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EXPERT REVIEWS

Adoptive T-cell therapy for B-cell malignancies

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The success of allogeneic hematopoietic cell transplantation (HCT) for B-cell malignancies is evidence that these tumors can be eliminated by T lymphocytes. This has encouraged the development of specific adoptive T-cell therapy, both for augmenting the anti-tumor effect of HCT and for patients not undergoing HCT. T cells that are capable of recognizing antigens expressed on malignant B cells may be recruited from the endogenous repertoire or engineered to express tumor-targeting receptors. Critical insights into the qualities of T cells that enable their persistence and function *in vivo* have been derived, and obstacles to effective T-cell-mediated tumor eradication are being elucidated. These advances provide the tools to translate adoptive T-cell transfer into reliable clinical therapies.

KEYWORDS: adoptive T-cell therapy • allogeneic hematopoietic cell transplantation • central memory T cell • chimeric antigen receptor • immunoglobulin idiotype • graft-versus-host disease • graft-versus-tumor effect • minor histocompatibility antigen • T-cell receptor • tumor antigen

The potential to use immune-based therapies for human malignancies is attractive because of the specificity of antibody and T-cell recognition. The most profound advances have been made in the development of antibodies as therapeutics, and several monoclonal antibodies that recognize molecules on the surface of cancer cells are being used in human cancer therapy. Antibodies that target CD20 and CD52 are now routinely employed in standard therapeutic regimens for subsets of patients with B-cell malignancies [1–3]. The development of effective T-cell therapy for human malignancy either through vaccination or by adoptive T-cell transfer, which refers to the isolation, expansion and reinfusion of tumor-reactive T cells, has been substantially more challenging. The difficulties in developing T-cell-based immunotherapies are due, in part, to the inability of current vaccines to reproducibly elicit effective tumor-reactive T-cell responses, and the complexity of deriving and expanding tumor-reactive T cells *ex vivo* that have the capacity to persist and function following adoptive transfer. Despite these obstacles, the exquisite ability of T cells to distinguish diseased from normal cells has encouraged the continued investigation of strategies to employ T cells as therapeutic agents.

There is evidence from allogeneic hematopoietic cell transplantation (HCT) that advanced B-cell malignancies are susceptible to a T-cell-mediated graft-versus-tumor (GVT) effect, although GVT activity cannot yet be reproducibly separated from graft-versus-host disease (GVHD) [4–7]. The demonstration that B-cell tumors are recognized by T cells has provided optimism that donor T cells specific for tumor-associated antigens might be isolated, expanded and administered to the patient to augment the GVT effect; or that autologous T cells might be elicited or engineered to recognize tumor-associated antigens, without the need for allogeneic HCT. Engineering of tumor reactive T cells can be accomplished by gene transfer techniques that introduce a T-cell receptor (TCR) with specificity for peptide fragments of intracellular proteins displayed on class I and class II MHC molecules expressed by the tumor, or a chimeric antigen receptor (CAR) that consists of a single-chain antibody fragment (scFv) specific for a B-cell surface molecule linked to the ζ-chain of the CD3/TCR complex [8,9]. This review will discuss the rationale and theoretical framework for developing adoptive T-cell therapy for B-cell malignancies, the obstacles that have been encountered, and the directions that are currently being taken for clinical translation.

GVT effect of allogeneic HCT in B-cell malignancies

Allogeneic HCT provides a potentially curative therapy for a variety of hematologic malignancies, including many B-cell tumors. Originally,

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allogeneic HCT was developed as a method of rescuing patients from the lethal toxicity of high doses of myeloablative chemoradiotherapy administered to achieve greater tumor-cell killing than could be achieved with conventional doses of chemotherapy [10], and was used as a treatment of last resort for patients with refractory leukemia, including B-lineage acute lymphoblastic leukemia (B-ALL). Consistent with the prediction from murine models, that immune recognition of tumor cells could contribute to tumor eradication, human allogeneic HCT for B-ALL was accompanied by a T-cell mediated GVT effect [11–13]. A GVT effect of allogeneic HCT has subsequently been confirmed in other B-cell malignancies, including chronic lymphocytic leukemia (B-CLL), multiple myeloma (MM) and non-Hodgkin lymphoma (NHL) [14–18]. The myeloablative chemotherapy regimen contributes substantially to tumor control but it is now realized that the curative potential of this procedure is a result of the immunologic elimination of malignant cells. This is evident from the strikingly lower relapse rate and increased leukemia-free survival rate in patients receiving an allogeneic HCT compared with syngeneic HCT [12,13]. There are several factors that limit the success of allogeneic HCT and efforts to improve outcome are focused on reducing toxicity due to conditioning and GVHD, and augmenting the GVT effect (Table 1).

Donor lymphocyte infusions for B-cell malignancies

A critical role for donor T cells in the GVT effect was demonstrated by studies by Kolb *et al.* who investigated the use of donor lymphocyte infusions (DLIs) in patients with leukemia relapse after allogeneic HCT [19]. Durable complete remissions were achieved in 10–40% of patients with B-ALL, B-CLL, MM and lymphomas [20–22]. This compares with response rates of up to 70% in patients with relapsed chronic myeloid leukemia (CML) and it has been hypothesized that the superior ability of CML cells to differentiate into antigen presenting dendritic cells (DCs) and prime anti-tumor T-cell responses may be responsible for these differences in outcome [19,23]. To improve the outcome of patients with B-cell malignancies receiving DLIs, strategies such as the administration of pre-DLI chemotherapy or the use of *ex vivo*-activated donor lymphocytes are being explored. The induction of GVHD remains a major complication associated with the use of donor lymphocytes. Although some patients receiving DLI achieve complete remissions in the absence of clinically evident GVHD, most responding patients develop acute and chronic GVHD suggesting the antigens that are recognized on tumor cells are often shared with other tissues. The risk of GVHD can be attenuated in a subset of patients by using gradually escalating doses of DLI but this strategy is most effectively applied in slowly progressive malignancies [24–26].

Table 1. Factors that limit the success of allogeneic hematopoietic cell transplantation.

