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Immunotherapy: opportunities, risks and future perspectives

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Abstract

This review is intended to reflect upon the current status and perspectives of cell-based immunotherapy at a time when the promise of extensive pre-clinical research has been translated into encouraging clinical responses. However, some of these have also been complicated by significant adverse reactions. As the field moves towards definitive late stage trials, with a growing interest from pharmaceutical companies, we realize that novel cell therapy strategies pose questions that are familiar to traditional drug development, along with new considerations due to the potential of T cells to persist long term and to expand after adoptive transfer. These questions address the safety of the product, the efficacy, the mode of action, and the anticipation of risks. From different perspectives, we intend to address exciting opportunities and safety concerns in current concepts of cellular immunotherapy.

Keywords

clinical trials; gene therapy; immunotherapy; safety; T cell receptor; treatment efficacy

Introduction

Helen E. Heslop & Martin Hildebrandt

The International Society for Cell Therapy (ISCT) 2014 Annual Meeting provides us with an opportunity to review the current status and perspectives of cell-based immunotherapy. This comes at a time when the promise of extensive pre-clinical research has been translated

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into encouraging clinical responses with several immunotherapy strategies (1–8). However, some of the most impressive clinical responses have also been complicated by significant adverse reactions (1,2,4,9). As the field moves toward definitive late-stage trials, with a growing interest from pharmaceutical companies, it is a timely moment to reflect on the risks and benefits inherent in studies with complex biological products.

Novel cell therapy strategies pose questions that are familiar to traditional drug development, along with new considerations regarding the potential of T cells to not only persist long-term up to 10 years (10,11) but to expand after adoptive transfer. These questions address the safety of the product, the efficacy, the mode of action and the anticipation of risks. Unfortunately, these questions will have even greater weight after the occurrence of unexpected adverse events with severe and life-threatening consequences (9,12,13). Nevertheless, the prediction of hazards will be informed by previous events that are analyzed in a thoughtful and structured manner. As an example, the adverse effects surrounding a first-in-man clinical trial of a superagonistic T-cell–activating antibody (14) cast a spotlight on points to be considered when approaching cell-based immunotherapies, including

- Species specificity *in vitro* and *in vivo*
- New agents potentially having new mechanisms of action
- The recognition of agonistic effects
- The potency when compared with a natural ligand
- A potential inactivation of physiological checkpoints
- An amplification of an induced effect
- Scientific rationale for the pre-clinical development and clinical trial
- Approriate dosage, preferably the lowest among several calculated starting doses
- Scientific merit of a first-in-man trial versus safety of the participants.

These recommendations (15) also have implications for cell-based immunotherapies as potential high-risk medicinal products and encourage a broad exchange of information to discuss and to adjust future trials that are based on emerging data on safety profiles of different products. The ISCT 20th Anniversary Annual meeting, and the plenary session on Immunotherapy presented here, is such a forum for an exchange on both the promise of cell therapy and on unexpected events observed by the groups actively engaging in similar research.

The authors who contributed to this review and to the plenary session must be thanked for summarizing current results and delineating future perspectives in cell-based immunotherapy so thoughtfully: Karl Peggs, London, will review the current status of pathogen-specific adoptive T-cell therapies that have now progressed to definitive licensing studies; Lutz Uharek, Berlin, will summarize the history and current status of redirected T cells and natural killer (NK) cells targeting tumors; Catherine Bollard, Washington, will

focus on anti-tumor T-cell therapies generated by stimulation with virus or tumor antigens. From different perspectives, they summarize three exciting areas of immunotherapy.

Pathogen-specific adoptive T-cell therapies

Karl Peggs

The basic concepts underpinning pathogen-specific adoptive T-cell therapies are relatively straightforward. Deficits primarily in number, but to some degree also function, of pathogen-specific T cells underlie the increased propensity to infection or reactivation of a variety of viruses after allogeneic hematopoietic stem cell transplantation. Adoptive transfer and engraftment of cells from an appropriate donor source followed by expansion *in vivo* can theoretically hasten the restoration of immunity and reduce the infective burden. This is technically easiest when the original stem cell graft donor has pre-existing immunity to the pathogen of interest. In these cases, direct selection of pathogen-specific T cells, or expansion of such cells in *ex vivo*, allows generation of a therapeutic product. Pathogens for which the greatest experience in clinical application exists include cytomegalovirus (CMV), Epstein-Barr virus (EBV) and adenovirus.

