchyma, but not the spleen, triggered homing properties on induced $CD8^+$ T cells to the mucosa. This process influenced the effectiveness of tumour growth control.[18] Importantly, HLA class I presentation of exogenous antigens to $CD8^+$ T cells, thereby inducing them to become CTLs (a process called cross-priming) seems to be mainly carried out by specialized dendritic cells, which in humans primarily involve the $CD141^+$ (BDCA-3) and $CD103^+$ subsets.[19,20] In addition to their role as antigen-presenting cells, both myeloid and plasmacytoid subsets of natural dendritic cells have been shown to kill tumour cells directly.[21]

Another promising strategy is to prime the immune system to the target antigen(s) by vaccination with a recombinant viral vector or DNA or mRNA vaccine and then boost with a second vaccine that incorporates the same antigens, but in a different vector (for example, vaccinia viral vector followed by fowlpox viral vector). Such heterologous prime-boost strategies generally provide strong cellular immune responses[22] and improved tumour control in preclinical studies.[23,24]

Very little data are available on how to compare different adjuvant strategies in humans. The multicomponent nature of active immunotherapies makes their evaluation difficult, as effects of adjuvants might vary with immunization schedule, route of administration and immune status.[25]

Box 1 | Antigens and adjuvants

The variety of tumour antigens that are recognized by T cells provides a range of potential targets for active, antigen-specific cancer immunotherapy. Adjuvants are needed to potentiate cellular immune responses to the antigen. Active immunotherapies often combine multiple antigens and/or adjuvants with the aim of generating a robust immune response.

Antigens

Shared antigens

- Cancer–testis antigens: BAGE, GAGE, MAGE, NY-ESO-1
- Differentiation antigens: CEA, gp100, Melan-A, PSA, tyrosinase
- Overexpressed antigens: HER2, hTERT, p53, survivin

Unique antigens

- Oncogene-associated antigens: β-catenin-m, HSP70-2/m, KRAS

Shared antigens with unique mutations

- Glycans: GM2, MUC1

Adjuvants

- Cytokines/endogenous immunomodulators: GM-CSF, IL12
- Microbes and microbial derivatives: BCG, CpG, Detox, MPL, poly I:C
- Mineral salts: Alum
- Oil emulsions or surfactants: AS02, MF59, Montanide™ ISA-51, QS21
- Particulates: AS04, polylactide co-glycolide, virosomes
- Viral vectors: Adenovirus, vaccinia, fowlpox

Abbreviations: AS, adjuvant system; BAGE, B melanoma antigen; BCG, bacillus Calmette-Guérin; CEA, carcinoembryonic antigen; CpG, cytosine-phosphate diesterguanine; GAGE, G antigen 12B/C/D/E; GM2, ganglioside GM2; GM-CSF, granulocyte-macrophage colony-stimulating factor; gp100, glycoprotein 100; HER2, human epidermal growth factor receptor 2; HSP70-2/m, heat shock-related 70 kDa protein 2 mutated; hTERT, telomerase transcriptase; MAGE, melanoma antigen-encoding gene; Melan-A, melanoma antigen recognized by T cells 1; MPL, monophosphoryl lipid A; MUC1, mucin-1; NY-ESO-1, cancer–testis antigen 1; PSA, prostate-specific antigen; QS-21, a plant extract derived from *Quillaja saponaria* that enhances the immune responses to antigens targeted by vaccines.

Overcoming immune suppression

Tumours can escape immune suppression through various mechanisms that are only partially understood. In addition to reduced immunogenicity and antigen loss, tumours often produce excessive amounts of immunosuppressive mediators such as adenosine, kynurenines (by means of indoleamine 2,3-dixoxygenase), prostaglandin E2 (PGE2), transforming growth factor-β (TGF-β) and VEGFA. For example, TGF-β inhibits the activation, proliferation and differentiation of T cells,[26] and suppresses the activity of CTLs[27] and dendritic cells, while inducing differentiation of immunosuppressive T_{REG} cells.[28]

The tumour microenvironment attracts increased levels of immunosuppressive leucocytes including T_{REGS}, myeloid-derived suppressor cells (MDSCs) and tumour-associated macrophages. A number of strategies have been used to deplete these cells in expectation that this will enhance antitumour immune responses. Treatment with low-dose cyclophosphamide has been shown to transiently reduce T_{REG} levels and enhance tumour-reactive T-cell responses when used alone[29] or in combination with active immunotherapy.[30] Other standard therapies, chemotherapies or radiotherapies, or treatment with targeted agents can also modify the tumour cell microenvironment.

The dynamic relationship between T_{REG} and effector cells is not straightforward or fully understood. Whereas high levels of T_{REG} cells in tumours have been correlated with poor prognosis across a range of cancers,[31–33] in a study of T_{REG} infiltration in tumours from patients with colon cancer, higher levels of T_{REG} were found to be associated with better overall survival and progression-free survival (PFS).[34] In a phase III study, in which patients with melanoma were randomly assigned to treatment with high dosed of IL-2 either alone or in combination with gp100 peptide vaccine, greater increases in levels of T_{REG} cells were seen in patients who showed a clinical response than in those with no response.[35] Therefore, the presence of T_{REG} cells might generally be interpreted as an indication of an ongoing T-cell response against the tumour, and the balance between proinflammatory and anti-inflammatory responses will determine the outcome.

Human T_{REGS} include at least two subsets with distinct effects. On the one hand, inducible T_{REG} cells (iT_{REG}) are induced to differentiate in the periphery and mediate tumour-associated immune suppression by contact-independent mechanisms including the production of immunosuppressive factors such as TGF-β.[36] On the other hand, the natural T_{REG} cells (nT_{REG})—which are responsible for maintaining self-tolerance and preventing autoimmunity—are produced in the thymus and act through contact-dependent mechanisms.[36] Further understanding of T_{REG} characteristics and functions might facilitate selective depletion of T_{REG} subsets.

