

# Adoptive cell transfer: a clinical path to effective cancer immunotherapy

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**Abstract** | Adoptive cell therapy (ACT) using autologous tumour-infiltrating lymphocytes has emerged as the most effective treatment for patients with metastatic melanoma and can mediate objective cancer regression in approximately 50% of patients. The use of donor lymphocytes for ACT is an effective treatment for immunosuppressed patients who develop post-transplant lymphomas. The ability to genetically engineer human lymphocytes and use them to mediate cancer regression in patients, which has recently been demonstrated, has opened possibilities for the extension of ACT immunotherapy to patients with a wide variety of cancer types and is a promising new approach to cancer treatment.

**Adoptive cell therapy (ACT).** The administration of a patient's own (autologous) or donor (allogeneic) anti-tumour lymphocytes following a lymphodepleting preparative regimen.

**Capillary leak syndrome**  
The loss of intravascular fluid into soft tissues and lung.

**Objective clinical response**  
The Response Evaluation Criteria in Solid Tumours (RECIST) defines an objective response as a 30% reduction in the sum of the longest diameters of measurable lesions comparing post-treatment with pretreatment values. The World Health Organization criterion defines an objective response to be a 50% reduction in the sum of the products of perpendicular diameters of measurable lesions. In both criteria no new lesions can appear.

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Adoptive cell therapy (ACT) has emerged as the most effective treatment for patients with metastatic melanoma. ACT-based immunotherapy was first described in 1988 (REF. 1), but the decisive improvement in efficacy came in 2002 with the introduction of an immunodepleting preparative regimen given before the adoptive transfer, which could result in the clonal repopulation of patients with anti-tumour T cells<sup>2</sup>. Of patients with metastatic melanoma refractory to all other treatments, 50% will experience an objective response, some with complete responses<sup>3</sup>. Responses can be durable and are seen in all organ sites, including the brain. Recent studies demonstrating that normal human lymphocytes can be genetically engineered to recognize cancer antigens and mediate cancer regression *in vivo* has opened opportunities for enhancing and extending the ACT approach to patients with a wide variety of cancer types<sup>4</sup>. These studies provide a valuable guide to the immunological principles that form the basis of effective immunotherapies for patients with cancer.

**The role of ACT in human cancer immunotherapy**  
Current efforts in the immunotherapy of human solid cancers fall into three main categories.

**Non-specific immunomodulation.** This is mediated by the administration of the T-cell growth factor interleukin 2 (IL2) and can activate endogenous tumour-reactive cells *in vivo* and reproducibly cause the regression of some human solid cancers<sup>5-7</sup>. The durability of the cancer regressions induced by IL2 led to its approval by

the US Food and Drug Administration for the treatment of patients with metastatic renal cancer in 1992 and metastatic melanoma in 1998. Although IL2 administration leads to toxicity owing to a capillary leak syndrome, experience with the administration of this cytokine has resulted in treatment-related mortalities of <1%<sup>8</sup>. More recently, antibody-mediated blockade of a cell surface inhibitory molecule, cytotoxic T-lymphocyte-associated 4 (CTLA4), has resulted in objective clinical responses in 10–20% of patients, but again only consistently in those with metastatic melanoma or renal cancer, suggesting that these two tumour types are exceptional in their ability to naturally generate endogenous anti-tumour cells of sufficient avidity and in sufficient numbers to mediate cancer regression when appropriately stimulated *in vivo*<sup>9,10</sup>. Investigations are underway to evaluate other general immune modulators such IL15, anti-transforming growth factor- $\beta$  (anti-TGF $\beta$ ) and anti-programmed death 1 (anti-PD-1) antibodies.

**Active immunization approaches (cancer vaccines).** These are based on immunizing cancer patients against their autologous cancers using either whole cells, proteins, peptides or a wide variety of immunizing vectors. The identification of a large number of human cancer antigens beginning in 1991 fuelled a resurgence of interest in this area<sup>11,12</sup>. Currently, only rare and highly sporadic regressions of solid cancers have been achieved using active immunization<sup>13</sup>. Several recent findings have further tempered enthusiasm for this approach. Even though up to 30% of circulating anti-melanoma CD8<sup>+</sup> T cells could be induced by immunization of

**At a glance**

- Adoptive cell therapy (ACT) is a treatment that uses a cancer patient's own T lymphocytes with anti-tumour activity, expanded *in vitro* and reinfused into the patient with cancer.
- ACT using autologous tumour-infiltrating lymphocytes is currently the most effective treatment for patients with metastatic melanoma and can mediate objective tumour regressions in 50% of patients.
- Lymphodepletion before ACT is an important component of the treatment because it eliminates T regulatory cells and eliminates lymphocytes, which compete with the transferred cells for homeostatic cytokines such as interleukin 7 (IL7) and IL15.
- ACT can be effective in treating selected patients with post-transplant lymphoproliferative diseases (PTLD) resulting from Epstein–Barr virus, which can cause PTLD during the immunosuppressed state.
- Recent studies have shown that genetic modification of lymphocytes using retroviruses that encode T-cell receptors can convert normal lymphocytes into lymphocytes with anti-cancer activity. The adoptive transfer of these lymphocytes into patients with metastatic melanoma can mediate tumour regression.

patients with melanoma, tumour progression can occur, suggesting that the cells induced are of low avidity and/or subject to inhibition by endogenous factors<sup>14</sup>. Although increases in anti-tumour T cells have been suggested in some active immunization protocols in patients with cancers other than melanoma, it has been possible to isolate and grow only rare anti-tumour T cells from these tumour types, again suggesting that T-cell precursors reactive with non-melanoma antigens are present at low frequency<sup>13</sup>.

**ACT.** This approach involves the identification *ex vivo* of autologous or allogeneic lymphocytes with anti-tumour activity, which are then infused into cancer patients, often along with appropriate growth factors to stimulate their survival and expansion *in vivo*. ACT has substantial theoretical and practical advantages over the approaches discussed above. It is necessary to identify only a small number of anti-tumour cells with

the appropriate properties that can then be expanded to large numbers *ex vivo* for treatment. *In vitro* tests can identify the exact populations and effector functions required for cancer regression, which can then be selected for expansion. The cells can be activated in the laboratory free from endogenous inhibitory factors and thus can be induced to exhibit the required anti-tumour effector functions. Perhaps most importantly, it is possible to manipulate the host before cell transfer to provide an optimal environment for the transferred cells. This approach has proved to be highly effective for the treatment of cancer in experimental animals as well as in cancer patients.