Obstacle	Strategy
Toxicity due to the conditioning regimen	Employ reduced-intensity conditioning regimens
Graft-versus-host disease	Improve drug regimens for immunosuppression Targeted suppression of alloreactive T-cell activation or function Remove alloreactive T cells from the donor stem cell graft
Tumor relapse	Adoptive transfer of tumor-reactive T cells Vaccination to elicit tumor-reactive T-cell responses *in vivo*

Reduced-intensity conditioning regimens for allogeneic HCT

Allogeneic HCT employing myeloablative conditioning is restricted to younger and medically fit patients to avoid excessive mortality from toxicities related to chemoradiotherapy. The median age at diagnosis for B-CLL and MM is over 60 years and these patients often have comorbidities due to their age, underlying malignancy or prior chemotherapy. This limitation to the use of allogeneic HCT was overcome by the development of reduced-intensity conditioning (RIC) regimens that use low doses of chemoradiotherapy to immunosuppress the recipient sufficiently to prevent rejection of the donor stem and T-cell graft, and enable a GVT effect to mediate tumor eradication [27,28]. RIC-HCT has been effective in several indolent B-cell malignancies, including B-CLL, MM and lymphoma, although aggressive lymphoma and B-ALL are less responsive [5–7,29]. Similar to the observations made in patients receiving myeloablative HCT, the anti-tumor efficacy of RIC-HCT is highly correlated with GVHD, which often requires long-term immunosuppressive therapy and is a major cause of morbidity and mortality. Therefore, the development of approaches that could be incorporated with HCT, such as the adoptive transfer of T cells that can specifically target antigens that are preferentially or selectively expressed on the tumor to augment the GVT effect without inducing GVHD, is a high priority of current research efforts.

Minor histocompatibility antigens as targets for the GVT effect

In the context of an allogeneic HCT from an HLA-identical donor, T-cell recognition of minor histocompatibility antigens (minor H antigens) is responsible for GVHD and has been implicated in the GVT effect. Minor H antigens result from genetic polymorphisms between the HCT recipient and the corresponding HLA-identical stem cell donor [30]. The most common mechanism for generating a minor H antigen is a non-synonymous single nucleotide polymorphism (SNP) that results in a peptide presented by HLA-molecules on recipient cells to which the HLA-identical donor is not tolerant and can be recognized as 'non-self' by donor $CD8^+$ and $CD4^+$ T cells [31]. Other mechanisms for generating minor H antigens including alternative splicing of peptide fragments, and differential protein expression as a consequence of gene deletion have also been identified [32,33].

Reduced-intensity conditioning-HCT relies almost exclusively on immune elimination of tumor cells and provides an opportunity to dissect the specificity of tumor-reactive T cells that develop

after HCT, and identify minor H or tumor-associated antigens that are the targets of the GVT response. In a study at the Fred Hutchinson Cancer Research Center (FHCRC) we analyzed the temporal kinetics and specificity of T cells that develop after non-myeloablative-HCT for B-CLL because large numbers of tumor cells could be stored pre-transplant and used to assess recognition by donor T cells that developed in the recipient post-transplant [34]. We found that $CD8^+$ and $CD4^+$ T-cell responses directed against minor H antigens expressed by B-CLL developed in all patients that achieved sustained tumor regression and correlated with the clearance of tumor cells from peripheral blood, bone marrow and lymph nodes. By contrast, patients with progressive B-CLL after HCT failed to develop T cells that recognized leukemic cells, even though they often developed GVHD, suggesting the failure to eradicate the tumor in these patients was because alloreactive T cells only recognized antigens that were not shared by target tissues of GVHD and the tumor (Figure 1) [34]. The kinetics with which tumor-reactive T cells developed in responding patients after RIC-HCT varied from several weeks to 1 year, however once established, these T cells persisted long-term, indicating that immunologic memory was established. Analysis of the specificity of $CD8^+$ T cells that recognized B-CLL in patients that achieved a complete remission demonstrated that multiple minor H antigens were being targeted, including those that were broadly expressed on both leukemic cells and nonhematopoietic tissues [34].

A focus of current work in the field of allogeneic HCT is the identification of minor H antigens that are limited in their expression to normal and malignant hematopoietic cells, and are absent on GVHD target tissues. Several minor H antigens that meet this prerequisite have been discovered, including HA-1, ACC-1 and LRH-1 [31,35,36], which are selectively expressed on all hematopoietic cells; and PANE-1, HB-1 and CD19, which are exclusively expressed on B-lineage cells and could be used to target B-cell malignancies [37–39]. However, the utility of an individual hematopoietic-restricted minor H antigen as a target for T-cell therapy is determined by the frequency of the HLA allele that presents the antigen and the requirement that the patient and donor be mismatched for the minor H antigen, in the appropriate direction. For HA-1, the most extensively studied minor H antigen, the disparity rate is estimated to be 4.2–8.5% in HLA-$A2^+$ sibling pairs and 6.8–12.2% in HLA-A2 matched unrelated pairs [40]. These data illustrate a problem for the translation of minor H antigen specific T-cell therapy into clinical practice with the panel of minor H antigens that are presently known, since only a few patients could actually benefit from this strategy. This obstacle will only be resolved by the discovery of additional minor H antigens. The HapMap project has identified over 10^4 SNPs in the human genome that confer amino acid sequence changes in coding regions and this database has enabled the use of genome association studies for minor H antigen discovery, which should rapidly expand the number of minor H antigens at our disposal [41]. A second obstacle for targeting minor H antigens is the need to derive and expand minor H antigen-specific T cells from the donor for adoptive transfer in every case. A potential solution discussed later in this review is the isolation of TCR genes from high-avidity minor H antigen-specific T cells, construction of gene transfer vectors and engineering of primary donor T cells for transfer in suitable patients.

Adoptive immunotherapy with T cells specific for nonpolymorphic tumor-associated antigens

Ideally, nonpolymorphic antigens expressed on B-cell tumors could be targeted using autologous T cells to circumvent the need to find a suitable donor for allogeneic HCT and the complications associated with HCT. In our analysis of T-cell responses that developed after