Most of the early demonstrations of proof of concept relied on an *ex vivo* expansion step, making more widespread clinical application more challenging. Nevertheless, these studies showed that it was technically possible to expand T cells with specificity for either EBV or CMV, and more latterly adenovirus, and that after adoptive transfer these cells appeared to expand, control viral infection and then contract but persist as a memory population providing longer-term immunity (16–18). Refinements over subsequent years included the development of culture conditions, allowing more rapid cell expansion (6,19–24) and increasingly sophisticated strategies allowing direct selection of virus-specific T cells when donor immunity is present and precursor frequencies are maintained at reasonably high levels. These included selection according to secretion of cytokines after re-stimulation with viral peptides (notably interferon [IFN]γ) (25–27), upregulation of cell surface activation markers or more direct selection on the basis of binding of class I human leukocyte antigen (HLA) multimers loaded with immunodominant viral peptides (28–30). Each of these approaches produces a therapeutic product that differs either relatively subtly or in some cases more dramatically in terms of cellular composition (eg, CD4 versus CD8, pauci-clonal versus poly-clonal), purity, antigen specificity and functional characteristics. Application in subsequent phase I-II studies has also introduced further variation in terms of cell doses administered, timing of administration after transplantation and indication for intervention (eg, prophylactic, pre-emptive or for clinically “resistant” infection). The result is that we have a series of relatively small clinical studies performed with the use of differing therapeutic products that give broadly similar messages. In the patients included in these studies, administration of the cellular therapeutic results in reconstitution of (presumed) donor-derived immunity related to expansion of transferred populations; this “immunity” appears to be functionally capable of clearance of a variety of viral pathogens, with establishment of longer-term T-cell memory and durable immunity in the absence of subsequent enhanced immune suppression, and the antigen-specific T-cell populations

appear to have a low risk of inducing significant toxicities, including graft-versus-host disease (GVHD) (31).

In most cases, results have been compared with outcomes in historical control cohorts, either formally or in the context of the discussion of the results. Although this is not unreasonable for phase I–II studies, it does highlight some of the difficulties in interpretation of the data. Notably, clinically significant active GVHD is an exclusion criterion in all studies. It is well established that CMV infection rates are higher in patients with GVHD, and infection episodes are likely to be more prolonged and clinically more problematic in these cases. Thus, there is a selection bias occurring for exclusion of those who are likely to have the greatest problems. Furthermore, it is only possible to administer a cellular therapeutic when one can be generated. Low frequencies of virus-specific T cells in the donor graft are reported to correlate with poorer post-transplant immune reconstitution but will also probably correlate an increased risk of failure to generate a product. Because very few of the study reports detail how frequently there was a failure to generate a therapeutic product, we can surmize that there is at least some selection bias occurring. These considerations highlight the pressing need for randomized confirmatory studies.

These considerations form the basis for two randomized confirmatory studies currently being performed in the United Kingdom assessing the utility of CMV-specific T cells: one in the sibling donor setting (Immunoprophylactic Adoptive Cellular Therapy Study or IMPACT) and one in the unrelated donor setting (Alternate Donor Study of Pre-Emptive Cellular Therapy Study or ASPECT). Both include only CMV-seropositive recipients receiving T-cell–depleted grafts because this patient group has (i) a very high incidence of CMV infection (ie, high clinical need and less chance of an unused preselected product in a prophylactic or pre-emptive study) and (ii) a low baseline incidence of GVHD (ie, reducing again the chance of an unused pre-selected product and providing a baseline for assessing potential toxicity of the cellular therapy). Patients are excluded if they develop grade >1 acute GVHD before the indicated time point for cellular therapy. The control groups receive standard viral polymerase chain reaction–based surveillance, with standardized criteria for intervention and stoppage of antiviral drugs. The active intervention arms receive CMV-specific T cells selected either by HLA streptamers (IMPACT/ASPECT) or by gamma-catch technology (IMPACT) according to HLA type.