Treatments that target other immunosuppressive cells in the tumour microenvironment (such as all-trans retinoic acid, sunitinib, gemcitabine and cyclooxygenase-2 inhibitors, as well as cyclophosphamide) could also enhance the efficacy of active immunotherapy and limit the number and functions of MDSCs.[37]

Modulation of immune checkpoints

Immune checkpoint mechanisms that normally prevent excessive and uncontrolled immune responses operate at the cell surface through lymphocyte inhibitory receptors including, but not limited to, CTLA-4 and programmed cell death protein 1 (PD-1).[38] Similar mechanisms probably also limit the clonal expansion of T cells following vaccination, providing a rationale for combining vaccines with checkpoint inhibitors. PD-1 is normally transiently induced following immune activation, but chronic antigen exposure, as is the case in cancer, can lead to persistently high levels of PD-1 and T-cell anergy or exhaustion.[39] Expression of PD-1 ligand 1 (PDL-1) by tumour cells can suppress CTL activity and has been correlated with poor prognosis.[40,41] Surface receptors acting as checkpoints are amenable to modulation with monoclonal antibodies and fusion proteins.[42,43]

The US approval of ipilimumab for the treatment of metastatic melanoma in March 2011 provided a proof of principle for targeting immune checkpoints in cancer treatment.[17] The main mechanism of action relies on releasing effector T cells and possibly on depleting T_{REG} cells in the tumour microenvironment.[44] Significant improvements were seen in overall survival in two phase III trials in metastatic melanoma when ipilimumab was used alone or in combination with dacarbazine (survival of 11.2 months versus 9.1 months in previously untreated patients; $P<0.001$)[45] or with a gp100 peptide vaccine (survival of 10.0 months versus 6.4 months in

patients with relapsed or refractory disease; $P<0.001$).[17] A number of antibodies targeting PD-1 are in development (for example, nivolumab and MK-3,475), and new early-phase clinical studies have yielded promising results with PD-1 blockade alone[46,47] and in combination with CTLA-4 blockade.[48]

Experience from clinical studies

Active immunotherapies are being extensively studied in clinical trials across a range of malignancies (Table 1 and Supplementary Table 1). In this section, we focus on late-phase clinical studies and newly published data.

Prostate cancer

As a landmark for active immunotherapy, sipuleucel-T (trade name Provenge®, Dendreon Corporation, Seattle, USA) was approved in April 2010 by the FDA for the treatment of asymptomatic and minimally symptomatic metastatic castration-resistant prostate cancer (mCRPC) and is currently the only FDA-approved therapeutic cancer vaccine. Sipuleucel-T is an autologous cell vaccine prepared by culturing peripheral blood mononuclear leucocytes from the patient with a recombinant fusion protein incorporating a prostate cancer antigen, prostatic acid phosphatase (PAP), and granulocyte-macrophage colony-stimulating factor (GM-CSF). The GM-CSF component facilitates targeting of the PAP antigen to antigen-presenting cells leading to their activation. This procedure is sequentially repeated across three cycles, so that patients are infused with a complex mixture of different cells and macromolecules including T cells that were activated *ex vivo* and *in vivo* by the preceding doses of antigen.[49] Therefore, the product is in reality a mixture of a cell vaccine and a form of adoptive T-cell therapy.

Approval of sipuleucel-T was based largely on the results of the randomized, double-blind phase III IMPACT trial[50] that showed a median overall survival of 25.8 months in patients treated with sipuleucel-T ($n=341$) compared with 21.7 months in those treated with placebo ($n=171$), corresponding to a relative reduction of 22% in the risk of death ($P=0.03$). The time to objective disease progression was similar in the two groups (14.6 weeks and 14.4 weeks).[50] Sipuleucel-T is currently being tested in prostate cancer as single-agent therapy or in combination with hormone therapy in phase II and III trials (Table 1).[51–55]

PROSTVAC®-VF (also called PSA-TRICOM; Bavarian Nordic, Kvistgaard, Denmark) comprises two recombinant viral vectors, each encoding transgenes for PSA, and three immune co-stimulatory molecules, T-lymphocyte activation antigen CD80 (B7), intracellular adhesion molecule 1 (ICAM-1), and lymphocyte function-associated antigen 3 (LFA-3). A vaccinia-based vector is used for priming followed by boosts with a fowlpox-based vector, both administered with GM-CSF. In a phase II trial, a difference of 8.5 months in overall survival was reported for the vaccine-treated group compared with patients with minimally symptomatic CRPC in the control group who were vaccinated with empty vectors.[56] A phase III trial is currently ongoing.[57]

Replication-deficient recombinant adenovirus type 5 (Ad5) vectors are efficient *in vivo* gene delivery systems and useful adjuvants for the delivery of TAA-coding genes.[58] In an ongoing phase II study, patients with newly-recurrent prostate cancer are being treated with Ad5-PSA, either as a stand-alone intervention or subsequent to hormone deprivation therapy.[59] To date, 100% of patients have developed anti-PSA T-lymphocyte responses. In a second protocol, antigen-specific T-cell responses have been observed in 67% of patients with hormone-refractory disease, who are being treated with Ad5-PSA alone.[59]

Naked DNA vaccines are generally thought to elicit weaker antitumour immunity than vector-based approaches, owing to the absence of a concomitant antiviral inflammatory response. However, early dose-escalation trials encompassing multiple injections of a naked DNA vaccine encoding PAP alongside GM-CSF have demonstrated effective anti-PAP T-cell responses in selected patients with prostate cancer.[60] Booster immunizations induced long-term PAP-specific T-lymphocyte responses in a subset of patients achieving a PSA doubling time of >200%.[61] This strategy is now under review in phase II clinical trials in combination with sipuleucel-T and/or GM-CSF.[62,63] In a phase I–IIa study, antigen-specific T-cell responses were reported in 26 of 33 (79%) patients with mCRPC following immunization with mRNAs (CV9103, CureVac) encoding four prostate-specific TAAs.[64] Phase IIb trials of CV9103[65] and a closely related mRNA vaccine (CV9104)[66] are ongoing.