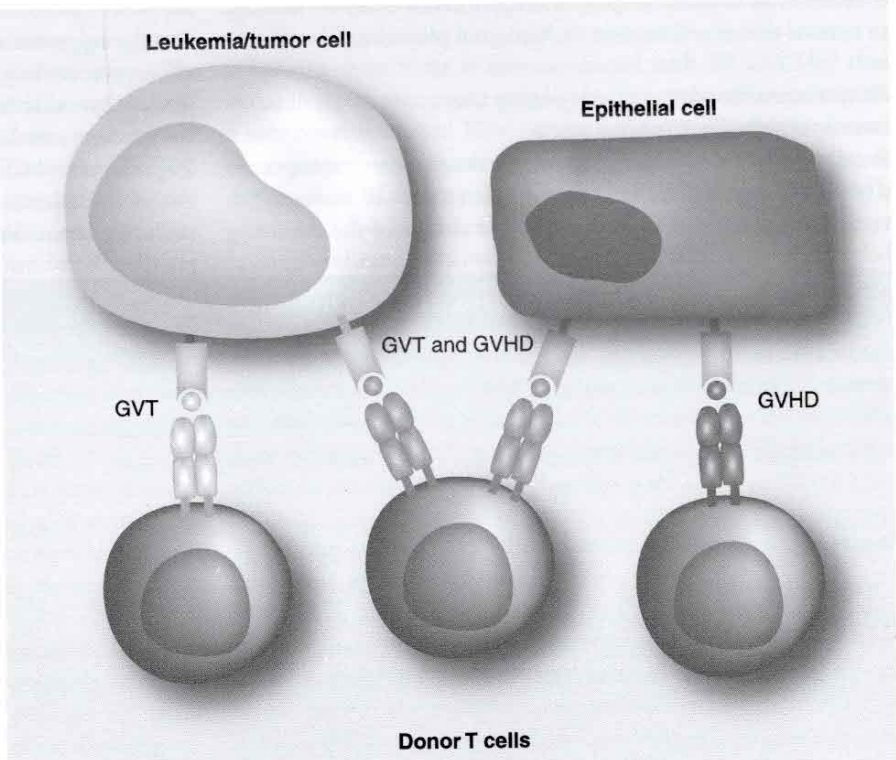

Figure 1. Expression of minor H antigens on tumor cells and epithelium dictate GVHD and GVT activity mediated by donor T cells. Minor H antigens that are selectively expressed on tumor cells but not on epithelium can serve as targets for a selective GVT response in the absence of GVHD. T cells specific for minor H antigens expressed on both tumor and epithelium can mediate both a GVT effect and GVHD, those specific for minor H antigens on tumor but not epithelium mediate a selective GVT response, while those specific for minor H antigens on epithelium but not on tumor only mediate GVHD.
GVHD: Graft-versus-host disease; GVT: Graft-versus-tumor.

allogeneic RIC-HCT for B-CLL, we were surprised to find that a proportion of T-cell clones were specific for nonshared and shared determinants that are only expressed on the recipient B-CLL, and not on Epstein–Barr virus (EBV)-transformed B cells [34]. Additional studies are in progress to characterize these putative tumor-specific antigens that are targeted as part of the anti-B-CLL response after transplant, and to determine whether they represent tumor-specific mutations or nonpolymorphic proteins. Several candidate tumor-specific proteins have already been identified in distinct subtypes of B-cell malignancies and are being pursued as targets for T-cell therapy. These include the immunoglobulin idiotype (Id), the bcr–abl protein in Philadelphia chromosome-positive B-ALL [42], and EBV-associated proteins that are expressed in EBV-positive Hodgkin disease (HD), Burkitt's lymphoma (BL), and EBV-induced lymphoproliferations that develop in severely immunocompromised patients. In addition to tumor-specific determinants that are restricted in their expression to the B-cell tumor, many proteins have been found to be overexpressed or aberrantly expressed in malignant B cells and to be present at much lower levels in normal tissues (Table 2). Tolerance to many of these tumor-associated self-proteins is not complete and it has been possible to isolate T cells that selectively lyse tumor cells. Whether such T cells can be used for adoptive therapy without excessive damage to normal tissues will require evaluation in pilot clinical trials.

Tumor-specific antigens: targeting the immunoglobulin idiotype

B-cell malignancies derive from a single B-cell clone that expresses a unique immunoglobulin (Ig) molecule on the cell surface. The variable regions of the heavy and light chains of the tumor Ig contain determinants that are specific for the malignant clone and are referred to as Id. The Id is a specific target for immunotherapy and passive immunotherapy with custom-made monoclonal anti-Id antibodies inducing an anti-tumor effect in up to 66% of patients, and long-lasting tumor regressions achieved in some cases [43]. However, this approach failed to completely eradicate all malignant B cells, and some patients relapsed owing to outgrowth of a mutated tumor Id-variant that had lost the epitope recognized by the monoclonal antibody [43,44]. T-cell recognition of Id-derived epitopes has also been demonstrated, and Id-specific T cells were shown to eradicate B-cell tumors in murine myeloma models [45].

Based on the partial efficacy of passive antibody therapy in humans and animal model data demonstrating anti-tumor activity of Id-specific T cells, it was logical to evaluate vaccination regimens that might elicit both antibody and T-cell responses. Clinical trials have focused on patients with follicular lymphoma (FL), which is sufficiently indolent to provide time to produce the vaccine and to develop an immune response after vaccination. Studies by Kwak *et al.* showed that immunization with autologous Id protein generated Id-specific humoral and cellular immune responses in patients with FL [46]. In subsequent trials, adjuvant cytokines and Id-loaded DCs were incorporated into vaccine regimens in an effort to improve immunogenicity and efficacy [47,48]. In a Phase I/II study by Bendandi *et al.*, in which granulocyte macrophage colony-stimulating factor was added to the vaccine, anti-Id antibody responses were induced in 15 out of 20 patients and $CD8^+/CD4^+$ T-cell responses were induced in 19 out of 20 patients. A subset of these patients achieved sustained molecular remissions of their lymphoma, providing evidence for an anti-tumor effect of vaccination [48]. A subsequent Phase II study in patients who had relapsed after initial chemotherapy, confirmed the immunogenicity of this approach and suggested a clinical benefit of vaccination based on a prolonged duration of complete response to reinduction chemotherapy [49]. Both humoral and cellular immune responses may be important in anti-tumor effects following anti-Id vaccination, although a fraction of patients who achieved complete remission after Id vaccination did so in the absence of detectable antibody responses [48]. A recent retrospective analysis of FL patients who had received Id vaccinations addressed the question whether the superior clinical outcome associated with an anti-Id immune response also resulted in an improved overall survival (OS). The study showed that the generation of an antibody response correlated with an improved OS at 10 years, whereas the generation of an anti-Id T-cell response was surprisingly not associated with improved OS [50]. This could reflect

Table 2. Nonpolymorphic antigens in B-cell malignancies.