Although such studies may prove the value of virus-specific T cells in a model transplant setting, many questions will remain unanswered. For example, what is the role of such therapies in the T-replete setting, in which problematic CMV infection more generally occurs in association with GVHD? How well will such cells perform in patients with slightly greater evidence of GVHD, both in terms of toxicity and in terms of function in the setting of enhanced immune suppression, for example, corti-costeroid use? How will the products be best integrated into transplant practice—as prophylaxis, as pre-emptive therapies or only in those with resistant or prolonged infections? What is the best approach when the donor is CMV-naive, for example, cord blood or seronegative donor? Nevertheless, it is hoped that the current studies will provide a more solid basis for further evaluation of these questions.

With respect to the issue of treating patients without available seropositive donors, there are a number of potential solutions being evaluated. The use of “third-party” virus-specific cells offers the intriguing possibility of being able to generate a bank of cell lines that would be rapidly available for use directed by a “best available HLA match” algorithm (wherein the transferred cells must recognize the pathogen in the context of a shared HLA allele). Potential issues here pertain to possible alloreactivity in either a graft-versus-host direction, resulting in third-party GVHD if the cells engraft robustly, or in the opposite host-versus-graft direction, resulting in rejection of the adoptively transferred populations before they can exert the desired effect. Nevertheless, proof of concept exists in the setting of EBV-related post-transplant lympho-proliferative disorders (32) and has been demonstrated more recently for a CMV, EBV and adenovirus after hemopoietic stem cell transplantation (HSCT) (33). No significant toxicities have been reported. The lack of detectable persistence of transferred cells raises interesting questions regarding the mechanism of therapeutic effect that should be addressed in future studies. One possible mechanism is that the third-party cells engender more rapid reconstitution of second-party immunity derived from the T cells of the original stem cell donor and that the third-party cells are acting either directly as a cellular vaccine or indirectly through a brief burst of lysis of virally infected host cells. Clearer resolution of the value of this approach should be provided in randomized controlled studies. An alternative strategy relies on induction of primary immune responses *ex vivo*, with subsequent expansion and adoptive transfer (34). Finally, although currently limited in terms of widespread application by technological constraints and costs, proof-of-concept studies evaluating genetically modified T cells transduced to express virus-specific T-cell receptors are currently being undertaken.

Whereas such mono-pathogen–specific therapies can address the more frequently occurring viral infections, it is well recognized that severely immuno-suppressed patients are at risk from multiple pathogens. Therapeutic products with multiple specificities may therefore be advantageous in certain clinical situations (35). An even broader repertoire of immune reconstitution against both known and unknown pathogens may be achievable by transfer of memory T-cell populations depleted of the naive compartment that contains most of the alloreactive potential of the graft (36). The results of ongoing clinical studies evaluating such products are keenly anticipated.

The role of redirected T cells and NK cells in tumor medicine

Lutz Uharek

In 1909, Paul Ehrlich proposed that the immune defense system can identify and eliminate nascent tumor cells. Exactly 100 years later, this principle was successfully applied to treat patients with refractory leukemia by use of their chimeric antigen receptor (CAR)-modified T cells (1,2,9). Previous attempts to exploit lymphocytes for tumor therapy were either associated with significant side effects, as graft-versus-host disease (GVHD) in the case of allogeneic T cells, or turned out to be very cumbersome as the use of tumor-infiltrating lymphocytes. Now we have the technology to expand and process T cells and NK cells in a way that allows specific targetting of a huge variety of antigens and cell surface molecules. Is this the beginning of a new era of tumor medicine in which cytotoxic drugs will be

replaced by individually designed cellular products? What are the realistic chances and what are the risks of this approach? Which are the central questions that still must be adressed in preclinical studies?