GVAX vaccine for prostate cancer (GVAX-PCa) is an active immunotherapy comprising two irradiated allogeneic prostate cancer cell lines, which constitutively express GM-CSF. Despite early indications of clinical safety and efficacy in patients with mCRPC,[67–69] two phase III trials were prematurely terminated owing to lack of therapeutic effect (VITAL-1 trial) and increased mortality (VITAL-2 trial).[70,71]

Breast cancer

A number of HER2-derived peptides have been investigated in clinical trials in combination with additional immunostimulatory agents, such as GM-CSF, cyclophosphamide or poly-ICLC (also known as Hiltonol™; a stabilized formulation of polyriboinosinic polyribocytidylic acid [poly I:C]). Active immunotherapy directed against HER2 might have a great benefit only in women with breast tumours that expressed HER2 at low levels. Administration of HER2-derived peptide E75 (also known as NeuVax™) alongside GM-CSF in patients at high risk for relapse was shown to prolong disease-free survival (DFS) in a subset of patients who expressed low levels of HER2.[72] A phase III clinical trial is currently underway to test the efficacy of E75 with GM-CSF in preventing recurrence in patients with early-stage, node-positive breast cancer and low-to-intermediate HER2 expression.[73] A phase II study is also currently ongoing to test the efficacy of combination immunotherapy using the E75 vaccine added to passive immunotherapy with the monoclonal antibody trastuzumab.[74]

Table 1 | Active immunotherapies in phase III development*

Immunotherapy	Targeted antigens	Adjuvants/ immune modulators	Study population	*n*	Outcomes	References
Prostate cancer						
Autologous cell vaccine: sipuleucel-T, Provenge®	PAP	GM-CSF	Metastatic, castration-resistant prostate cancer	512	OS: 25.8 months vs 21.7 months (HR 0.78; *P*=0.03) PFS: 3.7 months vs 3.6 months (HR 0.95; *P*=0.63) T-cell response in 73.0% vs 12.1% of patients	50–55
Allogeneic tumour cell vaccine: GVAX	Tumour cell	GM-CSF	Castration-resistant prostate cancer	626	OS: 20.7 months vs 21.7 months with docetaxel plus prednisone (HR 1.03; *P*=0.78)‡	70, 194
Allogeneic tumour cell vaccine: GVAX	Tumour cell	GM-CSF	Castration-resistant prostate cancer	408	OS: 12.2 months in combination with docetaxel vs 14.1 months docetaxel plus prednisone (HR 1.70; *P*=0.0076)§	71, 195
Breast cancer						
Peptide vaccine: Theratope	Sialyl-Tn	KLH	Metastatic breast cancer, in remission after first-line chemotherapy	1,028	Median OS: 23.1 months vs 22.3 months (*P*=0.916) With concomitant endocrine therapy, OS: 39.6 months vs 25.4 months (*P*=0.005) Median TTP: 3.4 months vs 3.0 months (*P*=0.353) With concomitant endocrine therapy: 10.6 months vs 6.3 months (*P*=0.078)	76, 77
Lung cancer						
Peptide vaccine: tecemotide (L-BLP25)	MUC1	Liposomal monophosphoryl lipid A plus cyclophosphamide	Unresectable stage III NSCLC; after chemo-radiotherapy	1,239	Median OS: 25.6 months vs 22.3 months (HR 0.88; *P*=0.123); OS with concurrent chemotherapy: 30.8 months vs 20.6 months (HR 0.78; *P*=0.016); OS with sequential chemotherapy: 19.4 months vs 24.6 months (HR 1.12; *P*=0.38)	79–81, 197
Peptide vaccine: GSK1572932A	MAGE-A3	Liposomal AS15	Completely resected stage IB–II NSCLC	182	Trial terminated owing to failure to meet primary end points of extended DFS. Not possible to identify gene signature predicting benefit	85, 86
Allogeneic tumour cell vaccine: belagenpumatucel-L, Lucanix™	Tumour cell	Anti-TGF-β	Stage IIIB–IV NSCLC	532	Median OS: 20.3 months vs 17 months (HR 0.94; *P*=0.594) Non-adenocarcinoma: 19.9 months vs 12.3 months (HR 0.55; *P*=0.036)	93, 198
Melanoma						
Peptide vaccine	gp100	IL2 plus Montanide™ ISA51	Locally-advanced stage III or stage IV melanoma	185	OS: 17.8 months vs 11.1 months (*P*=0.06) PFS: 2.2 months vs 1.6 months (*P*=0.08) T-cell responses in 7 of 37 (19%) patients Higher levels of $CD4^+foxp3^+$ cells in patients with clinical response (*P*=0.01)	35, 198
Peptide vaccine: GSK 2132231A	MAGE-A3	QS-21	Resected melanoma	1,349	Failed to meet primary end point of DFS; ongoing for end point of DFS in patients with predictive gene signature	100
Pancreatic cancer						
Peptide vaccine: GV1001	Telomerase	GM-CSF	Locally-advanced and/or metastatic pancreatic cancer	1,062	OS: 8.4 months (concurrent with chemotherapy) and 6.9 months (sequential chemotherapy) vs 7.9 months with chemotherapy alone (NS)	113, 199, 200
Colorectal cancer						
Autologous tumour cell vaccine: OncoVAX®	Tumour cell	BCG	Resected stage II–III colon cancer; after resection	254	42% reduction in the risk of recurrence and/or death (*P*=0.032); greatest effect in stage II disease with 60% reduction in risk of recurrence and/or death (*P*=0.007) and 54% reduction in risk of death	121
Haematological malignancies						
Autologous anti-idiotype vaccine	Idiotype	KLH	Advanced follicular lymphoma, with complete response after chemotherapy	177	PFS: 23.0 months vs 20.6 months (*P*=0.256) ≥1 blinded vaccination: 44.2 months vs 30.6 months (*P*=0.047)	130, 201

*Trials listed on Clinicaltrials.gov website; accessed on 19th September 2013. Survival data are medians and comparisons are for active treatment versus placebo or control, unless otherwise stated. ‡Study terminated early following futility analysis. §Study terminated early owing to excessive death in vaccine arm. Abbreviations: BCG, bacillus Calmette-Guérin; DFS, disease-free survival; GM-CSF, granulocyte-macrophage colony-stimulating factor; gp100, glycoprotein 100; HR, hazard ratio; KLH, keyhole limpet haemocyanin; L-BLP25, BLP25 liposome vaccine; MAGE-A3, melanoma-associated antigen 3; MUC1, mucin-1; *n*, number of patients; NS, not significant; NSLC, non-small cell lung cancer; OS, overall survival; PAP, prostatic acid phosphatase; PFS, progression-free survival; QS-21, a plant extract derived from *Quillaja saponaria* that enhances the immune responses to antigens targeted by vaccines; RT, radiotherapy; TGF-β2, transforming growth factor β2; TTP, time to progression; vs, versus.