Antigen class	Example	Tumor expression	Ref.
Immunoglobulin idiotype	CDR region of Id	Individual Id expressed in each B-cell malignancy	[51]
Viral antigens	EBV latent proteins (e.g., EBNA-1, 3, LMP-1,2)	EBV-LPD, Hodgkin disease	[65,68]
Chromosome translocation	Bcr/abl	Philadelphia chromosome-positive B-ALL	[42]
Overexpressed proteins	Bcl-2	B-CLL	[149]
	Fibromodulin	B-CLL	[74]
	Mdm2	B-CLL	[73]
	RHAMM/CD168	B-CLL	[150]
	Survivin	B-CLL	[75]
	DKK1	MM	[78]
	HM1.24	MM	[77]
	PRAME	MM, B-CLL	[150]
	SPAN-XB	MM	[76]
	WT-1	MM, B-ALL	[80,81]
Aberrantly expressed proteins	Cancer-testes antigens (e.g., NY-ESO-1, LAGE, MAGE)	MM	[80]

B-ALL: B-cell acute lymphoblastic leukemia; B-CLL: B-cell chronic lymphocytic leukemia; CDR: Complementarity determining region; EBV: Epstein–Barr virus; Id: Idiotype; LPD: Lymphoproliferative disease; MM: Multiple myeloma.

the sensitivity of assays to detect Id-reactive T cells compared with those to evaluate humoral immunity. Characterization of anti-Id T-cell reactivity has mapped the immunogenic epitopes within the hypervariable complementarity determining regions of the Ig heavy chain [51]. Of interest, *in vitro* studies have also suggested that Ig framework-derived peptides can function as cytotoxic T-cell epitopes [52], although mapping of the specificity of responses in vaccinated patients has yet to reveal recognition of framework regions.

The potential benefit of Id vaccination in FL has now been investigated in three Phase III studies [53]. The results of these trials have not yet been published but preliminary information that has been released indicates that only one trial showed a significant benefit in disease-free survival for vaccinated patients [53]. This study included 177 previously untreated patients with FL who achieved a complete remission after induction chemotherapy. The median disease-free survival for patients who received Id vaccination was 33.8 months versus 21.1 months in the control arm. This advantage in disease-free survival has not been observed in the two other studies [53]. The publication of data from these trials is awaited with interest, and may provide insight into what factors contribute to the differences in outcome following Id vaccination.

Compared with the success of Id vaccination in FL, the induction of anti-Id immunity in MM and aggressive lymphoma has been more challenging, and clinical responses have only been observed in a minority of patients [54,55]. The neutralization of anti-Id-antibodies by circulating myeloma protein and immune-escape mechanisms, such as the secretion of TGF-β and IL-10, by myeloma cells may compromise the priming and/or function of anti-Id T-cell responses [56]. Additionally, studies in murine myeloma models showed that Id-specific T cells are progressively deleted from the T-cell repertoire with increasing serum levels of myeloma protein [56,57]. Even if reactive T cells can be elicited, the functional phenotype may be critical in anti-tumor activity, with cytotoxic T lymphocytes and Th1 cells inhibiting tumor growth and Th2 cells promoting tumor progression [58].

Idiotype-specific T cells can be generated *in vitro* through stimulation with peptide-pulsed or Id-transduced autologous DCs, as well as through genetic introduction of chimeric anti-Id receptors, but efforts to employ adoptive T-cell transfer to target Ids have been limited to animal model studies [59,60]. The requirement to produce a customized Id product from each patient, to use as a reagent for antigen presentation, combined with the complexity of reproducibly deriving a T-cell product for adoptive therapy from each patient makes such an effort challenging. The use of vaccination with Id-protein or Id-loaded DC to elicit Id-specific T cells that could then be more readily isolated and expanded *ex vivo* may provide a solution to the latter problem, and facilitate studies to determine if vaccination combined with adoptive T-cell transfer could improve upon the results achieved with vaccination alone [55]. Furthermore, vaccination with tumor lysate-loaded DCs may represent a strategy to induce or reactivate a T-cell response with reactivity against multiple tumor antigens, including the Ig–Id [61].

Targeting EBV-associated B-cell malignancies with T-cell therapy

The lymphotropic EBV was first discovered in cultured lymphocytes from BL [62], and has also been detected in HD. The oncogenic potential of EBV is evident in severely immunocompromised HCT and solid-organ transplant patients who develop EBV-driven B-cell proliferations, termed EBV-associated lymphoproliferative disease. These tumors develop as a consequence of inadequate T-cell surveillance, and express the full range of latent cycle EBV antigens, including Epstein–Barr nuclear antigen (EBNA)-3A, -3B and -3C proteins, which serve as targets for T cells in immunocompetent donors. Indeed, EBV-associated lymphoproliferative disease that occurred in recipients of T-cell depleted allogeneic HCT can be successfully treated by the adoptive transfer of unselected lymphocytes from the respective EBV-seropositive donor, although this approach is complicated by GVHD [63]. To avoid GVHD, Rooney *et al.* isolated EBV-specific T cells from the peripheral blood of HCT donors by repeated *in vitro* stimulation and demonstrated that adoptive transfer of only the EBV-reactive T cells promoted regression of established EBV-driven lymphomas, and prevented the development of tumors when administered prophylactically [64]. The introduction of a marker gene into a subset of transferred EBV-specific T cells was used to track infused T cells and demonstrated that the cells persisted long-term *in vivo* [64].

Epstein–Barr virus is also associated with BL and HD that occur in immunocompetent individuals, but these tumors express a very restricted set of EBV antigens, that presumably facilitates their escape from host immunity. EBNA-1 is the only EBV protein expressed in BL but is not recognized efficiently by $CD8^+$ T cells [65,66]. HD also expresses EBNA-1, and the weakly immunogenic latent membrane proteins (LMP-1) and -2. Although LMP-1 and -2 are weakly immunogenic, strategies for isolating and expanding autologous T cells specific for these antigens from patients with HD have been developed [67,68]. Pilot studies in which T cells targeting these EBV antigens have been expanded and adoptively transferred to patients with HD have shown that the EBV-reactive T cells migrate to tumor sites and result in tumor regression in a subset of patients [69].

Targeting nonmutated tumor-associated antigens derived from self-proteins

Microarray studies that have compared the gene-expression profile of malignant B cells to their respective normal counterparts have uncovered highly expressed genes in the various histologic types of B-cell tumors [70–72]. Algorithms that predict peptide binding to MHC have identified potentially immunogenic peptide epitopes that might serve as targets for T-cell therapy in several overexpressed proteins, including MDM2, fibromodulin and survivin in B-CLL [73–75]; NY-ESO-1, SPAN-XB, DKK1, HM1.24 and WT-1 in MM [76–80] and WT-1 in B-ALL [81]. Tumor-reactive $CD8^+$ T cells that recognize these and other self-peptides have been isolated from normal donors using *in vitro* culture techniques [73–78,82,83]. However, it is often difficult to isolate T cells with high affinity to these self-antigens from patients who have been pretreated with cytotoxic chemotherapy that affects T-lymphocyte

numbers and function. Additionally, it remains to be determined whether targeting antigens that are also expressed in some normal tissues with T-cell therapy may lead to undesired toxicity.