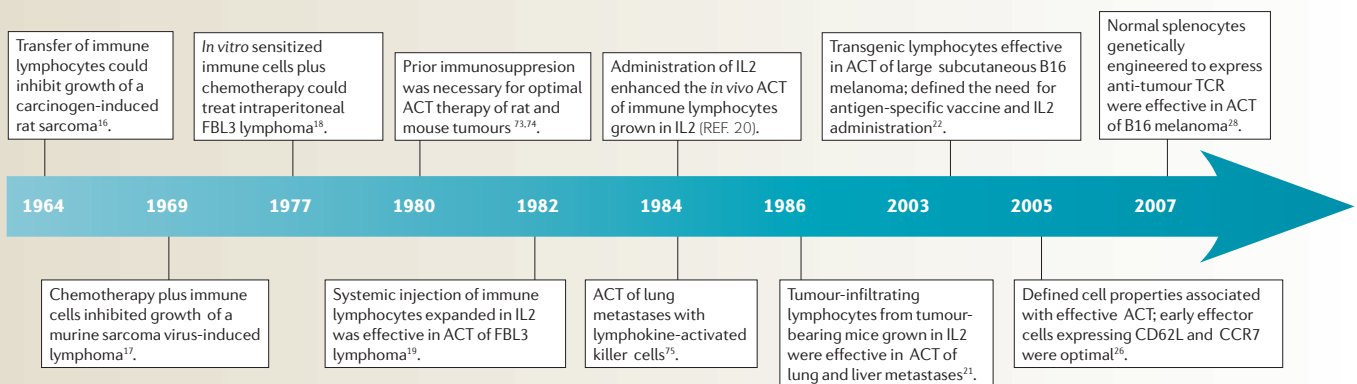
**Adoptive cell transfer in animal models**

Shortly after the demonstration that the cellular arm of the immune system was responsible for tissue rejection<sup>15</sup>, attempts were made to treat established rodent tumours by the transfer of immune cells. Examples of the development of ACT in animal models are summarized in TIMELINE 1. As T lymphocytes could not be grown *in vitro*, early efforts at ACT in the 1960s were limited to the use of cells obtained directly from immunized animals. Studies by Alexander and colleagues in the mid-1960s showed that sarcomas a few millimetres in size could be treated in rats by the administration of large numbers of lymphocytes from immunized syngeneic animals<sup>16</sup>. Extensive studies by Fefer and colleagues beginning in 1969 showed that intraperitoneal instillation of immune lymphocytes along with chemotherapy could effectively treat mice bearing intraperitoneal virus-induced lymphomas<sup>17</sup>. The ability to expand populations of anti-tumour immune cells using *in vitro* sensitization techniques in the mid-1970s freed these studies from the constraints imposed by the need for fresh cells from immunized hosts<sup>18</sup>. Eberlein *et al.* took this a step further by showing that the intravenous injection of immune cells grown in culture in IL2 could treat disseminated tumours in mice<sup>19</sup> and subsequent studies

**Avidity**  
The relative intensity of reactivity of lymphocytes when interacting with antigen.

**Allogeneic**  
Inter-individual genetic variation at the MHC locus. In a partially matched transplant, for example, some MHC antigens are shared by donor and recipient, but in addition the donor has some MHC antigens that the recipient does not.

Timeline 1 | Selected highlights in the development of ACT in animal models



ACT, adoptive cell therapy; IL2, interleukin 2; TCR, T-cell receptor.

**Lymphodepletion**

Lymphodepletion before ACT uses total body irradiation or cytotoxic drugs to deplete the lymphoid compartment of patients.

**Central memory cells**

A subset of antigen-reactive lymphocytes with markers such as CD62L and CCR7 that indicate a less differentiated phenotype.

**Antigen-presenting cells (APC)**

A subset of cells that have characteristics enabling them to efficiently present antigenic epitopes to lymphocytes (for example, dendritic cells).

showed that the concurrent administration of IL2 could further enhance the effectiveness of these IL2-dependent cells *in vivo*<sup>20</sup>.

The need for immunization of lymphocyte donors limited the application of this approach until 1986 when it was shown that tumour-infiltrating lymphocytes (TIL) from non-immunized mice bearing sarcomas or melanomas could be expanded *in vitro* in IL2 and used to successfully treat established lung and liver tumours<sup>21</sup>.

More recently, T-cell receptor (TCR) transgenic mice, all of whose lymphocytes express anti-gp100 tumour antigen TCRs, have provided a constant source of anti-tumour T cells that are valuable for defining host factors and cell properties associated with effective ACT of large, vascularized, subcutaneous tumours in mice<sup>22</sup>. The importance of host immunosuppression as a part of ACT was shown to be due to both the elimination of T regulatory cells<sup>23</sup> as well as the elimination of 'cytokine sinks' that compete with the transferred cells for homeostatic cytokines, such as IL7 and IL15, that are produced by host stromal cells<sup>24</sup>. The greater the degree of host lymphodepletion the more effective was the treatment<sup>25</sup>. The characteristics of the transferred cells themselves had a profound effect: anti-tumour T cells with a CCR7<sup>+</sup>, CD27<sup>+</sup>, CD28<sup>+</sup>, CD62L<sup>+</sup> phenotype that is characteristic of central memory cells were more effective than highly differentiated cells that lost these markers<sup>26</sup>. Immunization of the host with a vaccine expressing a tumour antigen recognized by the transferred cells enhanced their therapeutic effect<sup>22</sup>. Antigen-presenting cells (APCs) also have a role in effective ACT as the injection of APC-stimulating molecules such as Toll-like receptor agonists enhanced the efficacy of treatment<sup>27</sup>. More recently, it was demonstrated that normal murine splenocytes transduced with a retrovirus encoding an anti-melanoma TCR resulted in the generation of lymphocytes that recognized the melanoma *in vitro* and, when adoptively transferred, could mediate tumour regression *in vivo*<sup>28</sup>.

