

# Use of tumour-responsive T cells as cancer treatment

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The stimulation of a tumour-specific T-cell response has several theoretical advantages over other forms of cancer treatment. First, T cells can home in to antigen-expressing tumour deposits no matter where they are located in the body—even in deep tissue beds. Additionally, T cells can continue to proliferate in response to immunogenic proteins expressed in cancer until all the tumour cells are eradicated. Finally, immunological memory can be generated, allowing for eradication of antigen-bearing tumours if they reoccur. We will highlight two direct methods of stimulating tumour-specific T-cell immunity: active immunisation with cancer vaccines and infusion of competent T cells via adoptive T-cell treatment. Preclinical and clinical studies have shown that modulation of the tumour microenvironment to support the immune response is as important as stimulation of the most appropriate effector T cells. The future of T-cell immunity stimulation to treat cancer will need combination approaches focused on both the tumour and the T cell.

## Introduction

T lymphocytes can be found infiltrating human tumours and have been associated with both an improved or poor prognosis. Investigations suggest that this contradiction can be explained by close analysis of the phenotype of the tumour-infiltrating T lymphocyte. T cells can be broadly classified as cytotoxic CD8+ T cells, which can directly kill an antigen-expressing cell or cytokine-secreting CD4+ T cells. The CD4+ T-cell response can elicit both immune stimulatory or immune inhibitory effects. Specific CD4+ T-helper (Th) cell phenotypes are crucial for the expansion and persistence of tissue-destructive CD8+ T cells. Th1 CD4+ T cells secrete type I cytokines such as interferon (IFN)  $\gamma$ , resulting in the activation of antigen-presenting cells, which stimulate a CD8+ T-cell response.<sup>1</sup> Th2 CD4+ T-cells secrete type II cytokines, such as interleukin 4 (IL4), in response to antigen. Th2 CD4+ T cells can limit the activation of antigen-presenting cells and enhance humoral immunity as well as the influx of innate immune cells such as eosinophils and granulocytes.<sup>2</sup> The newly identified Th17 CD4+ T cell secretes IL17, eliciting tissue inflammation implicated in autoimmunity.<sup>3</sup> Finally, CD4+FOXP3+ T regulatory (Treg) cells will inhibit the development of an adaptive T-cell response directed against self-molecules, especially self-tumour antigens, via secretion of immunosuppressive cytokines such as IL10 or direct inhibition of antigen-presenting cells.<sup>4</sup>

## Role of T-cell subsets in tumour growth

The interplay of specific T-cell phenotypes is highlighted in an analysis<sup>5</sup> of more than 400 colon cancers for tumour-infiltrating lymphocytes (TIL). With tissue microarrays, investigators showed that a high density of CD8+ effector memory T cells in the tumour parenchyma was associated with increased survival. In a more detailed study of gene-expression patterns in a subset of tumours from 75 of the patients with colon cancer,<sup>6</sup> data suggested that an upregulation of genes related to Th1 adaptive immune response was associated with a decreased risk of relapse.<sup>6</sup>

Conversely, the presence of Treg cells seems to correlate with a poor prognosis in several tumour types in large

population-based studies. After assessing tumours in more than 300 patients with hepatocellular carcinoma, investigators showed both improved disease-free and overall survival in patients whose tumours had low numbers of Treg cells and high numbers of activated CD8+ T cells, measured by granzyme B staining, compared with patients whose tumours had high numbers of Treg cells and low numbers of activated CD8+ T cells.<sup>7</sup> Intratumoural Treg cells were assessed by immunohistochemical staining in more than 200 patients with invasive breast cancer.<sup>8</sup> Individuals with high numbers of Treg cells in their tumours had a shorter relapse-free and overall survival than did those with low Treg-cell infiltrate. These population-based analyses, using large numbers of well-defined cases, provide new insight into the role of T cells in cancer progression.

Direct evidence that tumour-specific T cells can induce an antitumour response is shown by the infusion of T cells used to treat patients with cancer. Donor lymphocyte infusions, used once patients with haematological malignant diseases have relapsed after allogeneic transplantation, have become a standard of care resulting in durable complete remissions in many individuals.<sup>9,10</sup> The clinical response is presumably due to a graft-versus-tumour effect.<sup>11</sup> Infusion of tumour-specific T cells in solid tumours has also met with some success. Transfer of autologous T cells derived from TIL resulted in a 51% response in 35 treated patients with refractory metastatic melanoma.<sup>12</sup> Although responses were not durable, evidence of substantial immunological remodelling of the tumour was seen, resulting in immune escape.

## Search strategy and selection criteria

We searched Medline (1990–2008) using search terms such as “T cell” and “cellular immunity” in combination with terms such as “cancer”, “tumor”, and “immune therapy”. We largely selected publications from the past 5 years, but did not exclude highly regarded older publications. We also searched the reference lists of articles identified by this search strategy and selected those we judged relevant. Review articles are cited to provide readers with more details and references if needed. Our reference list was modified on the basis of comments from peer-reviewers.