Efforts to target self-proteins expressed on B-cell tumors by adoptive therapy have initially focused on WT-1 and NY-ESO-1, which are only expressed at low levels in very few normal tissues but are highly expressed in tumor cells of some patients. WT-1 is expressed at high levels in myeloid leukemias and B-ALL and at very low levels on $CD34^+$ hematopoietic stem cells. WT-1-specific T-cell responses have been elicited in normal donors and implicated in the GVT effect against B-ALL after allogeneic HCT [83,84]. A WT-1 peptide vaccine increased the frequency of WT-1-tetramer-positive T cells and induced an anti-tumor response in a subset of leukemia patients without adverse events [85]. Techniques for the isolation of T cells specific for multiple epitopes in WT-1 from normal donors have been described [82,83], and clinical trials of adoptive T-cell therapy targeting WT-1 have been initiated.

NY-ESO-1 is a member of the cancer testis (CT) family of proteins that are normally only present in the testis and the placenta, but are upregulated in a variety of tumor types, including a subset of patients with MM and melanoma. NY-ESO-1 was found to be the most immunogenic CT antigen and both spontaneous and vaccine-induced immune responses against NY-ESO-1 have been reported in cancer patients [86,87]. A clinical trial of adoptive T-cell therapy for melanoma with autologous $CD4^+$ NY-ESO-1-specific T-cell clones resulted in a remission in one out of nine patients, illustrating the potential for T cells of this specificity to mediate anti-tumor activity [88]. These results have implications for the development of adoptive T-cell therapy for MM, since many members of the CT antigen family, including NY-ESO-1, are expressed in myeloma cells and these proteins have been implicated in tumor progression [89].

Genetic modification: endowing T cells with receptors that target tumor antigens

Although polymorphic and tumor-associated antigens have been identified for B-cell malignancies and mediate anti-tumor activity *in vitro* or in preclinical models, their translation into clinical trials is hampered in many cases by the failure to reproducibly isolate high-affinity T cells from patients or healthy donors that are specific for these antigens. The promise of adoptive T-cell therapy will only be realized if methods for the reproducible production of tumor-reactive T cells for therapy are developed. The genetic modification of T cells provides a potential solution for this problem, since T cells can be engineered to express a TCR capable of recognizing the desired antigen with high affinity or to express an artificial CAR that is specific for a tumor cell surface molecule (Figure 2).

T-cell receptor gene transfer

The specificity of T-cell recognition is provided by the TCR, and the transfer of *TCR*-α and -β genes into recipient T cells can endow the cells with the antigen specificity of the introduced TCR [90]. Thus, one approach to derive T cells for therapy of B-cell tumors is to introduce TCR genes from clones with specificity for a viral, minor H antigen, or nonpolymorphic tumor antigen into T lymphocytes of a patient [8,91–94]. The *TCR*-α and -β genes can be derived from the rare T cells that have been isolated with high affinity for the desired antigen, or from transgenic mice that express human HLA alleles and have been immunized with the human protein to generate a high-affinity murine T-cell response from which the murine HLA-restricted TCRs can be cloned [93].

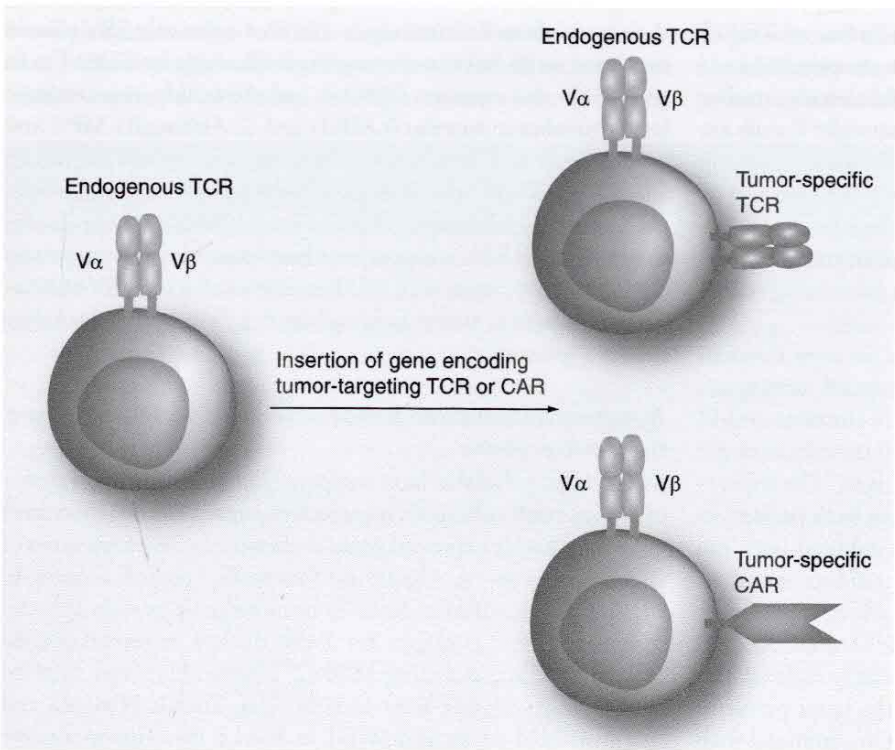

Figure 2. Gene transfer can retarget primary T cells to recognize tumor cells. Schematic demonstration of the engineering of bispecific T cells by inserting the α- and β-genes of a TCR specific for a tumor-associated antigen, or a gene encoding a CAR constructed of a single-chain antibody fragment fused to TCR signaling domains. CAR: Chimeric antigen receptor; TCR: T-cell receptor.

Gene transfer into T cells

The majority of TCR transfer studies have employed γ-retroviral vectors (RV) based on murine leukemia virus or murine sarcoma virus, or self-inactivating lentiviral vectors (LVs) [95,96]. A characteristic of RV is that cell division is required for vector integration following infection [97]. By contrast, LVs have the ability to transduce a variety of slowly or even nondividing cells, including unstimulated T cells in the absence of TCR activation [98]. For the