ACT is currently the most effective immunotherapy capable of mediating the rejection of large tumours in mice and optimization of the treatment in mouse models has had an important role in the design of ACT approaches in the human.

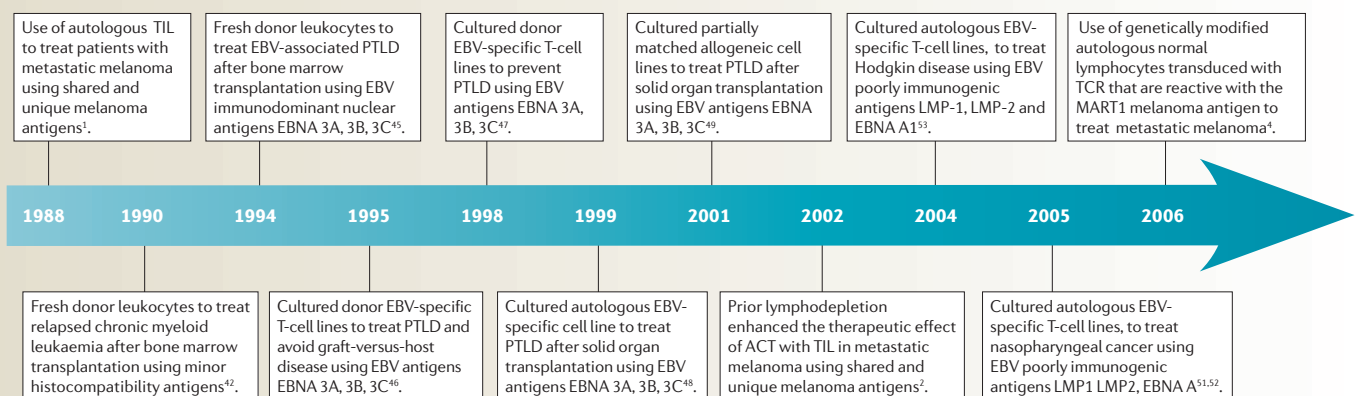
**ACT in metastatic melanoma**

Selected highlights in the development of ACT in patients with cancer are shown in **TIMELINE 2**.

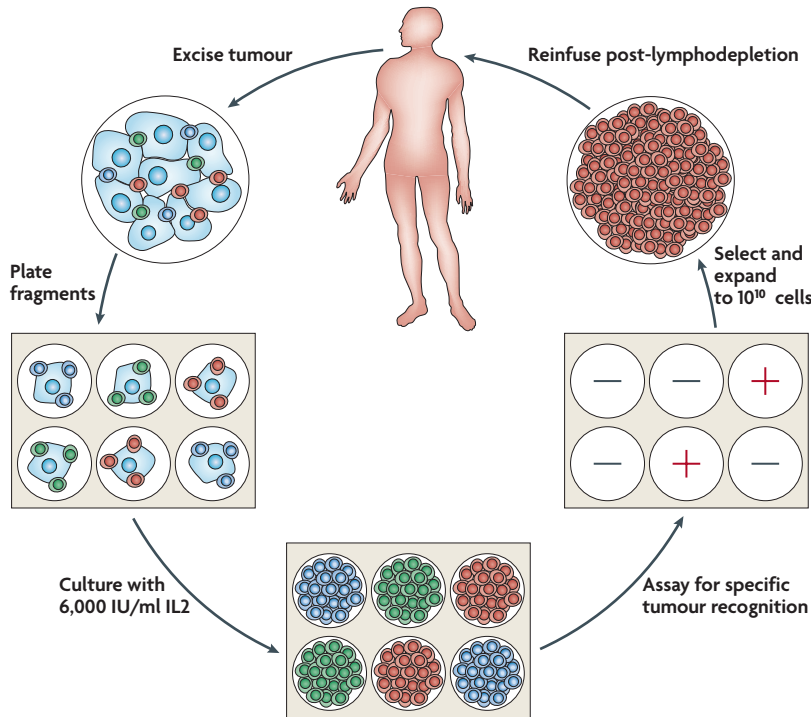
An important step in the development of human ACT was the finding in 1987 that lymphocytes infiltrating melanomas could be grown in IL2 and exhibit major histocompatibility complex (MHC)-restricted recognition of the autologous melanoma<sup>29</sup>. Over 50 different antigenic epitopes either unique to the autologous tumour or widely shared among melanomas were characterized using these TIL<sup>12</sup>. Studies using improved culture methods capable of generating up to 10<sup>11</sup> TIL showed that melanoma-specific activity could be detected in TIL from 81% of 36 consecutive patients<sup>30</sup>.

The infusion of autologous TIL grown from the resected tumour nodules of patients with metastatic melanoma represents the clearest example of the effectiveness of ACT for the treatment of patients with a metastatic solid cancer and has helped to elucidate the cellular and host characteristics required for effective treatment in humans. The original reports of this approach, first published in 1988 (REF. 1) and summarized in 1994 (REF. 31), described an overall objective response rate of 34% in 86 patients treated with autologous TIL plus high-dose IL2 (REF. 31). Patients received two cycles of treatment separated by 2 weeks. Eighty-eight percent of patients received >10<sup>11</sup> cells in the first cycle. Response rates were similar in patients treated alone (31%) or with low-dose cyclophosphamide (35%) and in patients who had not received prior IL2 (34%) or in patients refractory to prior IL2 therapy (32%). In this trial, TIL were administered regardless of their *in vitro* activity, although a retrospective analysis revealed that there was a highly

Timeline 2 | **Examples of ACT in patients with cancer**



EBV, Epstein-Barr virus; PTLD, post-transplant lymphoproliferative disease; TCR, T-cell receptor; TIL, tumour-infiltrating lymphocytes.