Is CAR or T-cell receptor technology superior?

Currently, two approaches for redirecting T-cell specificity are used: (i) T-cell receptors (TCRs), in which variable α- and *β*-chains are cloned from T cells with specificity against a tumor antigen (37), and (ii) CARs, in which tumor antigens were recognized through antibody single-chain variable fragments (scFvs) linked to intracellular signaling domains (38). The TCR approach was the first demonstration of redirected T-cell specificity (39). It relies on the natural way of T-cell function and has the major advantage that a large number of mutated intracellular proteins can be targeted. Its major difficulties are a low cell surface expression of TCRs and so called “mispairing,” which occurs by formation of TCRs formed by of one endogenous and one transduced TCR chain.

In contrast to TCR technology, the CAR technology requires no antigen processing and is HLA-independent. The major restriction preventing its broader application is the limited number of suitable antibody-targeted tumor surface antigens. Other problems of so-called “third-generation” CARs include cytokine release induced by low-avidity “off-target” binding and immunogenicity (the ScFv portion is generally mouse-derived), which may result in immune responses and early clearance of CAR-engineered T cells.

What are immunological acceptance criteria for T-cell and NK-cell–based biopharmaceuticals?

The optimal target antigen has the following properties: it is immunogenic, it is completely tumor-specific with no significant expression on normal tissues, it is highly expressed on all tumor cells (including tumor stem cells), it is essential for survival or proliferation of the tumor cell, it has multiple epitopes and it is expressed on the tumor surface. For clinical applications, it is not necessary that all of the above-mentioned criteria are fulfilled. The importance of each criterion will depend on the balance between aspects of safety, reliability and effectiveness in a given clinical situation.

In addition, the functional quality is determined by its ability to lyse tumor cells expressing a particular marker, the affinity with which the introduced receptor binds its antigen, the level of receptor expression on the cell surface, the *in vivo* expansion and persistence of the T cells, the lack of off-target toxicities. Full functionality is only ensured if both the afferent function (tumor recognition) as well as the efferent function (tumor kill) are operative. Assays to measure T-cell activation on exposure to patient-derived tumor tissue may help to determine the individual effectiveness *in vitro* (40).

The assessment of off-target toxicity is critical for a product without self-limiting properties. For CARs, it is relatively easy to determine whether the antibody shows cross-reactivity and binds to the surface of tissues not expressing the targeted antigen. Similar tests for cross-

reactivity of TCR-based bio-pharmaceuticals are more difficult to establish because all relevant HLA types must be considered.

Which is the optimal technology for gene transduction?

Retroviral or lentiviral vectors can be used to transfer TCR or CAR coding genes into T cells. Both yield a high level of stable transgene expression and permanent gene expression. The most important advantage of retroviral vectors is long-term experience in clinical trials (11). Whereas retroviral transduction can be performed only on efficiently dividing cells, lentiviral vectors are also capable of integrating into non-dividing cells. An additional albeit theoretical advantage is their lower risk of damaging insertions. It is therefore very likely that lentiviral vector systems will be used more often. Recently, transposon systems such as Sleeping Beauty (41) have been developed as simple and inexpensive methods for a stable non-viral genetic modification. In contrast to viral vectors, they do not have an intrinsic capacity to cross the cellular membranes and must be delivered either by different non-viral strategies or by vector systems. Transposons have the advantage that they do not require cell pre-activation, have a low immunogenicity and have a relatively large cargo capacity. Their major disadvantage is limited clinical experience and little knowledge about their oncogenic potential *in vivo*.

How to improve the effectiveness of redirected T cells and NK cells

Five different approaches to improve the effectiveness of redirected T cells and NK cells are currently discussed: (i) *in vitro* genetic engineering to modify T-cell and NK-cell features (transduction of interleukin [IL]-2 gene, induction of anti-apoptotic proteins, introduction of specific chemokine receptors), (ii) *in vitro* cytokine activation (IL-2, IL-7, IL-15 and IL-21), (iii) pre-selection of T-cell and NK-cell subsets (EBV- or CMV-specific T cells, central memory T cells, mature/activated NK cells), (iv) modifying the host environment by preconditioning before cell transfer or (v) by supportive treatment after T-cell transfer.