genetic modification of T cells, this may be an advantage since TCR activation and sustained proliferation required for RV transduction may impair the ability of transduced T cells to persist after adoptive transfer and reduce their anti-tumor activity. Both RV and LV preferentially integrate into transcriptionally active genes, and entail a risk of insertional mutagenesis and transformation of modified T cells, although LVs are considered to be less genotoxic [99,100]. The development of lymphoproliferative complications due to dysregulated proto-oncogene expression in a gene therapy trial that used engineered hematopoietic stem cells in patients with X-linked severe combined immunodeficiency (SCID), highlighted the risk related to cell transformation as a consequence of gene insertion and has led to the development of self-inactivating vectors with reduced mutagenic potential (Box 1) [101–103]. Compared with hematopoietic stem cells, mature T cells have been found to be more resistant to oncogene transformation and so far, no case of malignant transformation has been reported in adoptive immunotherapy trials that employed T cells genetically modified with suicide genes or gene markers [104]. Nonviral gene-delivery systems, such as electroporation into T cells have also been developed, however, the expression that can be achieved with plasmid DNA may vary considerably among T cells and is only transient after electroporation of RNA [105,106]. A potentially attractive nonviral system for stable gene transfer is the sleeping beauty (SB) transposon technology. SB is a synthetic transposable element that has been generated from the ancestral Tc1/mariner-like transposon in fish [107]. DNA transposons encode a transposase flanked by inverted terminal repeats that contain transposase binding sites. Any gene of interest flanked by the inverted terminal repeats can undergo transposition into the host genome via a cut and paste mechanism. SB tranposase has been shown to enable stable gene transfer into primary human T cells and its utilization for *TCR* gene transfer is anticipated [108].

The only experience using *TCR* gene transfer to generate T cells for adoptive therapy of human cancer has been in metastatic melanoma. In the first reported study, two out of 15 patients experienced objective tumor regressions after receiving autologous T lymphocytes engineered to express a TCR specific for the melanocyte differentiation antigen MART-1 [92]. Although this study established the therapeutic potential of genetically modified T cells for the treatment of human malignancies, issues were identified that need to be addressed to improve the results. These include safe methods for gene delivery that achieve adequate and stable TCR transgene expression, strategies for minimizing pairing of the introduced TCR chains with the endogenous TCR chains that might result in a deleterious specificity, and introducing the TCR into T cells that can establish a durable functional T-cell response *in vivo* after adoptive transfer.

Achieving adequate TCR expression, pairing & avidity

T-cell receptor transgene cassettes that allow the coupled transcription of both genes under control of the same promoter yielding a single coding mRNA molecule have been developed. These vectors utilize an internal ribosomal entry site or virus-derived sequences that encode self-cleaving peptides, such as 2A to promote coordinate translation of the mRNA into two separate α- and β-chain proteins. Recent studies that compared both features favored 2A elements, which allowed translation of the TCR-α and -β chains at an almost equimolar ratio and provided optimal expression of the TCR [96].

A significant concern with TCR transfer is the potential for cross-pairing of transferred with endogenous TCR chains, which could result in the formation of hybrid receptors, with potentially autoreactive specificity. Preferential pairing of the two introduced TCR-α and-β chains can be achieved by replacing the human constant regions of the TCR-α and-β chains with murine constant regions, or by incorporating cysteine residues in positions that promote disulfide bonds between the introduced chains [109]. As an alternative method, the introduction of TCR-α and -β chains into γδ T cells has been proposed to prevent TCR mispairing. However, the low frequency of γδ T cells in the peripheral blood and their preferential homing to the mucosa of the GI tract make them less attractive for adoptive immunotherapy of B-cell malignancies [110].

T-cell receptor chains specific for self-proteins are often of low affinity, and it may be necessary to improve affinity for therapeutic efficacy. This can be accomplished for a TCR of known specificity through directed *in vitro* evolution, enabling the engineering of TCRs with the ability to bind HLA-bound peptides at picomolar concentrations [111], or by high-throughput screening of TCR that have been mutated randomly to identify higher affinity pairs.

Chimeric antigen receptors

In 1997, the monoclonal anti-CD20-antibody rituximab was approved as the first therapeutic antibody for cancer treatment and has since revolutionized the treatment of CD20-positive B-cell tumors. The combination of rituximab with standard chemotherapy has significantly improved response and survival rates compared with chemotherapy alone [112,113]. The potential

Box 1. Potential genotoxicity of gene transfer into somatic cells.

Gene transfer into somatic cells can trigger oncogenesis as a consequence of the upregulation of cellular proto-oncogenes. Insertional mutagenesis has resulted in the development of leukemia in a subset of patients with X-linked severe combined immunodeficiency who were treated with hematopoietic stem cells that had been corrected by retrovirus-mediated gene transfer. Mature T cells have been shown to be relatively resistant to oncogene transformation as a result of retroviral insertion [104], and vector design may further reduce the genotoxic risk from vector integration and improve the safety of gene-modified T-cell therapy of malignancy. Nevertheless, this issue remains a concern for the clinical translation of therapeutics with gene-modified T cells. An additional approach to improve safety is to concurrently introduce a mechanism for conditional cell suicide, either using a drug that activates a death program or a monoclonal antibody to invoke killing by antibody-dependent cellular cytotoxicity [142–145].

to combine the specificity of monoclonal antibodies with the advantages of a durable cellular immune response spurred the development of CARs that could be expressed in T cells. CARs typically consist of a scFv that incorporates the heavy and light variable chains (VH and VL, respectively) of a monoclonal antibody that recognizes a tumor cell molecule fused to the ζ-chain of the CD3/TCR complex to trigger T-cell activation and cytotoxicity. To improve activation after engagement of the CAR, a costimulatory signal can be provided by the addition of CD28 or other costimulatory signaling domains to the ζ-chain [114,115]. An important advantage of CAR-modified T cells is their ability to recognize tumor cells without the requirements of MHC restriction, removing obstacles, such as defects in antigen processing and low levels of MHC expression on malignant cells, that might limit the efficacy of conventional or TCR-modified T cells.

Several B-cell lineage surface molecules are retained on B-cell tumors and represent attractive targets for CAR-modified T-cell therapy. The feasibility of adoptive immunotherapy with autologous CD20-specific CAR-modified T cells has been studied in patients with NHL and mantle cell lymphoma. In a trial of seven patients, two maintained a previous complete response, one achieved a partial response and four had stable disease [116]. Modified T cells persisted for up to 5–9 weeks after infusion in some patients but at a very low level. Improving the magnitude and duration of the response achieved by adoptive transfer will be important to improve efficacy, particularly for the treatment of patients with a larger tumor burden. Other surface molecules that are expressed on B-cell tumors have been targeted with CAR-modified T cells in preclinical studies, including CD19 and CD22 [117,118], and clinical trials with CD19-redirected T cells are now in progress in several laboratories for B-ALL, B-CLL and lymphoma.