**Figure 1 | The generation of anti-tumour T cells used for adoptive cell therapy.** A tumour is excised and multiple individual cultures are established, separately grown and assayed for specific tumour recognition. Cultures with high anti-tumour reactivity are expanded to large numbers (>10<sup>10</sup> cells) and reinfused into the cancer patient following the administration of a conditioning lymphodepleting chemotherapy. IL2, interleukin 2.

significant correlation between clinical response and the ability of the TIL to lyse the autologous fresh tumour ( $P = 0.0008$ )<sup>32,33</sup>. Traffic of indium-111-labelled TIL to tumour deposits correlated with clinical response ( $P = 0.022$ ). Shorter times in culture and shorter doubling times were also positively associated with response ( $P = 0.0001$  and  $0.03$ )<sup>33</sup>, in accord with the findings in mouse models that increased proliferative potential was an important property of clinically active cells.

Several shortcomings became apparent in these initial studies of ACT using TIL. Persistence of the transferred cells *in vivo* was short. Studies using retroviral insertion of the neomycin phosphotransferase gene to mark TIL and sensitive PCR assays to detect the transgene revealed that at 1 week barely 0.01% of cells in the circulation were the transferred cells<sup>34</sup>. Of the 29 patients that exhibited an objective clinical response, five were complete though only two were ongoing at 21 and 46 months. The median duration of the partial responders was 4 months<sup>31</sup>.

Emerging information from murine models of ACT emphasized the need for prior lymphodepletion to eliminate regulatory T cells as well as normal endogenous lymphocytes that compete with the transferred cells for homeostatic cytokines<sup>23,24,35</sup>. Studies using highly selected tumour reactive CD8<sup>+</sup> clones administered following lymphodepletion did not result in objective tumour regression, suggesting that the polyclonal nature of tumour reactivity and possibly the presence of CD4<sup>+</sup> cells were necessary to mediate tumour rejection<sup>3,36</sup>. This led to a new generation of ACT clinical protocols with

altered techniques for cell growth and with profound host lymphodepletion before cell transfer<sup>2,3</sup>.

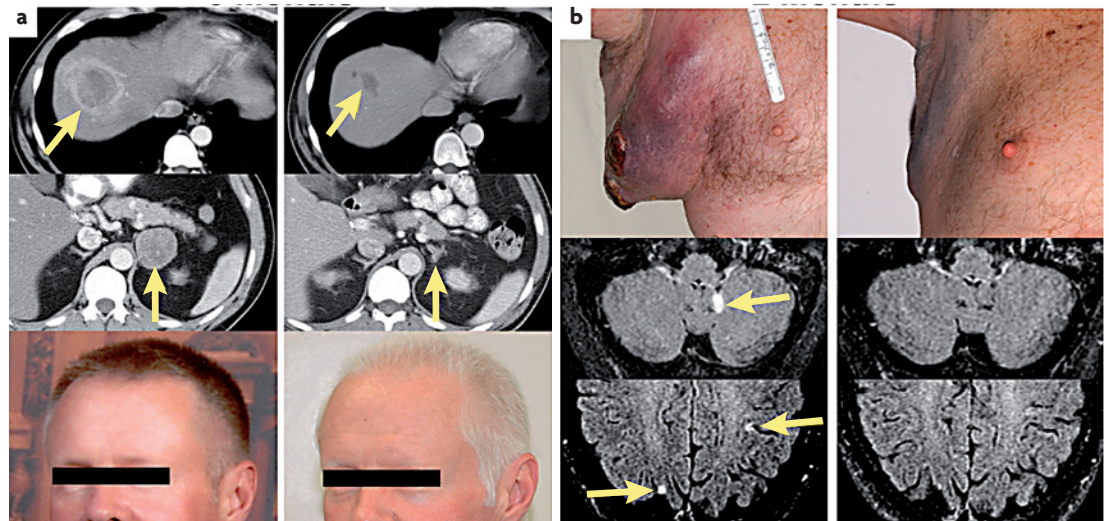
In the latest Surgery Branch, National Cancer Institute trials multiple TIL cultures were initiated from freshly resected metastatic melanomas (a mass of  $\geq 1\text{cm}^3$  was required) and as soon as anti-tumour activity could be detected *in vitro* against specific unique or shared antigens, cells underwent a rapid expansion using the T-cell-stimulating antibody OKT3 and IL2 (FIG. 1). Approximately  $5 \times 10^{10}$  cells were infused immediately following a non-myeloablative preparative regimen consisting of 60 mg/kg cyclophosphamide for 2 days followed by 5 days of fludarabine at 25 mg/m<sup>2</sup>. Twenty-five patients each also received either 2 Gy or 12 Gy plus the chemotherapy regimen. IL2 was administered for 2–3 days at  $7.2 \times 10^5$  IU/kg every 8 h.

Objective responses by standard response evaluation criteria in solid tumours (RECIST) were seen in 21 of 43 patients (49%), who received no total body irradiation, in 13 of 25 patients (52%) who received 2 Gy and 18 of 25 patients (72%) who received 12 Gy (TABLE 1). Patients with stable disease were not considered as responders as it is not possible to evaluate this criterion in the absence of randomized patient comparisons. Examples of anti-tumour responses in melanoma patients treated with ACT are shown in FIG. 2. Many of these responses at multiple metastatic sites are durable. None of the 10 complete responders have recurred at times from 8 to 63 months. Fifteen of the partial responders have ongoing responses from 4 to 64 months. The actuarial 3-year survival of patients receiving ACT with the chemotherapy non-myeloablative regimen alone or with 2 Gy total body irradiation is 25% and 42% respectively, compared with 14% for the no lymphodepletion group. The trend for increasing survival as a function of increasing lymphodepletion is highly significant ( $P = 0.007$ ), but this should be interpreted with caution as this was not a randomized comparison<sup>79</sup>.

There were no added toxicities in these trials owing to the cell administration, although the expected toxicities of IL2 administration were seen most often owing to the capillary leak syndrome caused by IL2. Neutropenic fevers that subsided when white cells recovered were seen in several patients.