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	Targeted cancers	Antigen characteristics	Stage of investigation
Gangliosides	Several malignant diseases	Membrane glycosphingolipids	Phase III trial in melanoma
MUC1	Several malignant diseases	Cell surface glycoprotein	Phase III trial in non-small-cell lung cancer
PSA (proteasome subunit $\alpha$ )	Prostate	Secreted protein	Several phase II studies ongoing
Carcinoembryonic antigen	Several malignant diseases	Secreted oncofetal antigen	Phase II studies ongoing, phase III studies in combination with other antigens
Prostatic acid phosphatase	Prostate	Secreted protein	Phase III trial in prostate cancer
MAGE3	Melanoma	Cancer-testis antigen	Phase III trial in non-small-cell lung cancer
gp100	Melanoma	Glycoprotein	Phase III trial in melanoma
Tyrosinase	Melanoma	Enzyme implicated in melanin biosynthesis	Phase III trial in melanoma
HER2/neu	Breast, ovarian, prostate	Overexpressed growth factor receptor	Several phase II trials ongoing
Telomerase-related proteins	Several malignant diseases	Regulates telomere activity	Phase III trial in pancreatic cancer
Survivin	Several malignant diseases	Protein inhibiting apoptosis	Phase II studies
EGFR (epidermal growth factor receptor)	Non-small-cell lung cancer	Overexpressed growth factor receptor	Phase III study in non-small-cell lung cancer
NY-ESO	Several malignant diseases	Cancer-testis antigen	Phase II studies
P53	Several malignant diseases	Regulates apoptosis	Phase II studies

**Table 1: Examples of human tumour antigens targeted in clinical trials on <http://www.clinicaltrials.gov>**

### Mechanisms of immune escape

Unfortunately, tumours possess many mechanisms to escape immune recognition. Most defined cancer-associated immunogenic proteins, or tumour antigens, are non-mutated self-proteins; thus, systemic tolerance is a major mechanism of tumour immune escape. As described, Treg cells limit inflammation and prevent autoimmunity and are important in inhibiting self-antitumour immune responses. Other forms of peripheral tolerance include deletion, ignorance, and anergy. Deletion in the periphery is similar to central deletion, in which a cell expressing a T-cell receptor with high affinity for a self-antigen is deleted. When self-reactive T cells encounter self-antigen, the T cells can upregulate the death receptor Fas, resulting in T-cell apoptosis.<sup>13</sup> Ignorance is thought to occur when self-reactive T cells persist but are not activated by the antigen. T cells can also remain ignorant of an antigen if the antigen is not easily accessible to the peripheral blood or lymphatic system.<sup>14</sup> Anergy is a state of unresponsiveness, and is traditionally thought to occur when T-cell-receptor ligation happens in the absence of costimulation.<sup>15</sup> Immune receptor and costimulatory molecules have often been downregulated or lost in tumour cells.<sup>16</sup>

Tumours directly produce a local suppressive milieu that affects the activity of the infiltrating immune cells. For instance, tumours can downregulate various factors

in antigen processing (eg, MHC molecules).<sup>17</sup> Furthermore, tumours can secrete inhibitory cytokines, such as IL10 and transforming growth factor (TGF)  $\beta$ , which can aid recruitment of Treg cells and inhibition of dendritic-cell maturation.<sup>18</sup> Many tumours also express ligands that can interact with infiltrating T cells to provide negative stimulation, which inhibits or reduces the effector functions of specific cytotoxic T lymphocytes.<sup>19,20</sup> The tumour stroma can also limit the therapeutic efficacy of activated T cells by acting as a physical barrier as well as by elaborating cytokines and other soluble factors that promote cell growth and remodelling.<sup>21</sup> Immune-based treatments will probably be most effective at low or non-detectable levels of tumour burden.

### Antigen-specific cancer vaccines Human tumour antigens

Tumour antigens have been identified in nearly every human cancer, by virtue of these proteins being immunogenic in patients and not in volunteer controls. The development of highly quantitative assays to measure antigen-specific T cells allows a precise assessment of the endogenous tumour-associated T-cell response in patients. Patients with melanoma have been reported to have endogenous tumour-specific T-cell precursor frequencies as robust as 1:1000 CD8+ T cells.<sup>22</sup> Moreover, the numbers of antitumour T cells can be even higher in the metastatic deposits of patients.<sup>23</sup> The native tumour-specific T-cell response in patients with melanoma is effective and can result in extensive immunological editing of the tumour, facilitating outgrowth of clones that are resistant to immunological intervention.<sup>24,25</sup>

Patients with breast cancer, however, could have a more restricted T-cell response to tumours. Native T-cell immunity to three tumour antigens in breast cancer, HER2/neu, CEA (carcinoembryonic antigen), and MAGE3 (melanoma-associated protein 3) was assessed by intracellular cytokine staining, which measures cytokine-producing T cells after antigen stimulation by flow cytometry.<sup>26</sup> Both tumour antigen-specific CD4+ and CD8+ T cells were higher in patients with breast cancer than in controls (0.018–0.04% vs 0.0002–0.010%), although T cells derived from the patients did not secrete IFN $\gamma$  in response to tumour antigen stimulation. Moreover, the antigen-specific effector memory population, which might mediate a therapeutic response as previously described, was quite low. Many vaccine strategies are aimed at manipulating the tumour-antigen-specific immune response to correct or overcome defects in endogenous immunity.