Clinical trials of T-cell therapy using CAR-modified T cells should provide insight into potential limitations of this approach. One concern is that transferred T cells might be eliminated prematurely due to a host immune response to the murine VH and VL fragments of CARs that are derived from murine antibodies. Another issue identified in preclinical work is that the stoichiometry of antibody binding to tumor cells is different from that of the TCR/MHC interaction and might not provide for optimal T-cell activation or survival *in vivo*. Clinical trials are required to determine how significant these issues will be. Many molecules being targeted on B-cell malignancies by CAR-modified T cells are also expressed on normal B cells, and successful therapy is likely to result in a B-cell deficiency, unless strategies are developed for the conditional elimination of transferred T cells after tumor eradication is complete.

Qualitative properties of T cells required for effective adoptive therapy

The efficacy of adoptive T-cell therapy requires that the transferred tumor-reactive T cells home to sites of tumor, mediate effector functions in the tumor environment, and persist sufficiently long *in vivo* to eradicate the majority or even all of the malignant cells. Preclinical models and clinical trials have shown that modifying the environment into which T cells are transferred and selecting T cells with distinct properties for adoptive transfer are important for achieving optimal anti-tumor effects.

Homing of T cells to tumor sites

The ability of adoptively transferred T cells to migrate to and penetrate large tumor masses has been shown to be an impediment to effective therapy of solid tumors in murine models [119], and may reflect alterations in homing receptors expressed on T cells, dysregulated expression of leukocyte adhesion molecules on the tumor vasculature and biophysical properties of the tumor environment that may limit T-cell infiltration [120,121]. There are limited data on the migration of human T cells administered to patients with malignancy and B-cell tumors in particular. Adoptively transferred T cells specific for EBV antigens expressed on EBV-associated post-transplant lymphoproliferative disease or HD were readily identified in tumor biopsies, suggesting that homing may be less of an obstacle for tumors that originate in lymph nodes or bone marrow, particularly if high levels of transferred T cells can be achieved and sustained in the blood [69]. Many tumors produce chemokines to attract and educate cells to have immunosuppressive functions, thus, in situations where homing of effector T cells is limited, it may be possible to improve their migration to tumor sites by introducing chemokine receptors that respond to factors produced in the tumor environment [122].

Local suppression of effector function in the tumor environment

It is increasingly apparent that the tumor environment is hostile to the development and function of effector T cells as a consequence of the local recruitment of Tregs and myeloid suppressor cells, tumor cell expression of ligands for inhibitory receptors, and/or secretion of molecules that inhibit effector T-cell function. The precise immune evasion strategies employed by B-cell malignancies are beginning to be elucidated and will be instructive for designing modifications to adoptively transferred T cells or the host to overcome immune evasion. For example, PD-L1 is present in HD and MM and promotes anergy and apoptosis of T cells after binding to programmed death-1 (PD-1) [123,124]. PD-1 expression was elevated in tumor-infiltrating and peripheral T cells of HD patients and blockade of the PD-1 signaling pathway restored IFN-γ production of tumor-infiltrating T cells, suggesting that such an approach might improve the efficacy of adoptively transferred tumor-reactive T cells [124]. Other immune escape strategies have been identified in HD and MM, including the production of TGF-β and IL-10, and strategies to render T cells resistant to these negative regulators have been described [56,125]. Finally, loss of tumor antigen expression and downregulation of HLA and costimulatory molecules, occur in B-cell malignancies, suggesting that targeting multiple epitopes might be necessary to prevent the emergence of antigen loss variants under immune selective pressure provided by adoptive T-cell transfer [126].

Recent studies by Gribben *et al.* have identified direct effects on gene-expression profiles of $CD4^+$ and $CD8^+$ T cells induced by cell-to-cell contact with B-CLL cells [127]. The affected genes

are involved in cytoskeleton formation, vesicle trafficking and cytotoxicity, and the altered gene expression in T cells is accompanied by defective synapse formation with target cells, leading to impaired recognition of malignant B-CLL cells. The formation of a functional immune synapse between T cells and B-CLL could be partially restored *in vitro* with the immunomodulatory drug lenalidomide, suggesting its potential use to promote recognition of tumor cells by adoptively transferred T cells [128,129].

Improving persistence of adoptively transferred tumor-reactive T cells with cytokines

Studies in animal models of adoptive T-cell therapy for leukemia have demonstrated that transferred T cells must persist for long periods to mediate complete tumor rejection. Efforts to translate T-cell therapy to human malignancies have required the *in vitro* isolation and expansion of T cells, which may render them less fit to survive and function *in vivo*. One of the first strategies that improved T-cell survival after adoptive transfer was the administration of IL-2 [130]. Administration of both low-dose and high-dose IL-2 transiently improved the persistence of transferred T cells, however high-dose IL-2 was accompanied by significant side effects [131].

IL-7 and IL-15 are used less often than IL-2 for expanding T cells *in vitro* but have been shown to be critical for survival and proliferation of memory T cells *in vivo* [132,133]. IL-7 and IL-15 levels become elevated in lymphopenia and induce the proliferation of T cells to restore homeostasis [134]. It was therefore hypothesized that the induction of lymphopenia might lead to prolonged persistence of adoptively transferred T cells by reducing the competition for homeostatic cytokines, minimizing the influence of Tregs and other suppressors, and creating space in the lymphoid compartment of the recipient for the transferred T cells. The use of lymphodepleting chemotherapy alone or with irradiation prior to the transfer of melanoma-specific T cells resulted in better persistence and expansion of transferred T cells *in vivo*, as well as improved tumor infiltration and anti-tumor activity [135]. Further clinical studies will help to elucidate whether the administration of homeostatic cytokines alone may have a similar effect on transferred T cells and circumvent the toxicity associated with lymphodepleting chemoradiotherapy.

Selecting T cells with the intrinsic capacity to persist & function after adoptive transfer

Clinical trials of adoptive T-cell therapy for human viral diseases after allogeneic HCT using T-cell clones or polyclonal T cells derived from a healthy donor with prior exposure to the pathogen demonstrated that transferred T cells could persist long-term *in vivo* [64,136]. This proved not to be the case when autologous tumor-reactive T cells were isolated from cancer patients and used to treat human malignancies, and poor persistence of transferred T cells probably contributed to the lack of sustained anti-tumor efficacy [116,130,135]. An explanation for these apparently discrepant results is now emerging from work in animal models, which suggest that the distinct transcriptional programs of naive and subsets of memory T cells impart qualitative differences that are retained in effector progeny and influence cell fate after adoptive transfer (Box 2).