Several findings in this trial are of note. The transferred cells expanded *in vivo* and persisted in the peripheral blood in many patients, sometimes achieving levels of 75% of all CD8<sup>+</sup> T cells at 6–12 months after infusion<sup>2</sup>. Persistence of the transferred T-cell clonotypes correlated with cancer regression<sup>37</sup>. Eleven of 13 responding patients had  $\geq 5\%$  persistence of transferred clonotypes compared with only 1 of 12 non-responders ( $P = 0.001$ ). In accord with this, the telomere length of the infused TIL correlated with cancer regression<sup>38</sup>. The mean telomere length in TIL administered to responding patients was 6.3 kb compared with 4.9 kb in TIL given to non-responders ( $P < 0.01$ ). TIL clonotypes that persisted *in vivo* had mean telomeres of 6.2 kb compared with 4.5 kb in non-persisting clonotypes ( $P < 0.001$ ). Telomere length is related to the proliferative history of the cell. Each time the cell divides telomeres at the

**Non-myeloablative**  
Relatively modest to moderate doses of chemotherapy are given, not to attack the cancer, but just to suppress the immune system for a brief period of a week or so.



**Figure 2 | Examples of objective tumour regressions in patients receiving adoptive cell transfer of autologous anti-tumour lymphocytes following a lymphodepleting preparative regimen.** In each case the pretreatment scans and photos are shown on the left and the post-treatment on the right. **a** | A 45-year-old male with metastatic melanoma to the liver (upper) and right adrenal gland (middle) who was refractory to prior treatment with high dose  $\alpha$  interferon as well as high-dose interleukin 2 (IL2). He underwent a rapid regression of metastases and developed vitiligo (lower). **b** | A 55-year-old male with rapid tumour growth in the axilla as well as multiple brain metastases from metastatic melanoma that was refractory to prior treatment with high dose IL2 who underwent rapid regression of nodal and brain metastases.

**Effector phenotype**

A constellation of cell surface markers that indicate that lymphocytes have differentiated into a mature effector cell capable of recognizing antigen and lysing target cells or secreting cytokines when encountering antigen.

**Alloantigens**

An antigen that exists in alternative (allelic) forms in a species, thus inducing an immune response when one form is transferred to members of the species who lack it.

**Buffy coat cells**

The plasma layer containing enriched white blood cells that results when whole blood is centrifuged.

**Chronic phase**

Indolent phase of the disease in patients with chronic myeloid leukaemia.

**Blast crisis**

Aggressive acute phase of the disease in patients with chronic myeloid leukaemia.

**Graft-versus-host disease**

Inflammatory and tissue-destructive immune reactions that result from the attack on host tissues by infused allogeneic lymphocytes.

**Post-transplant**

**lymphoproliferative disease (PTLD).**

Neoplastic proliferation of lymphocytes that occurs in patients undergoing immunosuppression, often in preparation for bone marrow or organ transplantation; can occur in host or recipient cells.

ends of chromosomes shorten. Thus cells with longer telomeres have a greater proliferative potential.

In contrast to murine models, the antigen-reactive administered TIL were of effector phenotype, CD27<sup>-</sup>, CD28<sup>-</sup>, CD45RA<sup>-</sup>, CD62L<sup>-</sup>, CCR7<sup>-</sup> (REF. 39). By 2 months after transfer, however, circulating tetramer-positive cells had a less differentiated phenotype and were CD27<sup>+</sup>, CD28<sup>+</sup>, CD45RA<sup>+</sup>, IL7R<sup>-</sup> but remained CD62L<sup>-</sup>, CCR7<sup>-</sup>, suggesting that highly activated tumour-reactive cells in culture can re-express many of these differentiation markers *in vivo*<sup>39</sup>. Re-expression of CD27 in TIL is an indication of a less differentiated cell and, following removal from IL2 *in vitro*, correlates with the effectiveness of these cells *in vivo*. These findings are in accord with studies in HIV-positive patients showing that CD27 expression promotes the *in vivo* survival of administered T cells<sup>40</sup>.

The high rate of cancer regression seen in these trials convincingly demonstrates the anti-tumour efficacy of ACT therapy and the importance of transferring cells with a high degree of antigen recognition and a high proliferative potential. Other issues of importance that might account for the lack of response in some patients relate to the traffic of the cells to draining lymph nodes or to poor expression of antigens by the tumour. Opportunities for improving ACT for patients with cancer based on the genetic modification of T cells are considered later in this Review.

**Other examples of effective ACT therapy**

**The treatment of patients with cancers expressing viral or alloantigens.** When the target antigen on a tumour is 'foreign' to the host and the avidity of the T cell is high, as is the case for viral antigens or alloantigens, ACT can be very effective in destroying large tumours in humans.

Minor histocompatibility antigens such as HA1 and HA2 can represent strong 'foreign' targets for effective ACT in cancer patients receiving allogeneic haematopoietic stem cell transplantation (HSC)<sup>41</sup>. In 1990, Kolb *et al.* reported cytogenetic remissions in three patients with relapsed chronic myeloid leukaemia treated with buffy coat cells from the HSC marrow donor<sup>42</sup>. Later studies showed that infusion of donor lymphocytes could mediate complete molecular remissions in 70–80% of patients with relapsed chronic myeloid leukaemia in chronic phase, in 20–30% of patients in blast crisis and in a minority of patients with relapsed multiple myeloma following treatment with allogeneic HSC transplantation<sup>43</sup>. Cloned CTL lines have induced responses, although the apparent lack of persistence of these lines *in vivo* has limited their effectiveness<sup>44</sup>.

**The treatment of patients with cancers expressing viral antigens.**

ACT can mediate cancer regression in humans with Epstein–Barr virus (EBV)-related lymphomas, which occur in patients receiving immunosuppressive drugs. Approximately 90% of humans have lymphocytes latently infected with EBV and the cellular immune response against these viral proteins is essential for the control of infected cells. Approximately 1% of individuals undergoing allogeneic HSC transplants who receive immunosuppressive drugs to prevent graft-versus-host disease develop post-transplant lymphoproliferative diseases (PTLD) in cells of donor origin and as many as 20% of solid organ transplant patients can develop PTLD in cells of recipient origin. These lymphomas express latent EBV antigens, including the immunodominant EBV nuclear antigens (EBNA) EBNA-3A, EBNA-3B and EBNA-3C, that are targets for immunotherapy.