Tumour antigens have been described for many classes of tumour-associated proteins. Table 1 shows examples of common tumour antigens that are being therapeutically targeted in clinical trials. The mechanisms by which self-proteins become tumour antigens in a malignant cell are not completely clear. Several possibilities exist: healthy

proteins in tumour cells are altered because of gene mutation or aberrant post-translational modification, potentially rendering them more immunogenic, such as P53. Cellular peptides produced by mismatch repair deficiency in colorectal cancers that show microsatellite instability could induce proinflammatory cytokines and peptide-specific T-cell responses.<sup>27</sup> Incorrectly glycosylated carbohydrates are immunogenic in some tumours, such as MUC1 (mucin). Many overexpressed proteins (eg, HER2/neu) have also been reported as tumour antigens in patients. Abundance of a tumour-associated protein could increase the number of peptides available for complexing to MHC molecules and subsequent recognition by the immune system. Finally, molecular mimicry of peptide sequences derived from foreign organisms with self-tumour antigens could cause the immunogenicity of antigens, such as MART1 (melan A).<sup>28,29</sup> The immunogenic proteins listed in table 1 show that many human tumour antigens are shared between tumour types and are not uniquely expressed in any one tissue type. An exception is tumour-specific idiotypes that are present in many haematological diseases associated with cell-specific rearrangement of immunoglobulin genes, which are unique to the malignant disease.<sup>30</sup>

### Common methods of antigen-specific immunisation

Table 2 lists some of the most common methods of antigen-specific vaccination. Most early-phase studies of cancer vaccines are focused on immunisation of patients to generate a measurable antigen-specific immune response. One of the initial approaches is to use immunogenic peptide epitopes derived from self-antigens to stimulate a T-cell response. Such studies were made possible by an understanding of the interaction of peptides in human class I and class II molecules and the identification of aminoacid motifs that would predict peptide binding.<sup>31</sup> Use of individual peptides could allow the removal of tolerising segments of a self-protein.<sup>32,33</sup> Class I epitopes, which stimulate a cytotoxic-T-lymphocyte response, and class II epitopes, which stimulate a Th response, have been tested. Since clinical trials of cancer vaccines have been directed toward the adjuvant setting to prevent disease relapse rather than toward the advanced-stage setting to treat refractory disease, the immune responses elicited could be very robust.

Two examples underscore the extent of tumour-antigen-specific immunity that can be achieved by use of tetramer analysis, a highly quantitative assay that measures T cells with specific peptide-MHC complexes correlating with an immunising class I epitope. The first approach used an HLA (histocompatibility antigen)-A2-restricted class I peptide specific for the melanoma antigen, glycoprotein gp100. The peptide had been modified by an aminoacid substitution of an anchor residue that greatly enhanced binding in MHC class I molecules compared with the unmodified peptide.<sup>34</sup> Investigators vaccinated 30 patients who had resected

stage I–III melanoma with the modified gp100 vaccine, with or without adjuvant interferon. T-cell responses to both gp100 and cytomegalovirus were assessed in the context of HLA-A2 class I peptides. 28 of 29 patients developed immunity that was much greater than baseline. 28% of patients showed peptide-specific CD8+ T-cell response that was greater than 1% of their circulating T cells, similar to the cytomegalovirus response measured simultaneously.<sup>35</sup>

Immunological adjuvants can also enhance the immunogenicity of vaccines by potently activating specific subsets of antigen-presenting cells, such as dendritic cells. In a second approach, vaccine immunisation targeting the melanoma antigen MART1, with CpG oligodeoxynucleotides as an adjuvant (known to trigger toll-like receptors on dendritic cells), resulted in immunity that was more than a log-fold higher than a vaccine with a standard adjuvant.<sup>36</sup> When tested in eight patients with advanced melanoma, the addition of CpG could increase MART1-specific, HLA-A2-restricted, cytotoxic T lymphocytes to as high as 3% of all CD8+ T cells. Unfortunately, mere stimulation of a CD8+ restricted T-cell response might not be completely effective without CD4+ T-cell help to maintain responses over time and facilitate in-vivo augmentation of vaccine-induced cytotoxic T lymphocytes.<sup>37</sup>

Vaccines encompassing class II epitopes designed to elicit a Th immune response have also been tested. Vaccine-induced tumour-antigen-specific CD4+ Th1 cells can home in to tumours and secrete inflammatory cytokines, modulating the microenvironment to enhance the function of antigen-presenting cells.<sup>38</sup> The generation of tumour-specific immunity occurs indirectly via cross priming; thus, activation of antigen-presenting cells is crucial to generate an effective response.<sup>39</sup>

Increased processing of endogenous tumour cells can result in the development of immunity to many immunogenic proteins expressed in the tumour that could counter the development of antigen-negative variants. Vaccination with class II epitopes that showed high avidity binding across many class II alleles allowed immunisation of patients with breast cancer to the growth-factor receptor, HER2/neu.<sup>40,41</sup> Active immun-

Rationale	
Peptide	Subdominant or cryptic epitopes can be presented to elicit immunity to self-antigens
Protein	Usually tested with potent immunological adjuvants to enhance immunogenicity
Plasmid DNA	Stable transfection of skin or muscle cells would allow antigen presentation and processing
Dendritic cell	Use of potent antigen-presenting cells to present self-antigens
Viral or bacterial vectors	Method to introduce antigen into class I processing pathway to stimulate cytotoxic T lymphocytes as well as provide foreign stimulus to induce immunity

**Table 2: Common methods of antigen-specific vaccination**

isation with HER2/neu peptides in 38 patients with stage III and IV breast cancer generated IFN $\gamma$ -producing, HER2/neu-specific, CD4+ T cells that could effectively home in to tumours in vivo, and resulted in the development of epitope spreading in vaccinated patients.<sup>42</sup> The endogenous broadening of the immune response after effective immunisation leads to the development of new immunity both in HER2/neu and in additional tumour antigens expressed in breast cancer, such as P53.<sup>43</sup> Both CD4+ and CD8+ T-cell responses have been detected in patients who had been immunised with class II epitopes.<sup>44</sup> The ability to modulate the tumour microenvironment with the cellular response, generating epitope spreading to additional epitopes, has been associated with clinical responses after active immunisation in patients with melanoma.<sup>45</sup>