New peptide vaccine approaches in breast cancer include the WT1 (Wilms tumour protein) antigen, a transcription factor involved in cell proliferation, differentiation and apoptosis. Early data indicate that administration of WT1 emulsified with Montanide™ ISA 51 adjuvant can induce tumour regression and WT1-specific CTL expansion in patients with breast cancer.[75] Immunization with sialyl-Tn, an epitope found in a variety of glycoproteins conjugated with keyhole limpet haemocyanin (KLH) failed to prolong survival in a phase III trial of 1,028 women with metastatic breast cancer;[76] however a significant increase in time to progression and overall survival was found in a pre-stratified subset of women with metastatic breast cancer who received concomitant endocrine treatment.[77] Non-peptide approaches include immunization with vaccinia virus modified to express mucin-1 (MUC1) and IL2, which caused partial tumour regression in two of 31 patients with metastatic breast cancer.[78]

Lung cancer

In the phase III START trial,[79] 1,513 patients with unresectable stage III non-small-cell lung cancer (NSCLC) that had not progressed after primary chemoradiotherapy were randomly assigned to receive tecemotide (a therapeutic vaccine designed to induce immune response to cancer cells expressing MUC1) or placebo.[79] Tecemotide consists of a MUC1 lipopeptide combined with monophosphoryl lipid A in a liposomal delivery vehicle. A single, low dose of cyclophosphamide was administered before the first immunization. Tecemotide failed to significantly prolong survival in the overall population (with median overall survival of 25.6 months with tecemotide versus 22.3 months with placebo; HR 0.88, $P = 0.123$).[79] However, in a preplanned subgroup analysis for stratification variables, tecemotide improved survival in patients who had received concurrent chemoradiotherapy (30.8 months versus 20.6 months; HR 0.78, $P = 0.016$), but not in patients who had received chemotherapy and radiotherapy sequentially.[79] A similar phase III trial of tecemotide, INSPIRE,[80] is ongoing in Asian patients with unresectable stage III NSCLC[81] and a further phase III trial is being initiated in patients with unresectable stage III NSCLC who have completed concurrent chemoradiotherapy (START2).[82]

TG4010 vaccine also targets MUC1, but consists of a recombinant vaccinia virus encoding MUC1 and IL2. In a phase II study in 148 patients with stage IIIB–IV NSCLC, PFS at 6 months was 43% with TG4010 combined with chemotherapy versus 35% with chemotherapy alone ($P = 0.3$).[83] Median overall survival did not differ significantly between the treatment arms (10.7 months versus 10.3 months; $P = 0.59$), but there was evidence for a late separation in the survival curves favouring TG4010.[83] A phase IIB–III study of TG4010 added to chemotherapy in around 1,000 patients with stage IV NSCLC is ongoing.[84]

GSK1572932A is a vaccine that combines a melanoma-associated antigen-3 (MAGE-A3) peptide with the immune adjuvant AS15. Results of a phase II study in 182 patients with resected stage IB–II MAGE-A3-positive NSCLC suggested a trend towards improved outcomes with GSK1572932A compared with placebo, although none of the changes were statistically significant.[85] However, a large (around 2,200 patients) phase III study of GSK1572932A after adjuvant chemotherapy for resected stage IB–IIIA NSCLC (the MAGRIT trial)[86] has recently been terminated after it failed to meet its primary end point of extending DFS, as well as proving impossible to identify gene signatures predictive of which patients might benefit from the vaccine therapy.[87]

Immunization with GV1001—a 16-amino-acid peptide vaccine corresponding to the active site of human telomerase reverse transcriptase—and GM-CSF induced specific immune responses in up to 80% of patients with unresectable stage III NSCLC.[88] In a phase II trial, median PFS for patients with a GV1001-specific immune response was 371 days compared with 182 days for non-responders ($P = 0.2$). Median overall survival was significantly longer among immune responders (19.0 months versus 3.5 months for immune nonresponders; $P < 0.001$) in a phase I–II study in which GV1001 was combined with a second telomerase peptide (I540). A phase III study is in development.[89]

The CIMAvax EGF vaccine is designed to induce an antibody-mediated rather than cell-mediated immune response. Recombinant human EGF is fused with a carrier protein and combined with Montanide™ ISA 51 adjuvant. Median overall survival did not differ significantly from that achieved with best supportive care alone in a phase II study carried out in 80 patients with stage IIIB–IV NSCLC.[90] However, survival was improved in patients with a good antibody response to CIMAvax EGF vaccine (11.7 months versus 3.6 months; $P = 0.002$).[90] CIMAvax is licenced in Cuba for stage IIIB–IV NSCLC.

Belagenpumatucel-L vaccine (Lucanix™, NovaTx Corporation, San Diego, USA) consists of four NSCLC cell lines transfected with a TGF-β2 antisense gene. In a phase II study, survival was improved with a higher dose of belagenpumatucel-L (2-year survival of 52% versus 20% with high dose and low dose, respectively)[91] and median overall survival was longer in patients with a cellular and humoral immune response to the vaccine than in patients without a response (survival of 32.5 months versus 11.6 months; $P = 0.011$).[92] A new phase III trial in stage IIIB–IV NSCLC reported no significant difference in overall survival with belagenpumatucel-L compared with placebo in the overall study population. However, survival was prolonged in a number of subgroups, including those who had previously received radiotherapy, those starting belagenpumatucel-L treatment within 12 weeks of completing front-line chemotherapy and those with non-adenocarcinomas.[93]

Tergenpumatucel-L incorporates three lung cancer cell lines transfected with a murine α-1,3-galatosyltransferase gene and was associated with median overall survival of 11.3 months in a phase II study in 28 patients with metastatic or recurrent NSCLC.[94] Induction of IFN-γ secretion by antigen-specific T lymphocytes was associated with improved overall survival (21.9 months versus 7.2 months; $P = 0.044$).[94,95]

The anti-idiotype vaccine racotumomab is designed to stimulate an immune response against neu-glycolyl-containing gangliosides found on the surface of various tumours including NSCLC. In a phase II study of patients with stage IIIB–IV NSCLC, median overall survival was longer with racotumomab than with placebo (10.6 months versus 6.3 months; $P = 0.02$)[96] and a phase III trial is in progress.[97]