Table 1 | **Adoptive cell therapy in patients with metastatic melanoma**<sup>79</sup>

Treatment	Patients (n)	Response (n (%))		
		PR	CR	OR
No TBI	43	17 (39.5)	4 (9.3)	21 (48.8)
2 Gy TBI	25	11 (44.0)	2 (8.0)	13 (52.0)
12 Gy TBI	25	14 (56.0)	4 (16.0)	18 (72)

All patients received cyclophosphamide 60 mg/kg for 2 days then fludarabine 25 mg/m<sup>2</sup> for 5 days. Includes all patients who received expanded tumour-infiltrating lymphocytes (TIL) plus the full preparative regimen as a first TIL treatment. CR, complete response; OR, objective response; PR, partial response; TBI, total body irradiation.

In 1994 O'Reilly and colleagues demonstrated that the infusion of small numbers of normal non-irradiated donor lymphocytes (10<sup>6</sup>/kg) achieved complete responses in five patients with lymphomas occurring following the treatment of leukaemia with chemotherapy and T-cell-depleted allogeneic HSC grafts<sup>45</sup>. Shortly thereafter, Rooney *et al.* demonstrated that infusion of long-term cultured EBV-specific T-cell lines generated by repeated *in vitro* sensitization and expanded in IL2 could effectively prevent and treat PTLD and thus avoid the graft-versus-host disease that is caused by transfer of whole T-cell populations<sup>46,47</sup>. Donor-derived EBV-specific CTL lines were infused into 60 patients at high risk of developing EBV PTLD and none developed the malignancy, compared with 11.5% of historical controls. Five of six patients treated for overt PTLD experienced a complete regression. These long-term cultured cells could be detected in patients up to 3 years after infusion. These studies have been widely reproduced using EBV-specific donor cell lines to treat donor PTLD in patients undergoing allogeneic HSC transplantation.

PTLD in solid organ transplant patients is a more difficult problem as it is difficult to raise autologous EBV-specific cells in patients on high doses of immunosuppressive drugs and cells from the organ donor are rarely available<sup>48</sup>. Thus treatment of these patients has used EBV-specific cell lines from partially matched unrelated (allogeneic) donors<sup>49</sup>. Recently Haque and colleagues reported a multicentre clinical trial using a bank of 60 long-term allogeneic EBV-reactive T-cell lines to treat PTLD patients. Of 33 patients, 52% achieved a partial or complete response at 6 months after treatment<sup>50</sup>.

The success in treating EBV PTLD led to the use of autologous anti-EBV cell lines to treat patients with stage 4 nasopharyngeal cancer (NPC) that was refractory to conventional treatments. Virtually all poorly differentiated nasopharyngeal cancer express the less immunogenic EBV latent proteins, LMP-1 and LMP-2, as well as the EBV EBNA-1 antigen. The infusion into six patients of anti-EBV cell lines targeting these antigens led to two complete remissions ongoing at 11 and 23 months and one partial remission lasting 12 months<sup>51</sup>. In a second series two partial responses were seen in ten treated patients<sup>52</sup>. These weak EBV antigens are also expressed in the malignant Reed–Sternberg cells found in about 40% of patients with Hodgkin disease. ACT using autologous EBV-reactive cell lines mediated objective responses in 3 of 11 evaluable patients with Hodgkin disease<sup>53</sup>.

These studies demonstrating the effectiveness of ACT directed against EBV and allogeneic antigens, as well as studies showing that the infusion of IL2-expanded cytomegalovirus (CMV)-reactive autologous T-cell lines grown long-term in IL2 could prevent CMV infection in immunosuppressed patients<sup>54</sup>, have profound implications for the development of ACT approaches to the treatment of patients with solid cancers. Administration of an avid anti-tumour T cell targeting a highly expressed antigen can result in cancer regression.

**ACT using gene-modified lymphocytes**

The success of ACT for the treatment of patients with metastatic melanoma has formed a foundation on which to build improvements of this approach. TIL with high avidity for tumour antigens can only be generated from some patients with melanoma and a need exists for the generation of T cells with broad reactivity against shared cancer-associated antigens present on multiple tumour types. The ability to introduce genes into circulating human lymphocytes provides the flexibility to introduce antigen receptors as well as molecules that can provide the cell with enhanced properties required for effective ACT therapy (reviewed in REFS 55,56). Genes encoding TCRs can be isolated from high avidity T cells that recognize cancer antigens and retroviral or lentiviral vectors can be used to redirect lymphocyte specificity to these cancer antigens<sup>57-60</sup>. Mispairing of the inserted chains with endogenous TCR chains can be greatly reduced by insertion of murine constant region sequences or by insertion of cysteine residues that favour pairing of only the transduced chains<sup>61,62</sup>. High-affinity TCRs can be obtained from rare reactive human clones or from transgenic mouse cells following immunization against human cancer antigens, which thus avoids the tolerance that can limit the generation of these cells in the cancer patient. High-affinity TCR against a p53 epitope<sup>63-65</sup> and against carcinoembryonic antigen (M. R. Parkhurst, personal communication) that is present on common epithelial cancers have been generated in transgenic mice and used to redirect the specificity of human lymphocytes. Phage display techniques have been used to generate TCRs with 10<sup>6</sup>× the affinity of a natural TCR directed against the cancer–testis antigen NY-ESO-1, which is expressed on many common cancers<sup>66</sup>. Chimeric TCR that use the combining site of antibodies genetically fused to intracellular T-cell signalling chains such as CD3ζ can

**Reed–Sternberg cells**

Cells with a characteristic morphology that are thought to be the malignant cells in patients with Hodgkin lymphoma.