The first step in a cancer-specific, cellular immune response is to have antigen presented to T cells by the most potent antigen-presenting cells—dendritic cells. A common method used to harness power for cancer vaccines is to purify dendritic cells from monocytes derived from the peripheral blood and to load tumour-specific antigens onto the dendritic cells. The antigen-loaded cells are then injected into patients. This approach has resulted in several studies showing the generation of antigen-specific immunity as well as clinical responses, although only in a few patients.<sup>46</sup> Methods to improve dendritic-cell immunisation include: direct intranodal delivery to counteract the inability of most dendritic cells to migrate from the vaccine site to the draining lymph node to present antigen; systemic depletion of Treg before vaccination; and use of new compounds to enhance the activation of dendritic cells.<sup>47–49</sup>

#### Clinical efficacy of antigen-specific vaccines

The success in generation of measurable tumour-antigen-specific immunity after active immunisation in patients with cancer has led investigators to measure any clinical effect. When antigen-specific vaccines were used in established refractory disease, few clinical responses were recorded. A review<sup>46</sup> showed responses in more than 700 patients with melanoma after immunisation with various vaccines. Generally, vaccine preparations were associated with less than 10% clinical response in tumour-bearing patients.<sup>46</sup> Many factors could cause the lack of clinical benefit. Cancer vaccines are probably not effective in progressive refractory disease, because the immunosuppressive effects of the tumour microenvironment most likely prevent or greatly limit the development of immunity.<sup>50</sup>

Cancer vaccines have now been used to modulate disease that is stable after treatment for a maximum response or in the adjuvant setting, with the consideration that an evolving immune response could be more effective in preventing disease relapse. By targeting the *BCR-ABL* oncogene in chronic myelogenous leukaemia,

a known tumour antigen, investigators showed that patients who had stable disease persistent disease after treatment could benefit from immunisation. 16 patients with chronic myelogenous leukaemia were treated to maximum response with either imatinib or interferon and vaccinated with a BCR-ABL-peptide-based vaccine. Most of the treated patients had a clinically complete remission with active immunisation.<sup>51</sup> Such studies suggest cancer vaccines could have the most benefit in states of very low tumour burden or even in cancer prevention.<sup>50</sup> Finally, cancer vaccines targeting idiotypes have shown some success in several malignant diseases. Investigators immunised 33 patients with follicular lymphoma with an idiotypic vaccine after the patients had achieved a second complete remission. As second remissions are generally shorter than the first, the trial assessed remission duration as a measure of response.<sup>52</sup> The vaccine induced an antigen-specific immune response in most patients and immunity was associated with increased disease-free survival. Similar studies showing increased clinical responses in patients immunised with idiotypic vaccines have been reported in Hodgkin's disease and B-cell lymphoma.<sup>53–55</sup>

Some methods used to augment immunity could, in fact, inhibit the response. For example, IL2 is frequently studied as a vaccine adjuvant to support the expansion of T cells in vivo. A phase II study in 40 patients with stage IIB–IV melanoma showed that the use of low-dose IL2 dampened the immune response elicited with vaccination.<sup>56</sup> We now know that IL2 treatment can result in the expansion of Treg cells, which can inhibit the development of immunity.<sup>57</sup> Indeed, even homeostatic expansion occurring after induced lymphopenia can stimulate proliferation of Treg cells.<sup>58</sup> Even vaccination against tumour antigens, either peptides or tumour-cell lysate, have been reported, which induces proliferation of Treg cells and limits immunity.<sup>59</sup> Finally, the targeting of one antigen could result in effective immunity without complete tumour regression, as highlighted in a study of dendritic-cell vaccines loaded with HER2/neu antigen in patients with non-invasive breast cancer, ductal carcinoma in-situ. Investigators immunised 13 patients with four weekly vaccinations of dendritic cells pulsed with HER2/neu-specific class I and II peptides before definitive surgery.<sup>60</sup> At the time of removal of the lesion, seven of 11 assessable patients had some reduction in HER2/neu expression in their tumour, with some measurable decrease in disease bulk; however, the disease remained.

Thus, antigen-specific cancer vaccines can elicit immunity and have shown clinical benefit in mild disease states. Unfortunately, targeting of one antigen in the context of a heterogeneous tumour with the stimulation of functional immunity could generate antigen loss variants and immunoediting rather than complete tumour eradication.<sup>61,62</sup> Strategies that target different antigens or generate substantial epitope spreading might be needed to achieve the most effective clinical benefit.