Melanoma

Adoptive T-cell therapy with expanded cultures of tumour-infiltrating T lymphocytes has been associated with remarkable clinical responses in patients with metastatic melanoma.[98] Antigen-specific approaches have targeted TAAs including β-catenin, gp100, MAGE-A3, melanoma antigen recognized by T cells 1 (melan-A; also known as MART1), cancer–testis antigen 1 (NY-ESO-1) and survivin (also known as baculoviral inhibitor of apoptosis repeat-containing 5; BIRC5).[35,99–103] A gp100-specific vaccine formulated with a sequence-optimized peptide of the Montanide™ ISA 51 adjuvant and combined with systemic IL-2 improved clinical outcomes in a phase III trial carried out in 185 patients with locally-advanced stage III and stage IV melanoma.[35] The response rate was 16% with the vaccine combined with IL-2 versus 6% with IL-2 alone ($P = 0.03$). PFS was also significantly longer with the vaccine and IL-2 combination (2.2 months versus 1.6 months; $P = 0.008$). Median overall survival was 17.8 months with the gp100 vaccine and IL-2 compared with 11.1 months with IL-2 alone ($P = 0.06$).[103] A phase III study of the MAGE-A3 vaccine GSK 2132231A after resection of stage IIIB–C melanoma (the DERMA trial)[100] failed to meet one of its co-primary end points, a prolongation of DFS in the overall MAGE-A3-positive population. The study will continue until the second co-primary end point of DFS in a subset of patients with a gene signature shown to be predictive of response to MAGE-A3 immunotherapy is assessed.[104]

Adoptive cell therapy with autologous engineered T cells transduced with an anti-MAGE-A3 TCR induced substantial clinical regression in five out of nine patients with late-stage cancer (seven of whom had metastatic melanoma). Three patients experienced neurological toxicity, which resulted in death of two of them.[105] Further investigation suggested that the neurological toxicity is related to previously unrecognized expression of MAGE family antigens in brain tissue. Absence of toxicity with the vaccines might suggest existence of tolerance mechanisms that prevent the presence of high-avidity TCR, such as those used in the adoptive T-cell therapy trial.

In patients with metastatic melanoma treated with dendritic cells pulsed with a cocktail of melanoma-associated antigens (gp100, MAGE-A1, MAGE-A2 and MAGE-A3, MART-1 and tyrosinase) and KLH, survival was longer for patients who responded to immunization with a positive CTL response than for non-responders (21.9 months versus 8.1 months) and was longer for immunized patients than non-immunized matched control patients (13.6 months versus 7.3 months).[106] In a study of 29 patients with stage III–IV melanoma, loading of both MHC class I and class II epitopes of gp100 and tyrosinase onto dendritic cells enhanced the induction of antitumour responses, which suggested that activation of $CD4^+$ T helper cells could be used to increase cytotoxic $CD8^+$ T cell responses.[107] Survival was improved compared with matched control patients (PFS of 5.0 months versus 2.8 months, $P = 0.0089$; overall survival of 15.0 months versus 8.3 months, $P = 0.089$).[107]

Phase III evaluation of an allogeneic whole–tumour-cell vaccine administered with Bacillus Calmette–Guérin (BCG) as adjuvant after resection of metastatic melanoma was terminated early owing to lack of efficacy.[108] Studies of other allogeneic tumour-cell vaccines are ongoing in this setting.[109,110] A study comparing an irradiated, whole-tumour-cell vaccine with a dendritic-cell-based strategy for metastatic melanoma showed significantly longer survival for dendritic-cell-vaccine loaded with autologous antigens compared with immunization with the irradiated whole tumour cells (2-year survival of 72% and 31%, $P = 0.007$).[111]

In a phase II study of 25 patients with stage III–IV melanoma, the efficacy of a prime-boost strategy with recombinant NY-ESO-1-expressing poxviruses (vaccinia followed by fowlpox) was evaluated.[102] The proportion of patients with NY-ESO-1-specific $CD8^+$ T cell responses increased from 40% pretreatment to 88% after vaccination. Median overall survival was significantly longer for patients with a post-vaccination immune response than for those without (82 months versus 15 months, $P = 0.007$).

Pancreatic cancer

The active immunotherapies that are commonly investigated in pancreatic cancer include the telomerase peptide vaccine GV1001 and the allogeneic tumour-cell vaccine algenpantucel-L, which is an irradiated combination of two allogeneic pancreatic cancer cell lines transduced with murine alpha-1,3-galatosyltransferase. Two phase III studies with GV1001 were initiated based on promising early-phase trials,[112,113] but both studies had disappointing results. One of the studies was terminated early owing to lack of benefit[112] and in the other one, no significant survival benefit was seen when GV1001 was given with either concurrent or sequential chemotherapy and the overall response rate with sequential chemo-immunotherapy was lower than that for chemotherapy alone (8.9% versus 17.6%; $P = 0.001$).[113] In a phase II study of algenpantucel-L added to adjuvant chemotherapy (gemcitabine plus 5-fluorouracil) for resected pancreatic cancer, the overall survival of patients at 1, 2 and 3 years were 86%, 51% and 42%, respectively, suggesting an improvement in survival on the basis of comparison with historical control data.[114] Of note, the putative survival benefit with immunotherapy seemed to increase over time, rising from a 37% increase over the expected survival based on historical control data at 1 year to a 121% increase at 3 years. Phase III studies of algenpantucel-L added to the standard of care (chemotherapy or chemoradiotherapy) for borderline resectable and/or unresectable pancreatic cancer[115] and following surgical resection[116] are ongoing.

Box 2 | Key issues in implementing active immunotherapy in clinical practice

Clinical trial end points
- Clinical studies should take into account that the relation of dose to efficacy and safety might not be proportional, and that the optimal therapeutic dose might not be the maximum tolerated dose
- Trial design needs to reflect that clinical effects might take substantially longer to develop than with established anticancer therapies, including chemotherapy and radiotherapy, and might not involve substantial reductions in tumour size
- Immunotherapeutic trials should incorporate new measures of antitumour effects on the basis of assessment of immune response patterns

Multimodality treatment
- Rationale exists for combinations of active immunotherapies with debulking surgery, chemotherapy, radiotherapy, signal transduction inhibitors, adoptive T-cell therapy, cytokines, antivascular agents, immunostimulatory antibodies and inhibitors of immunosuppression
- Studies of active immunotherapy in combination with other treatment modalities should be pursued as a high priority, given the potential for synergistic enhancements
- Careful consideration of safety is essential before combining treatments in clinical studies, particularly the potential role for complex interactions in shaping an immune response

Patient selection
- Active immunotherapy might be most effective in early-stage or indolent disease and when tumour growth is controlled
- Diagnostic assessments that provide detailed information of immune functions should be identified and their correlations with clinical responses to active immunotherapy should be determined
- Preliminary evidence suggests that parameters that might act as markers of immune responsiveness relevant to active immunotherapy including tumour-specific T cells and autoantibodies as well as immune cell subpopulations (such as dendritic cells, T cell subsets including T_{REG} cells, macrophages) and chemokines in the circulation and tumour microenvironment should be included
- Treatments to overcome tumour-associated immunosuppression and restore responsiveness to active immunotherapy should be pursued

Use of suitable adjuvants
- Identification of the optimal combinations of antigens, adjuvants and delivery vehicles is an important priority to maximize the effectiveness of active immunotherapy
- Immunotherapy development must consider that adjuvant effects can vary according to factors including dose, immunization schedule, antigen, route of administration and host immune status
- Prime-boost strategies should be assessed when developing active immunotherapies

Abbreviation: T_{REG}, regulatory T cell.