**Tolerance**

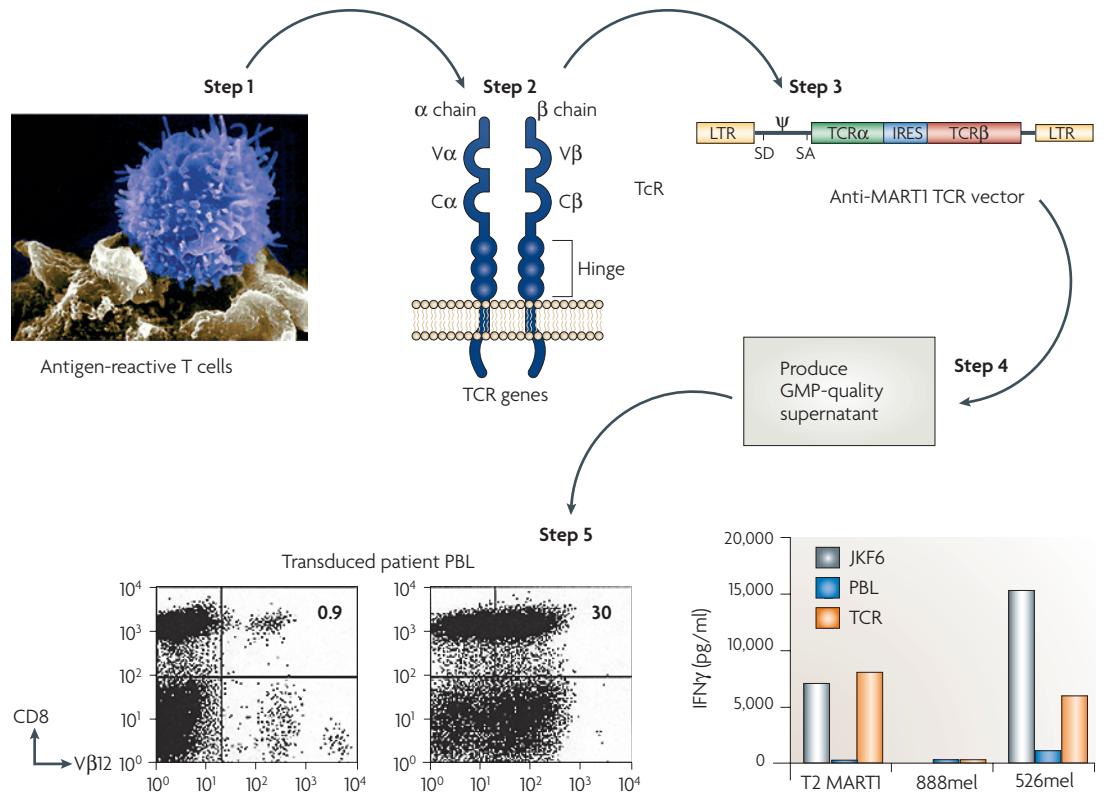
The process that ensures that B- and T-cell repertoires are biased against self-reactivity, reducing the likelihood of autoimmunity.

**Carcinoembryonic antigen**

A protein found in fetal gastrointestinal tissue that can be upregulated in some gastrointestinal cancers and can serve as a marker of tumour burden.

**Cancer–testis antigen**

A class of antigenic proteins present on some human cancers but not on adult normal tissues except for testes.



**Figure 3 | The steps involved in generating anti-tumour T cells by inserting genes encoding T-cell receptors.** Highly avid anti-tumour T cells are identified and the genes encoding their T-cell receptors (TCRs) are cloned and inserted into retroviruses. Retroviral supernatants are then produced under good manufacturing practice (GMP) conditions and used to insert the T-cell receptors into normal lymphocytes. Expression of the T-cell receptor is then compared in untransduced (UnTd) and transduced (Td) cells by fluorescence-activated cell sorting analysis and by recognition *in vitro* of HLA-A2\* 526 melanoma line and not the HLA-A2<sup>-</sup> 888 melanoma line. The effector cells were the anti-MART1 JKF6 line and untransduced (PBL) and transduced (TCR) lymphocytes. C, constant; IRES, internal ribosome entry site; LTR, long terminal repeat; SA, splice acceptor site; SD, splice donor site; V, variable. Steps 3 and 5 reproduced, with permission, from REF. 4 © American Association for the Advancement of Science (2006).

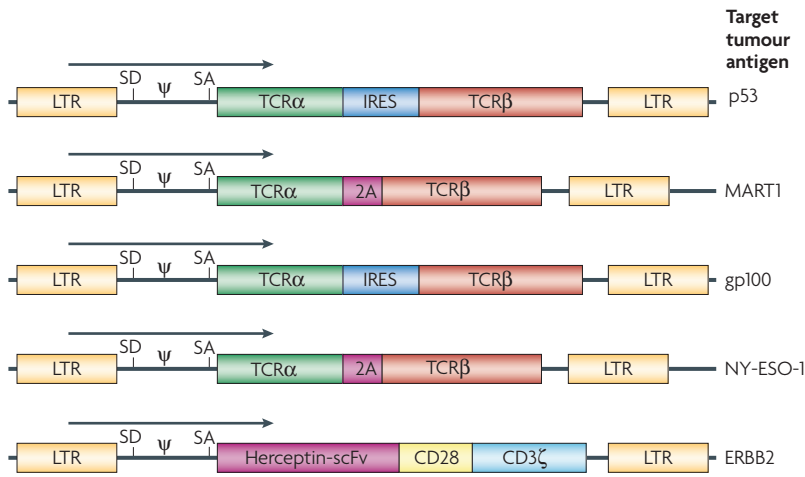
redirect the recognition specificity of lymphocytes to cell surface tumour-associated antigens and thus avoid the limitations of MHC restriction imposed by the use of  $\alpha$ - $\beta$  TCRs<sup>67–71</sup>.

The steps involved in this process are shown in FIG. 3. High-avidity T cells that are reactive with tumour antigens are identified in the human (often after extensive *in vitro* sensitization) or from transgenic mice immunized with human cancer antigens. The genes encoding the TCRs from these T cells are cloned and inserted into retroviruses. Retroviral supernatants are then generated under good manufacturing practice conditions that enable their use in humans. These retroviruses can be used to transduce human T cells that express the receptor and can be expanded *in vitro* for infusion into cancer patients.