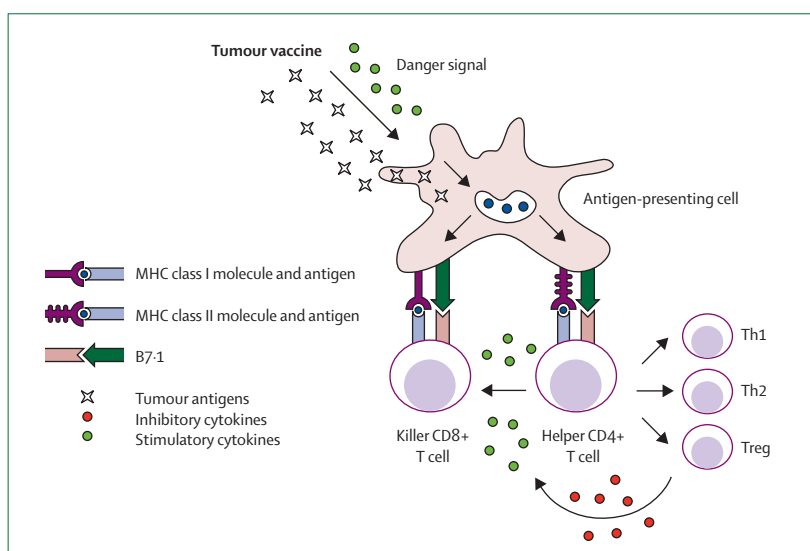
## Tumour-cell-based vaccines

A benefit of whole-cell tumour vaccines is that many tumour-associated antigens (known and unknown) are simultaneously delivered, eliminating the need to predetermine which antigens are the most immunogenic and mediate tumour rejection.<sup>63</sup> In the classic antigen-processing pathway, extracellular proteins are presented on MHC class II molecules to CD4+ T cells, whereas intracellular antigens are presented on MHC class I molecules to CD8+ T cells.<sup>64,65</sup> However, evidence has shown that exogenous or shed antigens can be taken up by dendritic cells and presented by MHC class I molecules to CD8+ T cells via so-called cross-presentation, generating effector cytotoxic T lymphocytes.<sup>66</sup> Thus, whole-cell vaccines could activate CD4+ and CD8+ T cells (figure 1). Several vaccine approaches have been developed to provide an unedited antigenic repertoire for immune recognition (table 3).<sup>67–69</sup>

## Autologous versus allogeneic tumour cells

Presumably, the best source of tumour cells for vaccine use would be from autologous primary tumours. To develop an autologous tumour-cell vaccine, patients would need to undergo surgical resection of their primary tumour, which would then be expanded *in vitro* and modified in some way to make the tumour more immunogenic. Autologous tumour-cell vaccines have been investigated as an adjuvant treatment after nephrectomy in renal-cell carcinoma.<sup>70</sup> 558 patients were randomly assigned to vaccine versus observation; 379 were assessable. After more than 5 years' follow-up, progression-free survival in the vaccine group was better than in the observation group. However, in a randomised phase III study of more than 400 patients with colon cancer, an autologous tumour-cell vaccine showed no clinically significant benefit.<sup>71</sup> Several technical problems with this vaccine approach have limited further study, including labour-intensive procedures needed to produce individual vaccines and difficulties in consistently expanding primary tumours *in vitro* to achieve the high cell numbers needed for immunisation.<sup>72,73</sup> Therefore, an alternative to the autologous vaccine is an allogeneic option, whereby the same tumour cell line, expressing shared tumour antigens, can be given to all patients.<sup>74</sup>

The success of allogeneic tumour-cell vaccines is based on the assumption that one or more tumour antigens expressed by vaccine cells are shared by most patients with that form of cancer, especially for melanoma.<sup>75,76</sup> The few phase III randomised trials done have met with limited success. The Southwest Oncology Group assigned 689 patients with high-risk node-negative melanoma to an allogeneic melanoma lysate vaccine versus observation. At a median follow-up of 5.6 years, no significant difference in disease-free survival was seen between the two groups.<sup>77</sup> However, in a prospective subset analysis, some patients had improved survival. 97 vaccinated



**Figure 1: Tumour vaccine cells genetically modified to produce danger stimuli (similar to granulocyte-macrophage colony-stimulating factor) to attract and mature antigen-presenting cells** Tumour-associated antigens are shed from dying vaccine cells to provide a source of antigen. Antigens can then be taken up by antigen-presenting cells, expressing costimulatory molecules such as B7-1, to be processed and presented to T cells. Phenotypes of CD4+ T cells can either enhance (eg, Th1) or inhibit (eg, Treg) the immune response.

patients with node-negative melanoma of specific HLA types had 89% 5-year survival compared with 59% for 78 controls ( $p=0.0002$ ).<sup>78</sup>

Other definitive trials of similar approaches have shown no survival benefit. More than 700 patients with melanoma were assigned to a melanoma-cell-lysate vaccine and surgery versus controls with surgery only. At 5 years' follow-up, no significant survival benefit in the vaccine group was recorded compared with controls.<sup>79</sup> A challenge would be to identify whether a biomarker could determine a subset of patients who might benefit from immunisation. Overall, the lack of success of these studies has led to strategies aimed at enhancing the immunogenicity of the approach.

## Genetically-engineered tumour cells

Tumour-cell vaccines can be genetically modified to enhance immunogenicity in many ways. The most

	Rationale
Autologous tumour and tumour lysate	Provide patients with own antigenic repertoire for immunisation
Allogeneic tumour and tumour lysate	Approach based on shared tumour antigens between individual tumours
Genetically-engineered tumour	Modifications to enhance whole-cell tumour immunogenicity
Tumour-derived peptides	Stripping peptides from autologous tumour cells to use as vaccine <sup>67</sup>
Dendritic-cell-based with RNA or DNA	Autologous dendritic cells with genetic material derived from autologous tumours or allogeneic tumours <sup>68</sup>
Heat-shock proteins	Chaperoning of antigenic peptides to MHC molecules inside cells and can be purified from cells as immunogens <sup>69</sup>