Another therapeutic target in pancreatic cancer has been the tumour-specific *KRAS* mutations that are present in 90% of pancreatic cancers. In a phase I–II trial, immunization with synthetic mutant RAS peptides and GM-CSF was assessed in patients with advanced-stage pancreatic cancer ($n = 38$) and survival was longer for immune responders compared with non-responders (148 days versus 61 days).[117] In a later study of long-term follow-up of 23 patients vaccinated against mutant *KRAS* following surgical resection for pancreatic adenocarcinoma, an immune response rate of 85% was reported.[118] The 10-year survival was 20% in vaccinated patients compared with 0% for a cohort of patients treated without vaccination.[118]

Colorectal cancer

The most widely studied TAA in colorectal cancer (CRC) is carcinoembryonic antigen (CEA). A new adenoviral gene delivery platform encoding the CEA antigen, Ad5 [E1-, E2b-]-CEA(6D), was shown to induce cell-mediated immunity in 61% of patients with advanced-stage CRC.[119] The efficacy of Ad5 vaccines is sometimes limited by pre-existing or induced Ad5-specific neutralizing antibodies;[119] however, overall survival (48%) at 12 months was similar across patients regardless of their pre-existing Ad5 neutralizing antibody titres.

Whole-cancer-cell immunotherapies have also shown some promise in CRC. Phase III trials with Onco-VAX® (Vaccinogen Inc., Maryland, USA), an irradiated, autologous tumour-cell vaccine with BCG adjuvant, in patients with stage I–IV colon cancer, found improvements in recurrence-free and overall survival, but largely limited to the patients with stage II disease.[120,121] A confirmatory phase IIIb trial has been requested by the FDA and is due to begin in patients undergoing resection of stage II-CRC.[122]

In a new phase II trial, survival was assessed in patients who were disease-free after CRC metastasectomy and perioperative chemotherapy, and who received autologous dendritic cells modified with a poxvector encoding CEA and MUC1 (known as PANVAC™).[123] Recurrence-free survival of 47% at 2 years was similar to that in patients receiving PANVAC™ plus GM-CSF (55%). Survival for all vaccinated patients was longer than survival for a comparator group of patients who did not receive active immunotherapy.[123]

Renal cell carcinoma

Active immunotherapies in development for renal cell carcinoma (RCC) include AGS-003, a personalized therapy comprising autologous dendritic cells transfected with patient-specific tumour cell RNA and a synthetic, truncated human CD40 ligand (CD40L). In a phase II study in patients with newly diagnosed metastatic RCC with an unfavourable prognosis, AGS-003 therapy was combined with the tyrosine kinase inhibitor sunitinib.[124] Median PFS was 11.2 months and median overall survival was 30.2 months, exceeding the values expected from historical data.[124] A phase III clinical trial is currently assessing AGS-003 when added to standard of care treatment with sunitinib for advanced RCC.[125]

IMA901 vaccine consists of nine peptides derived from antigens overexpressed in RCC. In a phase II study, overall survival of patients with metastatic RCC who had not responded to previous therapy with cytokines or VEGF-inhibitors was 67% when receiving a combination treatment of cyclophosphamide and IMA901 plus GM-CSF, and 54% when treated with IMA901 plus GM-CSF alone.[12] Single-dose cyclophosphamide administered 3 days before IMA901 plus GM-CSF was shown to reduce the number of T_{REGS} and improve overall survival in patients with multiepitope T-cell responses to IMA901, but had no survival benefit in patients with no immune response.[12] In a phase III trial of IMA901 in combination with sunitinib in metastatic RCC, scientists have completed recruiting patients and the study is currently in follow-up.[126]

Box 3 | Rationale for combination of active immunotherapy with other treatments

Combination with debulking surgery
- Reduce tumour-mediated immunosuppression[183] and quantity of malignant tissue to eradicate
- Active immunotherapy likely to be more effective in patients with minimal residual disease[184]

Combination with radiotherapy
- Modify the tumour microenvironment[185]
- Induce immunogenic cell death and release of tumour antigens
- Enhance tumour infiltration by effector T cells[186]
- Immune-activating activity of local radiotherapy might contribute to abscopal effects[187]

Combination with chemotherapy
- Induce immunogenic cell death[188]
- Reduce T_{REG} frequencies[147]
- Enhance T-cell maturation[189]

Combination with signal transduction inhibitors (such as rapamycin)
- Potentiate vaccine-induced generation of memory T cells[190]
- Increase susceptibility to cytotoxic effector cells[191]

Combination with adoptive T-cell transfer
- Prime T cells and amplify antitumour immune responses[192]

Combination with cytokines
- Enhance cellular immune responses through a variety of mechanisms
- Can include combination with GM-CSF, IL-2, IL-12, IL-15, IL-21

Combination with monoclonal antibody antagonists of immune checkpoints (such as anti-CTLA-4, anti-PD-1)
- Block key mechanisms that downregulate T cell immune responses[193]
- Combination with monoclonal antibody agonists of co-stimulatory pathways
- Promote T-cell survival and proliferation (such as OX40 or CD137)
- Enhance antigen presentation and T-cell activation (such as CD40)[181]

Combination with small molecule inhibitors of tumour-mediated immunosuppression
- Reversal of tumour-induced immunosuppression mediated by factors such as TGF-β,[177] STAT3[178] and IDO[179]

Abbreviations: CTLA-4, cytotoxic T-lymphocyte protein 4; GM-CSF, granulocyte-macrophage colony-stimulating factor; IDO, indoleamine 2,3-dioxygenase 1; PD-1, programmed cell death protein 1; TGF-β, transforming growth factor β.