Thus far, only some studies used immuno-magnetic selection techniques to restrict the T-cell or NK-cell pool. However, there is no doubt that the *in vivo* survival and effectiveness of adoptively transfused cells depends on subtype and differentiation status. In the past, most of the clinical trials have used effector memory T cells (TEMs) as a result of cell culture technologies leading to a rapid differentiation into late-stage effector cells (today mostly by utilization of a CD3/CD28 activation procedure). Although *in vivo* TEMs show more cytotoxicity when compared with central memory T cells, TEMs might not ensure long-term tumor surveillance. Gattinoni and colleagues (42) identified stem cell memory T-cells (TSCM) as a very attractive population for adoptive cell transfer because of their self-renewal capacity and their ability to generate TEM, central memory T cells and effector T cells *in vivo*.

Environmental variables, including the presence of regulatory T cells (Tregs) or myeloid-derived suppressor cells (MDSCs), can also be important for the success of adoptive T-cell transfer. Host preparative lymphodepletion, introduced by Dudley and colleagues (43), has been proposed to ensure optimal environmental conditions such as reduction of Tregs and MDSCs, decrease in endogenous lymphocyte competition for cytokines or access to antigen-

presenting cells. Systemic administration of cytokines such as IL-2, IL-7, IL-12 and IL-15 or IFN-γ after adoptive T-cell transfer has also been used to enhance T-cell and NK-cell effector function. However, such treatments are often associated with relevant side effects, and their potential advantages have not been formally demonstrated.

How to improve the safety of genetically engineered cells

The risk of side effects caused by on- and off-target toxicity has risen with increasing effectiveness of genetically modified T cells and NK cells. Therefore, different approaches to allow on-demand cell destruction by application of a substance that can switch on a suicide gene have been developed. Herpes simplex virus thymidine kinase (HSV-TK) can be regarded as a reference strategy (44); it is currently under investigation in a phase III clinical trial. Other strategies include inducible caspase 9 (iCasp9) (45) and application of CD20 (46).

Which tumors should be targeted, and what is the optimal administration schedule of the cell product?

The targeted tumor should be immunogenetic and sensitive to cell-induced apoptosis (either on the basis of *in vitro* data or experience from allogeneic DLI). There is still an urgent need to define and standardize reliable biomarkers and feasible tests to determine the sensitivity of tumor entities toward T-cell and NK-cell–mediated cytotoxicity. To allow the induction of an immune response, slowly growing tumors are preferred. Tumor entities, tumor stages, and clinical situations in which no alternative treatments are availabe should be chosen for early-stage trials.

Thus far, there are no conclusive data concerning the optimal number of TCR- or CAR-transduced cells. Regarding conventional anti-cancer drugs, dosing and application schedules will certainly depend on specific characteristics of the cell product and host factors, such as tumor recognition (immunogenicity) and sensitivity of the tumor toward cell–mediated (perforin) lysis, as well as dynamics of tumor proliferation and tumor sensitivity toward chemotherapy or other immunotherapies.

What kind of additional and supportive treatment can increase effectiveness and reduce risks?

Cytotoxic therapies (conditioning) before adoptive cell transfer can enhance effectiveness. The aim is (i) to “make space” for the newly administered cells, (ii) to reduce the number of host lymphocytes to prevent alloreactivity and rejection and (iii) to induce tumor cell apoptosis to reduce the number of tumor cells and increase their immunogenicity. Decisions on the type and intensity of the conditioning must consider the relative impact of each of these three goals in a particular clinical setting as well as the individual situation of the patient. This makes it very difficult to give general recommendations.

Side effects of T-cell transfer should be addressed by means of a careful risk assessment and establishment of risk-oriented prophylactic and therapeutic procedures. Because a

considerable variation in the magnitude of the induced immunological effect must be expected, massive tumor destruction with subsequent potentially lethal tumor lysis syndrome can occur. Therefore, prior tumor reduction, careful monitoring of markers for tumor destruction (LDH) and renal function as well as prophylactic application of allopurinol and adequate hydration is essential to prevent fatal outcomes.