**Table 3: Common methods of vaccination with unrestricted antigenic repertoires**

common methods include modification of tumour cells to express cytokines or T-cell costimulatory molecules. Cytokines have been shown to be important activators of dendritic cells and other antigen-presenting cells, and several cytokines have been investigated for enhancing dendritic-cell function. Studies in murine models have shown that incorporation of granulocyte-macrophage colony-stimulating factor (GM-CSF) into vaccinating tumour cells yields the greatest degree of systemic immunity compared with other cytokine genes, including IL2, IL4, tumour necrosis factor (TNF)  $\alpha$ , and IFN $\gamma$ .<sup>80</sup> Early clinical trials of GM-CSF-transduced tumour-cell vaccines have shown that antigen-presenting cells infiltrate the vaccine site.<sup>72</sup> In a phase I vaccine trial, allogeneic, GM-CSF-secreting, pancreatic-cancer cells were given in sequence with adjuvant radiation and chemotherapy. Investigators recorded pronounced responses of delayed-type hypersensitivity to autologous pancreatic tumour cells, but not to healthy pancreas cells, which had developed in three patients who received higher doses of the vaccine.<sup>81</sup> These patients had remained disease-free 7 years after diagnosis, suggesting a possible survival benefit. The surviving patients showed a post-vaccination immune response to a new pancreatic tumour antigen, mesothelin.<sup>82</sup>

The extent of T-cell activation or suppression is carefully controlled by several receptor-ligand interactions that T cells initiate with various types of professional and non-professional antigen-presenting cells.<sup>83</sup> The receptor-ligand interactions can result in the activation of T cells (eg, CD28 interaction with CD80 or 86), or inhibit T-cell function (eg, CD152 with CD80 or 86). Various costimulatory molecules have been engineered to be expressed by whole-tumour cells. Many costimulatory molecules expressed in a tumour cell seem to be more efficient than single molecules in eliciting tumour-specific T-cell immunity. Investigators used a modified vaccinia virus expressing a triad of costimulatory molecules including CD80, intercellular adhesion molecule (ICAM)-1, and leucocyte-function-associated antigen (LFA)-3 to infect chronic lymphocytic leukaemia cells. The tumour cells expressing the three T-cell activation molecules stimulated chronic lymphocytic leukaemia *in vitro*, which could lyse unmodified cells.<sup>84</sup>

Such vectors can also be used to transfect tumour cells *in situ*, thereby negating the need to generate cultured tumour-cell lines from patients. Investigators gave a vaccinia virus expressing CD80 intratumourally in 12 patients with metastatic melanoma,<sup>85</sup> three responded clinically, and melanoma-specific immunity was generated in tested patients. Presumably, the ability to elicit systemic tumour-specific immunity was due to the enhanced presentation of the melanoma antigens by the tumour cells that were successfully engineered to express the costimulatory molecule. Thus, although therapeutic proof of principle could be accomplished with genetically-engineered whole-cell tumour vaccines, this approach

could be adapted to modify tumour cells *in vivo* without *ex-vivo* culture.

### Adoptive T-cell treatment

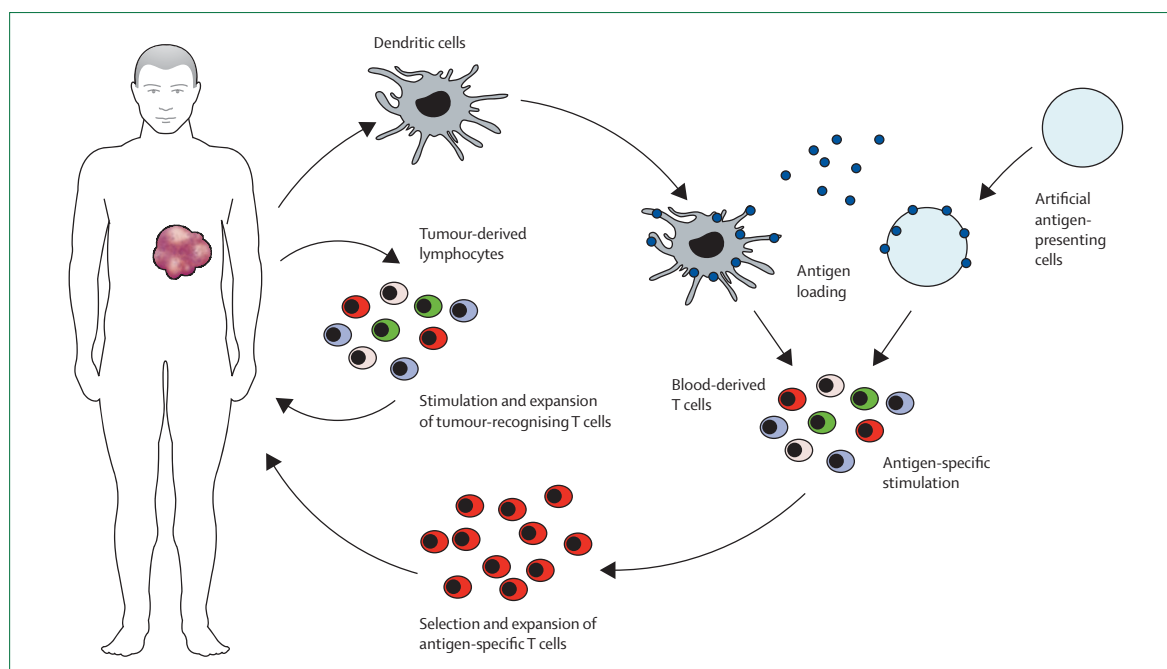
The main purpose of adoptive T-cell treatment is to augment a tumour-specific T-cell response above what is possible by vaccination alone. Infusion of tumour-competent T cells could allow levels of immunity to be capable of eradicating established disease. Anergic T cells can be rescued if removed from the tolerising milieu and stimulated *ex vivo*.<sup>86,87</sup> The rationale for adoptive T-cell transfer is based on attempts to circumvent or break tolerance using *ex-vivo* stimulated autologous T lymphocytes, allogeneic T-cell populations, or (more recently) human T cells engineered to attack tumours more effectively.