Haematological malignancies

Targets for antigen-specific immunotherapy of haematological malignancies include WT1, MAGE, MUC1 and the preferentially expressed antigen of melanoma (PRAME). In a phase II study, in which a WT1 peptide was administered with GM-CSF and KLH as adjuvants, 10 of 17 patients with untreated, relapsed, or refractory acute myeloid leukaemia (AML) had an objective response of stable disease, and four more patients had a clinical response after an initial period of disease progression.[127] High blast levels were associated with reduced immunogenicity, suggesting that efficacy is greater in patients with minimal residual disease.

In a phase I–II study of 10 patients with AML in haematological remission after chemotherapy, five patients had normalization of *WT1* mRNA expression levels following immunization with dendritic cells loaded with full-length WT1 protein.[128] Two of these five patients improved from partial to complete remission following vaccination, and clinical responses were correlated with induction of WT1-specific $CD8^+$ T cell responses.[128]

Anti-idiotype vaccines have been successful in some studies of follicular lymphoma, despite demonstrating weak immunogenicity in multiple myeloma.[129] Immunization with a hybridoma-derived autologous tumour immunoglobulin idiotype conjugated to KLH and with GM-CSF adjuvant prolonged median DFS by 13.5 months ($P = 0.047$) in a phase III study of patients with follicular lymphoma with a complete response after chemotherapy.[130] Specific immune responses in most patients (61%) following anti-idiotypic vaccination correlated with improved DFS in a series of 33 patients with a complete second response to chemotherapy after first relapse of follicular lymphoma. The duration of the second complete response was longer than the first complete response for immune responders, but was shorter than the first complete response for non-responders.[131] Other phase III multicentre trials that have not yet been published have yielded negative results. The apparent minimal efficacy of anti-idiotype vaccines and the development of next-generation B-cell-depleting monoclonal antibodies have reduced the interest in anti-idiotype approaches for indolent lymphoma.

MUC1-specific immunotherapy with tecemotide was assessed in a phase II trial in 34 patients with previously untreated, slowly-progressive, asymptomatic multiple myeloma or stage II–III myeloma in stable response or plateau phase following antitumour therapy.[132] Induction of MUC1-specific immune responses was seen in nearly half of the patients. No objective clinical response was noted, but the paraprotein concentration was reduced over time in 13 of 29 patients, predominantly in those patients with early-stage disease.

Implications for clinical practice

The experience from clinical trials of active immunotherapies highlights the potential efficacy of this therapy in the treatment of cancer and identifies a number of key issues that need to be addressed if this potential is to be achieved (Box 2). Firstly, active immunotherapies are recognized to work in different ways from chemotherapies and this difference needs to be taken into account in the design and interpretation of clinical studies.[133] Whereas chemotherapy has an essentially immediate onset of action, a full immune response to active immunotherapy can take several months to develop. Furthermore, chemotherapy usually involves the direct action of an agent on its target, whereas active immunotherapy acts indirectly by engaging an immune system, in which many components can have different effects in different situations. Consequently, the proportional pharmacodynamic relationships among the treatment dose, efficacy and toxicity, which are typical of chemotherapy, do not apply to many immunotherapies, and the optimal biological dose is often not the maximum tolerated dose.

Patient selection

As with other targeted therapies, only a minority of patients currently benefit from active immunotherapy but, for some patients, the benefits might be substantial. Patient selection is therefore an important issue. Initial assessments of active immunotherapies have generally

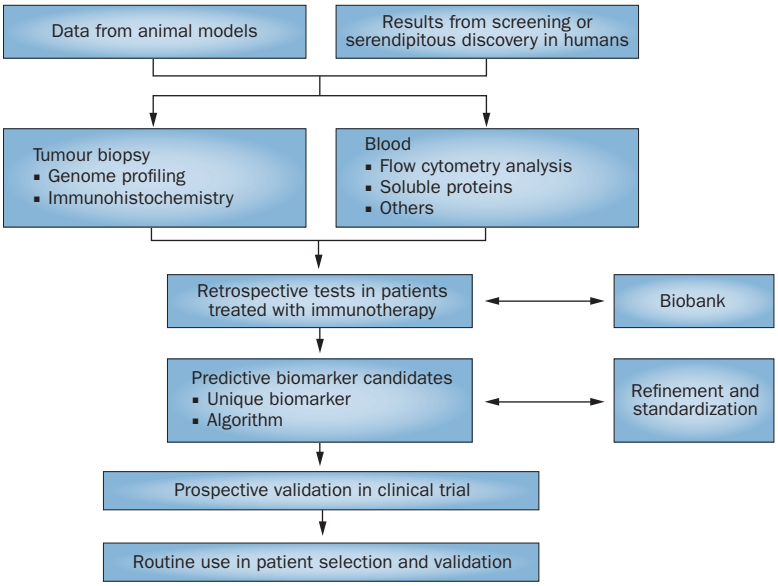


Figure 3 | Roadmap to the development of predictive biomarkers for active immunotherapy. The goal of developing predictive biomarkers is to select the patients who are most likely to benefit from a particular intervention. Potential biomarkers might be identified through a rationale or discovered serendipitously. For cancer immunotherapy, parameters measured in tumour biopsies might be of particular importance in predicting responses to immunotherapy, given the importance of the tumour microenvironment in modifying immune responses. Any potential biomarker must first be validated retrospectively. Promising candidates, either unique biomarkers or algorithms incorporating multiple predictive parameters, can then be optimised. Only after predictive power has been prospectively demonstrated in clinical trials can the biomarker be reliably applied to routine clinical practice.

been in patients with advanced-stage disease. However, rapid tumour growth and extensive tumour-related immunosuppression suggest that these patients might be least likely to benefit from this treatment. In a number of clinical studies in which active immunotherapy failed to prolong survival in the intention-to-treat population, subgroup analyses suggested clinical benefits in patients with early-stage or relatively indolent disease.[120,134–136] The potential benefit of using predictive biomarkers for patient selection is discussed below, but with regards to treatment with single active immunotherapies, patients with less-advanced disease are more likely to benefit.[137]

Immunotherapy in multimodal treatment

People with more-advanced malignancies might require active immunotherapy to be combined with chemotherapies, other immunotherapies and/or other immune-enhancing interventions in order to gain meaningful benefit (Box 3). Repeated exposure to tumour antigens is necessary, but not sufficient, to demonstrate therapeutic benefit. Through the combination of active immunotherapy with other antitumour and immune-modulating treatments, other immune-activating mechanisms such as appropriate dendritic-cell maturation, T-cell expansion and relief of tumour-associated immune suppression, which are key events to mount a robust antitumour immune response, could be possible.