The second clinical problem that must be anticipated is an anaphylactic reaction, probably directed against xenogeneic proteins of the transferred T-cell product (47). As the result of sensitization against these antigens, the risk of an anaphylactic shock increases after the second or following administrations and application of the product should take place in an environment in which patient monitoring and equipment for adequate emergency interventions are ensured.

The third critical side effect of T-cell–based therapeutics is the induction of a cytokine release syndrome (4), which often cannot be discriminated easily from an anaphylactic reaction because major aspects of clinical manifestation (hypotension) are similar. The release of cytokines, in particular of IL-6, IL-10 and IFN-γ, is not only caused by direct release by activated cytotoxic T cells but could also represent the consequences of a macrophage activation syndrome, which might be reversible with the use of IL-6R inhibitors. Prophylactic administration of steroids could also reduce the intensity of anaphylactic reactions and cytokine release syndrome but could negatively influence the effectiveness of the transferred cells and are therefore usually not administered. Standards for immediate diagnosis and treatment for all three major side effects should be incorporated into the study protocol.

How should safety aspects be considered in clinical trials?

Adverse effects are categorized as on-target effects (also referred to as target-related, exaggerated pharmacology or mechanism-based) or off-target effects (as a result of unspecific modulation of other targets). The discrimination between on- and off-target toxicity is important because high or unaccaptable off-target toxicity should be a subject of further improvements in cell engineering and production, whereas excessive or life-threatening on-target toxicity raises general questions concerning the choice of the antigen. However, in some cases, the level of on-target toxicity can be reduced by changing dose and application schedule.

Long-terminal repeats of the viral vector system can increase the expression not only of the transduced genes but also of neighboring genes. Inserted near an oncogene, retroviral vectors may thus drive oncogenesis. However, all oncogenic events have occurred during gene transfer to stem cells, and it appears that mature lymphocytes harbor only a very low risk for insertional mutagenesis and are resistant to retroviral transformation (48). Long-term safety data are availble over a time span of more than 10 years (11).

Results of clinical trials with TCR-modified T cells include cardiological (12,49) and neurological (50) toxicities. Whereas the cardiac toxicity was off--target and probably caused by the CDR2 mutations that enhance major histocompatibility complex binding of the TCR, resulting in recognition an epitope in titin (12), the neurological toxicity was “on-

target" in that the identical epitope was expressed in MAGE-A3 and MAGE-A12 (50). The previously unrecognized expression of MAGE-A12 in human brain underlines the importance of elaborated antigen-expression profiles, that is, on the basis of deep sequencing technology. No autoimmune symptoms that could be related to TCR "mispairing" have been reported thus far. Nevertheless, careful monitoring of GVHD-like syptoms is appropriate. In most trials with CAR-transduced T cells (mostly directed against B-cell antigens), no severe on-target side effects have been reported (51–54). However, especially T cells transduced with first-generation CARs often failed to persist, limiting the number of patients bearing CAR-modified T cells with long-term follow-up.

Concerning on-target toxicities, the example of CD19 demonstrates that even life-threatening side effects (cytokine storm, tumor lysis and long-lasting B-cell depletion) can be accepted in a particular clinical setting (2,4,9). Regarding every conventional drug, the magnitude and probability of side effects must be determined as accurately as possible and must be outweighed against the expected clinical benefit for each individual patient.

Conclusions

Innovative technologies to transfer tumor antigen specificity to lymphocytes are currently opening the door to a new field of anti-cancer therapies. Although the value of redirected T cells and NK cells still must be demonstrated in controlled studies, the substantial tumor regression seen in first clinical trials impressively demonstrates the potential of these next-generation cell products. In the future, it will be important to determine product, target and clinical requirements for the successful implementation of this new approach into treatment pathways. Only the combination of high-quality products, professional risk management with a careful evaluation and consideration of all relevant safety aspects and well-designed clinical trials will successfully ensure the transfer of redirected T-cell and NK-cell therapies from the bench to bedside.