### Autologous T-cell infusions

Most clinical trials testing adoptive T-cell therapy have focused on the use of the patient's own T cells. The cloning of tumour-antigen-stimulated autologous T cells has allowed the investigation of homogeneous T-cell populations with defined specificity, avidity, and effector function.<sup>88</sup> Clinical trials have shown some therapeutic efficacy when MART1 specific cytotoxic T lymphocyte clones mediated some tumour regressions after transfer in patients with metastatic melanoma.<sup>89</sup> The magnitude and persistence of the transferred immunity could be limited by this approach partly because T-cell cloning needs a long culture period and preferentially leads to the differentiation of T cells displaying an effector-memory phenotype (T<sub>EM</sub> cells). T-cell transfer experiments in mice have shown that T<sub>EM</sub> cells persist only for a short time, whereas the transfer of central-memory T cells (T<sub>CM</sub> cells) results in a long-term memory response and the differentiation of T<sub>EM</sub> cells on antigen stimulation.<sup>90,91</sup>

Furthermore, the *in-vivo* expansion of tumour-specific cytotoxic clones could be restricted because of the lack of CD4+ T-cell help. Indeed, the clinical experience from adoptive transfer of cytomegalovirus-specific T cells into patients at risk for cytomegaloviral disease after allogeneic bone-marrow transplantation showed that the long-term persistence of adoptively transferred CD8+ T-cell clones depended on the endogenous development of CD4+ Th cells recognising the same antigen.<sup>92</sup> The recognition of the need for T-cell help has resulted in the development of adoptive T-cell treatment approaches that attempt to create a tumour-specific immune response that is functional, endogenous, and polyclonal, and that implicates both CD8+ and CD4+ T cells. The help given by CD4+ T lymphocytes during the priming of CD8+ cytotoxic T lymphocytes confers a key feature of immunological memory—the capacity for autonomous secondary expansion after re-encounter with antigen.<sup>93,94</sup>

Initial attempts to treat patients with their own polyclonal tumour-specific T lymphocytes have focused on the non-specific expansion of peripheral blood lymphocytes



**Figure 2: Isolation and ex-vivo stimulation of antigen-specific tumour-reactive T lymphocytes for adoptive transfer**

Autologous lymphocytes are stimulated ex vivo with antigen-loaded antigen-presenting cells, such as dendritic cells. Enriched tumour-specific T cells are then re-infused for treatment.

with cytokines and has shown limited efficacy. Since the natural frequency of tumour-reactive T cells in the blood is often too low to be directly detectable, the number of antigen-specific peripheral blood T lymphocytes can be enhanced by repetitive in-vitro stimulations with antigen-presenting cells. One of the most common methods to stimulate T lymphocytes in vitro is the use of dendritic cells presenting the antigen of interest (figure 2). New approaches to polyclonal T-cell expansion attempt to replace autologous dendritic cells by artificial antigen-presenting cells with improved T-cell stimulatory properties.<sup>95,96</sup> An alternative method to repetitive, antigen-specific T-cell stimulation in vitro is the in-vivo immunisation of patients to enhance the starting numbers of antigen-specific T cells before ex-vivo expansion of the T cells. Vaccination might allow the in-vivo expansion of high-avidity tumour-specific T cells that could retain destructive function once expanded ex vivo.<sup>97,98</sup>

Tumour tissue or tumour-infiltrated lymph nodes are an alternative source to peripheral blood for the isolation of tumour-reactive T cells, because the tissues contain both CD8+ and CD4+ T cells that are specific for various tumour-associated antigens. Initial attempts to treat patients with ex-vivo stimulated TIL have met with limited success, largely due to the deficient effector functions of the TIL that had not been fully restored by ex-vivo stimulation.<sup>99,100</sup> Cultured T cells with strong stimuli such as CD3 antibodies and high doses of IL2 negatively affects the proliferative capacity of T cells and can even result in the outgrowth of Treg cells.<sup>57,58,88,101</sup> Recently, the transfer of ex-vivo activated autologous

TIL into patients with melanoma has elicited encouraging results, after immune suppressive factors were overcome by lymphodepletion of the recipients before TIL transfer.<sup>102</sup> Conditioning with a lymphodepleting chemotherapy led to the expansion and persistence of transferred T cells, which was associated with cancer remissions.<sup>12,102</sup> This approach takes advantage of the naturally-occurring homeostatic responses (when lymphopenia is induced) to promote the expansion of transferred T cells and subsequently restore the size of the original T-cell pool. The underlying mechanisms for lymphopenia-induced homeostatic proliferation are complex and include the removal of suppressive cells such as Treg cells or cytokine-consuming cells.<sup>48,103,104</sup>

#### Allogeneic T-cell infusions

Allogeneic transplantation can induce tumour regressions and even cure various malignant haematological diseases. One of the underlying mechanisms for this antitumour success is thought to be the graft-versus-leukaemia/lymphoma or graft-versus-tumour effect, which might be mediated by donor-derived T lymphocytes.<sup>105</sup> Donor lymphocyte infusions can re-induce complete remissions in various haematological malignant diseases when relapse occurs after allogeneic transplantation.<sup>106</sup> Depending on the HLA compatibility between donor and recipient, an HLA mismatch could exist between the patient's antigen-presenting cells and the donor's T cells. In HLA-mismatched individuals, peptides derived from tumour or self-antigens and then presented with the

patient's HLA molecules are treated as foreign by the donor T cells.<sup>107</sup> In HLA-identical individuals, donor T cells recognise peptides derived from polymorphic gene products, which are called minor histocompatibility antigens. The gene polymorphisms result in a pattern of HLA-peptide complexes that can differ between donor and patient, and therefore can lead to an enhanced immunogenicity of minor histocompatibility antigens. After donor lymphocyte infusions, the increase in the number of T cells specific for minor histocompatibility antigens has been shown to correlate with induction of complete remissions, thus supporting the hypothesis that these antigens can mediate graft-versus-tumour effects.<sup>108,109</sup>