The first clinical trial to successfully mediate the regression of human cancer by ACT using genetically engineered autologous lymphocytes has recently been published<sup>4</sup>. Sixteen patients were treated with a TCR that was reactive with the MART1 melanoma antigen isolated from highly reactive TIL. Two patients with metastatic melanoma who received ACT of their autologous

normal lymphocytes transduced with genes encoding this MART1 TCR underwent regression of liver and lung hilum metastases respectively and both are currently disease free over 2 years later. Two of 15 additional patients subsequently experienced objective tumour regressions (S.A.R., unpublished observation). TCRs with far greater affinity for the MART1 melanoma antigen have been identified and are now being evaluated in clinical gene therapy trials<sup>72</sup>. TCRs are now available against a broad array of cancer antigens present on common epithelial cancers and we have recently begun a trial treating patients with epithelial cancers using autologous T cells transduced with a TCR that recognizes a p53 epitope<sup>65</sup> (FIG. 4).

As mouse models clearly indicated that increased lymphodepletion could improve the efficacy of ACT<sup>12</sup> we are performing clinical trials in which patients receive non-myeloablative chemotherapy plus either 2 Gy or 12 Gy whole body irradiation. Other improvements in ACT are being studied (see TABLE 2 for selected examples). Murine models have shown that CD4<sup>+</sup>CD25<sup>+</sup>FOX3<sup>+</sup> regulatory T cells can inhibit ACT and thus the regimen can be modified to deplete CD4<sup>+</sup> cells or



**Figure 4 | Diagram of the retroviral constructs used to insert T-cell receptor (TCR) genes in T cells.** T cells can be engineered with two classes of receptor proteins that are capable of recognizing tumour-associated antigens. Naturally occurring TCRs require coordinated expression of an  $\alpha$  and  $\beta$  chain, which can be facilitated by an internal ribosome entry site (IRES) or by the use of a 2A fusion protein. A chimeric antigen receptor is an artificially constructed hybrid protein containing the antigen-binding domains of a single-chain antibody (scFv) linked to T-cell signal domains, such as CD28 and CD3 $\zeta$ . Vector-specific *cis*-acting sequences are the long terminal repeat (LTR) that contains the enhancer, promoter and polyadenylation sites, splice donor (SD) and splice acceptor (SA) sequences, and packaging signal ( $\psi$ ). The target antigen for each of these vectors is as indicated.

**The future of ACT**

In contrast to common epithelial cancers, melanoma appears to be a tumour that naturally gives rise to anti-tumour T cells. However, other cancers are equally susceptible as the targets of reactive T cells. The susceptibility of melanoma to ACT provides optimism for the application of ACT to common epithelial cancers using TCR gene-modified lymphocytes.

A major problem with the application of ACT is that it is a highly personalized treatment and does not easily fit into current modes of oncological practice. The treatment is labour-intensive and requires laboratory expertise. In essence, a new reagent is created for each patient and this patient-specific nature of the treatment makes it difficult to commercialize. Pharmaceutical and biotechnology companies seek off-the-shelf drugs, easy to produce, vial and administer. From a regulatory standpoint, ACT might be more appropriately delivered as a service rather than as a ‘drug’. Blood banks have been instrumental in providing CD34<sup>+</sup> haematopoietic stem cells for clinical studies and might be the ideal location for the generation of the anti-tumour T cells needed for ACT.

As modern science increasingly provides the physician with sophisticated information about the unique aspects of an individual cancer, changes in the modes of care delivery need to accommodate this. The ability to use this patient-specific information can lead to a new era of personalized medicine in which individual treatments, such as ACT, are devised for each patient.

Studies of ACT have clearly demonstrated that the administration of highly avid anti-tumour T cells directed against a suitable target can mediate the regression of large, vascularized, metastatic cancers in humans and provide guiding principles as well as encouragement for the further development of immunotherapy for the treatment of patients with cancer.

selectively eliminate T-regulatory cells. Lymphocyte function may be improved using antibodies or genetic approaches that block inhibitory signals on lymphocytes such as CTLA4, PD-1 or TGF $\beta$ . Increased persistence and function of the transferred cells may be accomplished by the administration of alternative cytokines such as IL15, or by stimulating the cells *in vivo* by the administration of a vaccine or by activating host APCs with Toll-like receptor agonists.

**Table 2 | Opportunities for improving ACT for the treatment of human cancer**

Method	Approach	Example refs
Genetic modification of lymphocytes to introduce new recognition specificities	$\alpha\beta$ TCR, chimeric TCR	4
Genetic modification of lymphocytes to alter function of T cells	Use of co-stimulatory molecules (CD28, 41BB); cytokines (IL2, IL15); homing molecules (CD62L, CCR7); prevention of apoptosis (BCL2)	76
Modify host lymphodepletion	Selective depletion of CD4 <sup>+</sup> cells or T regulatory cells	23
Block inhibitory signals on reactive lymphocytes	Antibodies to CTLA4 or PD-1	9
Administer vaccines to stimulate transferred cells	Recombinant virus encoding antigen	22
Administer alternative cytokines to support cell growth	IL15, IL21	77
Stimulate APCs	Use of Toll-like receptor agonists	27
Generate less differentiated lymphocytes	Alternate culture conditions and growth promoting cytokines <i>in vitro</i>	26
Overcome antigen escape variants	Use of natural killer cells	78

ACT, adoptive cell therapy; APC, antigen-presenting cell; CTLA4, cytotoxic T-lymphocyte-associated 4; IL, interleukin; PD-1, programmed death 1; TCR, T-cell receptor.

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## DATABASES

**Entrez Gene:** <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>  
 CCR7 | CD25 | CD27 | CD28 | CD34 | CD3ζ | CD4 | CD62L | gp100 | EBNA-1 | EBNA-3A | EBNA-3B and EBNA-3C | FOX3 | IL2 | IL2RA | IL7 | IL7R | IL15 | LMP-1 | MART1 | NY-ESO-1 | p53 | PD-1 | TIGB  
**National Cancer Institute:** <http://www.cancer.gov/>  
 chronic myeloid leukaemia | melanoma | multiple myeloma | nasopharyngeal cancer | renal cancer  
**National Cancer Institute Drug Dictionary:** <http://www.cancer.gov/drugdictionary/>  
 cyclophosphamide | fludarabine  
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