T-cell therapies for hematologic malignancies: use of a non–gene transfer approach

Catherine M. Bollard

Although hemopoietic stem cell transplantation has increased survival significantly for patients who have moderate risk disease and achieve a complete remission before HSCT, those with persistent disease continue to have a dismal prognosis despite HSCT of less than 10% survival at 2 years. Furthermore, patients who relapse after HSCT also have a poor prognosis, with less than 20% surviving 2 years despite a second HSCT. Even for patients who enter HSCT for high-risk malignancies in complete remission, the prognosis remains guarded, with less than 35% surviving to 5 years. Thus, despite the allogeneic graft-versus-leukemia (GVL) or graft-versus-tumor (GVT) effect, relapse represents the major cause of treatment failure in these patients, and novel therapies are critical for patients with high-risk leukemia or lymphoma with relapsed or refractory disease.

Approaches to harness and increase the GVL effect include use of donor lymphocyte infusions, suicide gene–transduced T cells and T cells selectively depleted of GVHD

reactivity. Although these approaches increase the GVL effect, they carry a risk of causing GVHD. Although tumor vaccines or dendritic cells loaded with vaccine can avoid GVHD, results have been largely unsuccessful after HSCT, in part because of impaired T-lymphocyte and B-lymphocyte immune reconstitution (55). More recently, T cells gene-modified to express a CAR directed to a specific antigen have been developed. Although the data from several groups are promising (1,2,4,9,52,56), CAR treatment has limitations: (i) tumors can downregulate or mutate the surface expressed CAR receptor and evade CAR cell attack; (ii) in hematologic malignancies, the paucity of suitable surface antigen expression has restricted CAR therapy largely to targeting CD19 on B-cell tumors; (iii) CAR infusions have significant inflammatory toxicities, which limit their safe application. Alternative and safer T-cell therapy strategies that overcome these obstacles and target a broad range of tumor antigens are thus still needed to overcome these obstacles.

Tumor antigens

The identification of antigens on the tumor cells that might be targets is a prerequisite for developing immunotherapeutic approaches. Many tumor antigens can also be weakly expressed in healthy cells from the same lineage, although they may be upregulated or dysregulated in the malignant cell. Successful eradication of tumor may thus be complicated by loss of healthy circulating cells expressing the antigen. Antigens used for anti-tumor immunity must be chosen carefully from examples that are (i) unique to tumors, (ii) highly expressed in tumor cells, minimizing the normal tissue damage, or (iii) expressed on healthy cells that may be deleted for some time without major complication (eg, B cells).

Targeting viral antigens

Many lymphomas are associated with viruses, presenting unique, often highly immunogenic epitopes as T-cell targets. Latent EBV infection is found in non-Hodgkin lymphoma, including Burkitt's lymphoma, NK-T lymphomas and lymphoproliferative disease (LPD) and a subset of Hodgkin disease (57). The mechanism of EBV-induced lymphomagenesis is well established in the EBV lymphoproliferative diseases in immunosuppressed individuals but is poorly defined in other EBV-associated lymphomas in which EBV may represent a passenger virus. Nevertheless, the presence of EBV in tumor cells presents a target for immunotherapy. Donor-derived EBV-specific T cells have been used for some time to prevent and treat EBV-associated lymphoma after HSCT (7). Targeting this highly immunogenic tumor achieves remarkable response rates without toxicity or GVHD even when the infused T cells are specific for multiple viruses (18). Adapting these immunotherapy approaches to type II latency tumors, however, is challenging because a more restricted array of subdominant EBV antigens is expressed and the frequency of clones recognizing latent membrane protein (LMP)1 or LMP2 antigens expressed on these tumors is low in polyclonal EBV cytotoxic T lymphocyte (CTL) lines generated with the use of LCL (58). EBV-associated Hodgkin disease and non-Hodgkin lymphoma that develop in the immune-competent host show type II latency in which viral gene expression is limited to LMP1 and LMP2, EBNA 1 and EBERs. Expression of a minimal subset of genes, which are weak targets for CTL activity, therefore allows the malignant cells to evade the immune system. Nevertheless, the subdominant EBV antigens EBNA1, LMP1 and LMP2 may serve