### Engineered tumour-specific T cells

The widespread application of autologous T cells for adoptive T-cell treatment is limited by the fact that the isolation and expansion of tumour-reactive T cells is not successful in every eligible patient. This problem might be overcome if the patient's primary T lymphocytes are grafted with a second T-cell receptor known to recognise a defined tumour antigen.<sup>110</sup> T-cell-receptor gene transfer into human peripheral blood T lymphocytes has been shown to be feasible, resulting in the generation of such transgenic T cells that can be reprogrammed toward tumour-associated antigens.<sup>111–113</sup> Allorestricted T cells with peptide-dominant binding can serve as a source for highly avid T-cell receptors against specific antigens, particularly self-antigens.<sup>107,114</sup> Clinical studies of the approach are being initiated, and early results suggest that the strategy is feasible with few side-effects.<sup>115</sup>

Although early studies of T-cell-receptor-engineered T cells do not show improved clinical response compared with the use of autologous expanded cells, methods are being developed to enhance the therapeutic efficacy of gene-modified cells. Since the number of receptor molecules on the T cell has been shown to be important for function, systems that would further improve T-cell transfection efficiency are being developed.<sup>116,117</sup> Cotransfection of costimulatory or immune receptor molecules could also enhance function.<sup>116</sup> Furthermore, cotransfection of cytokines that are important for T-cell growth and proliferation might allow continued expansion of the transferred cytotoxic T lymphocytes *in vivo*.<sup>118</sup> Safety could be ensured by the inclusion of suicide gene proteins such as HSV-TK (herpes simplex virus thymidine kinase) or caspase 9, which would destroy transferred cells if toxic effects were excessive.<sup>118,119</sup>

### Future directions

Many compounds can modulate the environment and be useful in the development of combination immunotherapy to elicit tumour-specific immunity. For example, the *in-vivo* administration of an antibody can be used to block CTLA4 (cytotoxic T-lymphocyte-associated antigen 4), an inhibitory receptor that dampens the ability of T cells to

respond to antigen. The use of CTLA4 monoclonal antibodies alone can result in a 14% clinical response rate in advanced-stage melanoma.<sup>120</sup> However, removal of natural toleragenic pathways does have toxic effects. Clinically significant enterocolitis occurred in more than 20% of patients. Notably, the antitumour response was three times higher in those patients who developed enterocolitis than in those without toxic effects.<sup>120</sup>

At lower doses that do not induce lymphopenia or bone-marrow suppression, a growing number of chemotherapeutic drugs have been found to possess immune modulatory activities.<sup>121</sup> For example, doxorubicin is an anthracycline antibiotic that can inhibit topoisomerases I and II, as well as DNA and RNA synthesis. The drug has also been shown to affect both the innate and adaptive immune responses. Doxorubicin can affect monocyte and macrophage activity, predominantly in an antigen-independent manner.<sup>122</sup> Another commonly used drug, paclitaxel, is a dipertene plant product that binds to  $\beta$ tubulin, resulting in stabilisation of microtubules and G2/M-stage mitotic arrest. The immune effects of paclitaxel are mainly due to its lipopolysaccharide-like activity. Similar to lipopolysaccharide, paclitaxel can induce macrophages to secrete several proinflammatory cytokines, including IL1 $\beta$ , GM-CSF, TNF $\alpha$ , and nitric oxide.<sup>123</sup> This effect is thought to occur because paclitaxel can interact with toll-like receptor 4, triggering a danger signal and activating downstream signalling cascades, including MAPK (mitogen-activated protein kinase) and NF $\kappa$ B (nuclear factor  $\kappa$  B).<sup>124</sup> Cytoxin pretreatment has been shown to overcome tolerance in various preclinical and clinical models.<sup>125,126</sup> This effect could be due to the drug's ability to decrease the secretion of inhibitory cytokines (TGF $\beta$  and IL10) by splenocytes, relieving T-cell suppression.<sup>127</sup> Other reports also state that cytoxin can directly affect Treg cells.<sup>128</sup> In addition to modulating tolerance, cytoxin can also aid in the expansion and survival of CD8+ memory cells,<sup>129</sup> possibly by creating T-cell space after high-dose treatment.

Thus, both novel and standard compounds can be used to supplement treatments designed specifically to enhance tumour-specific cellular immunity. A combination of compounds that stimulate the appropriate T-cell populations as well as inhibit toleragenic mechanisms will probably yield the greatest clinical benefit.

#### Conflict of interest statement

MLD is an inventor on patents held by the University of Washington pertaining to T-cell therapies. EMJ has been a consultant for Amplimmune, where she provides advice on new agent development (pre-clinical advice to date); has been a consultant for Bristol-Myers Squibb in the past year; has licensed intellectual property to Anza Corp and has the potential to receive royalties in the future; and although she does not receive any financial support or has the potential for royalties, John Hopkins has the potential to receive royalties from Cell Geneys on the GVAX vaccine in the future. HB declares that she has no conflict of interest.

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