Discrete phenotypic and functional subsets of T cells have been identified, including naive (TN), effector (TE), central memory (TCM) and effector memory (TEM) T cells [137]. After TCR engagement by antigen *in vivo*, TN cells undergo proliferation and programmed differentiation, resulting in the generation of large numbers of TE cells, most of which die as antigen is cleared, leaving a small pool of functionally distinct TCM and TEM cells that persist long-term and respond to antigen re-exposure by differentiating into a new wave of TE cells [137,138]. It has generally been assumed that TE cells isolated from TN, TCM or TEM subsets would behave similarly and have equivalent efficiency when used for adoptive T-cell therapy. Recent data in a nonhuman primate model in which antigen-specific TE cells derived from either TCM or TEM were reinfused into animals has cast doubt on this assumption. In this study, T-cell clones derived from TCM, but not those derived from TEM cells were capable of persisting long term, reacquiring the phenotype of memory cells and populating memory T-cell niches *in vivo* after adoptive transfer [139]. These results demonstrate that some of the progeny of clonally derived TE cells retain intrinsic programming of the parental cell of origin and provide an explanation for the inconsistent persistence of transferred T cells when unselected T cells are used as the source to generate tumor-specific T cells for adoptive immunotherapy. Moreover, these findings suggest that selecting T cells from the TCM pool for the introduction of T-cell or chimeric antigen receptors that target B-cell malignancies may ensure more uniform persistence after cell transfer. An alternative approach that has been employed is to use T cells specific for a virus, such as EBV or CMV for genetic modification, since many of these cells will be derived from TCM. This strategy also enables signaling through the endogenous TCR to potentially amplify and maintain the transferred T cells *in vivo* and has been used in preclinical studies with EBV-specific T cells expressing an anti-CD30ζ CAR to target HD and with some success in clinical trials with EBV-specific T cells expressing a CAR specific for neuroblastoma [140,141].

Box 2. Distinct programming of T cells used for adoptive therapy.

It has been suggested previously that at least some memory T cells have properties of self-renewal and differentiation, attributes typically assigned to stem cells [146]. A recent study in mice demonstrated that the initial division of a naive T cell after antigen encounter is asymmetric and endows each of the daughter cells with distinct properties that dictate their ability to function as memory cells [147]. A putative memory T stem cell has also been described in a murine model of graft-versus-host disease [148]. Taken together, this work illustrates that even similar T-cell subsets exhibit distinct programming, and has focused greater attention on how properties of T cells other than tumor specificity might influence their efficacy in adoptive immunotherapy.

Expert commentary

The application of adoptive T-cell therapy for human malignancies, including B-cell tumors has long held attraction due to the potential for specific elimination of tumor cells without the toxicities associated with chemotherapy and radiation. There is convincing evidence from allogeneic HCT that B-cell malignancies are susceptible to elimination by T cells, however the translation of this knowledge into specific adoptive T-cell therapy has been impeded by the lack of readily available antigens to target in B-cell tumors, with the exception of Ig–Id or EBV antigens in the subset of malignancies that express EBV proteins. Efforts to identify polymorphic and nonpolymorphic tumor antigens expressed on B-cell malignancies that can be recognized by T cells have led to studies of T-cell therapy in preclinical models, and clinical trials of this approach have been initiated. The engineering of T cells to express a TCR or CAR that targets antigens and surface molecules that are broadly expressed by B-cell malignancies can overcome the obstacle of isolating tumor-reactive T cells from the endogenous repertoire, and has facilitated the initiation of clinical trials by several groups in both aggressive and indolent B-cell tumors. Critical insight has been derived into qualitative properties of T cells that ensure persistence after adoptive transfer. The evaluation of data from well-designed clinical trials and ongoing studies in animal models will determine the potential toxicity, efficacy and limitations of adoptive T-cell therapy for B-cell malignancies, and will direct subsequent studies that may lead to the establishment of adoptive T-cell transfer as a useful and broadly applicable modality.

Five-year view

The field of adoptive T-cell therapy is currently undergoing a significant resurgence, due in part to the proven efficacy of this approach in melanoma, the opportunities provided by molecular profiling of tumors to identify target antigens, and the feasibility of isolating or engineering T cells of defined specificity. Allogeneic HCT will remain a mainstay of therapy for B-cell malignancies owing to its proven curative potential, and efforts in several laboratories to identify polymorphic antigens expressed on B-cell tumors using genome-association studies will identify minor H antigens that can be targeted selectively with T-cell therapy to augment GVT reactivity without causing GVHD. If this endeavor is successful, the use of less toxic RIC regimens might be extended to younger patients with more aggressive B-cell tumors. Clinical trials of adoptive T-cell therapy for B-cell malignancies in the nontransplant setting, including those that use TCR- or CAR-modified T cells, have already been initiated and will be completed in the next 5 years. These trials will provide data on the safety and efficacy of this approach that will be used to design subsequent trials, and determine whether it is appropriate and how best to incorporate T-cell therapy into conventional therapeutic regimens. In patients who fail to respond to T-cell therapy, it will be important to analyze whether the failure of therapy reflects intrinsic properties of effector T cells, or systemic or local suppression of function in the tumor-bearing patient to provide insight into the subset of patients that can benefit most by T-cell therapy, and to derive strategies for improving outcome in patients less likely to respond.

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Key issues

- Indolent B-cell malignancies are susceptible to the T-cell mediated graft-versus-tumor effect of allogeneic hematopoietic cell transplant as a consequence of recognition of polymorphic and tumor-associated antigens.
- B-cell malignancies express or overexpress several nonpolymorphic proteins that have limited expression on normal tissues, and can be targeted by tumor-specific T cells present in the endogenous repertoire.
- Gene-transfer techniques can be used to endow T cells with tumor specificity, either through the expression of T-cell receptor genes that recognize tumor-associated peptides displayed on MHC molecules, or through the expression of single-chain antibody fragments that are specific for B-lineage surface molecules.
- Effector T cells derived from central memory precursors exhibit long-term *in vivo* persistence after adoptive transfer, migrate to memory T-cell niches and revert to the memory pool, suggesting that adoptive T-cell therapy for cancer employing central memory cells may have improved efficacy.
- T-cell homing to tumor sites and maintenance of effector function in the immunosuppressive tumor microenvironment will be critical for the success of adoptive T-cell therapy.

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• **Durable complete remissions can be induced following non-myeloablative hematopoietic cell transplantation (HCT) in a subset of patients with advanced chemotherapy-refractory B-cell chronic lymphocytic leukemia (B-CLL) and provides evidence for the susceptibility of B-CLL to the T-cell-mediated graft-versus-tumor (GVT) effect. Also demonstrates that the beneficial GVT effect and detrimental graft-versus-host disease (GVHD) cannot be consistently separated with current approaches and that alternative therapeutic modalities are required to confer anti-tumor reactivity in patients who do not respond to HCT.**

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