Many investigators have proposed a beneficial effect of chemotherapy or radiotherapy on immune response functions. Studies have shown that tumour cell death eliciting endoplasmic reticulum stress and release of molecules such as alarmins during unprogrammed cell death is immunogenic.[138] This mechanism might depend on pro-inflammatory effects and the release of a wide range of tumour-specific antigens amenable to cross-presentation by dendritic cells. Furthermore, mouse experiments suggest that immune-mediated effects are important to the effectiveness of radiotherapy and chemotherapy, and data in humans support this hypothesis.[139–141] Chemotherapy can modify the phenotype and function of T-cell populations; for example, by decreasing the number of T_{REG} cells or depleting the existing T-cell pool.[142,143] Chemotherapy-induced lymphopenia enhances homeostatic antigen-independent proliferation of naive lymphocytes by removing competition for survival factors such as IL7 and IL15.[144] Chemotherapy also affects the MDSC pool,[145] modifying the profile of chemokines and/or growth factors in the vicinity of the tumour.[146] Clinical evidence shows that chemotherapy could suppress immune inhibitory mechanisms in the tumour microenvironment.[147,148] Combinations of immunotherapies together with tumour-specific chemotherapies are currently being evaluated,[149,150] and combinations of radiotherapy and immunotherapy have shown promising results in early-phase clinical studies.[151,152]

Monoclonal antibodies neutralizing inhibitory immune check points such as anti-CTLA-4[17,45] and anti-PD-1/PDL-1[42,46,48] are in phase III clinical development either as monotherapy or in combination with standard of care. Such antibodies have shown evidence of synergy with cancer vaccines in mouse models,[153,154] and clinical results[155,156] suggest a potential for the combinations.

Other potential approaches to enhancing responses to active immunotherapy include ablation or reprogramming of tumour-associated macrophages (TAMs), which have a key role in maintaining the immunosuppressive milieu of the tumour microenvironment. CSF-1 seems to be a key mediator of tumour invasion and metastasis.[157] Elevated circulating levels of CSF-1 and high levels of expression of CSF-1 have been correlated with poor survival in patients with epithelial ovarian cancer,[158] and breast adenocarcinomas,[159] respectively. In a new study, CSF-1-positive macrophage stromal responses were associated with high tumour grade and lymphovascular invasion of endometrial carcinomas.[160] Monoclonal antibodies and tyrosine kinase inhibitors that block signalling through the CSF-1 receptor, and thus deplete macrophages,[161] have been shown to enhance antitumour immunity and infiltration of CTLs in response to chemotherapy in preclinical models.[162] Depletion of TAMs in human mammary adenocarcinoma tissue was shown to promote $CD8^+$ cytotoxic cell infiltration and enhance antitumour immunity. Monoclonal antibodies against CSF-1 and CSF-1R are under clinical investigation in a range of different tumour types.[163] Chemokine receptor antagonists might also have potential as anticancer therapies to prevent tumour infiltration by MDSC and T_{REG} cells.[164]

Predictive biomarkers

As mentioned earlier, patient selection is an important issue and biomarkers that provide an early indication of response or are predictive of clinical benefit are needed as a priority. Possibly, no single immune biomarker will prove adequate and algorithms that incorporate multiple biomarkers will be needed (Figure 3). The RAIDs (Rational molecular Assessment Innovative Drug Selection) project,[165] for example, is taking a multidisciplinary approach integrating genomic studies, protein arrays, viral genotyping and immunohistochemical investigations in an attempt to identify the best option of targeted therapy for patients with cervical cancer.

A diverse variety of potential biomarkers exists. Pretreatment circulating levels of proteins, DNA, tumour cells and immune cells are easy to assess, but levels in the tumour microenvironment are likely to be better predictors of response. Assessments of immune responses both before and on-treatment might also prove valuable in predicting long-term outcomes.

Pretreatment screening of patients for the presence of target TAAs is clearly important. However, it should be considered that—owing to epitope spreading—antigens other than the specified TAA might be important determinants of efficacy.[166] Sequencing of the whole genome of each tumour to identify unique, mutation-derived antigens could allow a choice of tumour antigens that is truly personalized for optimal immunogenicity and clinical efficacy.[167] Circulating biomarkers that correlate with tumour burden might predict response to immunotherapy, given that immunotherapy seems to be most effective in patients with early-stage or indolent disease. For example, lower baseline PSA levels have been shown to correlate with greater survival in patients with prostate cancer treated with sipuleucel-T.[136]

Gene and immune signatures are likely to be more insightful in assessing the function of a complex network such as the immune system, and would be expected to be more effective in predicting outcomes than single parameters. For example, a newly identified signature of 84 genes mostly associated with immune-related functions can predict response to MAGE-A3 immunotherapy in patients with melanoma.[104]

Conclusions

Active immunotherapy is emerging as an important addition to conventional cancer treatments, but many important questions remain. Optimal combinations of antigens, adjuvants and delivery vehicles need to be determined and effective strategies for overcoming tumour-associated immunosuppression should be developed. Combinations of complementary immunotherapies are clearly needed to induce robust and sustained antitumour responses, and this progress needs to be reflected in both industry-sponsored and investigator-initiated clinical development programmes. Extensive efforts must be made in the identification of new predictive biomarkers and their prospective validation in the real-life clinical setting.

Review criteria

A search for original articles published between 2000 and 2014 and focusing on therapeutic cancer vaccines was carried out in MEDLINE and PubMed. The search terms used were “cancer” and “vaccine” and “immunotherapy”, alone and in combination. All articles identified were English-language, full-text papers. We also searched the abstracts for key scientific congresses (such as ASCO and ESMO) over the past 5 years. References were also supplemented with key references from the authors’ personal libraries. We would to like to apologize to colleagues for not including interesting research in early clinical trials and preclinical models, but this was outside the intended scope of the paper and was not possible given limitations on space.

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Author contributions

All authors researched the data for the article and provided substantial contributions to discussions of its content, wrote sections of the article, and reviewed and approved the final draft of the full manuscript.

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