as targets for immunotherapy approaches. In a phase I dose-escalation study, we evaluated the use of autologous EBV-specific CTL for patients with EBV-positive Hodgkin disease and showed persistence of these cells for up to 1 year, with a response rate of 20% (59). However, the response rate was less impressive than that seen in EBV-LPD after HSCT and only seen in patients with relatively low tumor burden. This may have been due to a lack of specificity of the EBV-specific CTL for the immuno-subdominant LMP1 and LMP2 antigens present on the Hodgkin tumor. In addition, the tumor produces inhibitory factors such as tumor growth factor (TGF)-*β*, which affect CTL and antigen-presenting cell activity (60).

In a clinical trial, we generated LMP1- and LMP2-specific CTL lines in patients with EBV +ve Hodgkin disease or EBV-positive B-cell or T/NK cell non-Hodgkin lymphomas (3,61). Patients received doses of 4×10^7 CTL/m^2 to 1.2×10^8/m^2. No immediate toxicity was observed, and 28 of 29 patients without radiological evidence of disease who received CTL as adjuvant therapy after SCT or chemotherapy remain in remission and 13 of 21 patients with active relapsed disease had a tumor response, which was complete in 11 patients (3). Therefore, immunotherapy with autologous LMP-CTL was well-tolerated in patients with relapsed EBV+ve Hodgkin disease/non-Hodgkin lymphoma, and infused LMP-CTL cells accumulated at tumor sites and induced clinical responses. In a follow-up study, we are now attempting to overcome TGF-*β*–induced suppression and utilizing this approach after HSCT with the use of donor-derived LMP-CTL.

Targeting leukemia-associated antigens

In addition to allo-antigens, there are many leukemia- and lymphoma-associated antigens. They can be classified as (i) antigens common to many malignancies, for example, cancer-testis antigens MAGE, BAGE, GAGE and NY-ESO-1; (ii) antigens overexpressed by malignant cells, for example, Her2/neu, AFP, Telomerase, WT1, RHAMM and PRAME (62,63); (iii) antigens specific for hematopoietic lineages, for example, primary granule proteins proteinase 3 and cathepsin G in myeloid malignancies.

Lymphoma-associated antigen–specific T cells

We have devised several strategies that consistently expand leukemia- and lymphoma-directed T cells from healthy donors for targeting AML through WT1, PR3, NE and MAGE-A3; ALL through WT1, MAGE-A3, PRAME and survivin; and Hodgkin lymphoma/ non-Hodgkin lymphoma through PRAME, NY-ESO, survivin and MAGE A4. The resultant lines were predominantly $CD3^+$T cells (mean, 98%) with an effector memory phenotype. When these multispecific T-cell lines were co-cultured with primary leukemia blasts or lymphoma cells matched at one or more class I or class II HLA antigens, we found specific tumor recognition and elimination, even with single HLA class I or class II allele–matched target cells. We also expanded such TAA-specific T cells from more than 50 patients with ALL or lymphoma (Hodgkin lymphoma or non-Hodgkin lymphoma) and evaluated their cytolytic activity against autologous blasts. *Ex vivo*–expanded CTL lines had specificity for a median of two antigens (64–66). We also demonstrated that patient-derived multi–TAA-CTL could efficiently kill autologous tumors and that this effect was increased in the

presence of demethylating agents (67,68). This approach of infusing TAA-specific T cells before and/ or after vaccines might therefore be able to overcome the critical obstacle of vaccine therapy when there are limited immune responses as a result of increased immunosuppression after chemotherapy or SCT. Furthermore, infusion of briefly expanded polyclonal donor TAA-specific CTL donor T cells recognizing a broad number of TAAs to reduce immune escape may overcome the treatment failures associated with single-antigen–directed CD19-CAR cells.

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