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Planes, Trains and Automobiles: Perspectives on CAR T cells and other cellular therapies for hematologic malignancies

Saar Gill, MD PhD

Division of Hematology-Oncology, Department of Medicine, University of Pennsylvania Perelman School of Medicine

Abstract

Hematologic oncologists now have at their disposal (or a referral away) a myriad of new options to get from point A (a patient with relapsed or poor-risk disease) to point B (potential tumor eradication and long-term disease-free survival). In this perspective piece we discuss the putative mechanisms of action and the relative strengths and weaknesses of currently available cellular therapy approaches. Notably, while many of these approaches have been published in high impact journals, with the exception of allogeneic stem cell transplantation and of checkpoint inhibitors (PD1/PDL1 or CTLA4 blockade) the published clinical trials have mostly been early phase, uncontrolled studies. Therefore, many of the new cellular therapy approaches have yet to demonstrate incontrovertible evidence of enhanced overall survival compared with controls. Nonetheless, the science behind these is sure to advance our understanding of cancer immunology and ultimately to bring us closer to our goal of curing cancer.

Keywords

Chimeric Antigen Receptors; T cell receptor transgenic T cells; allogeneic hematopoietic cell transplantation; alloreactivity; natural killer cells; tumor-infiltrating lymphocytes

INTRODUCTION

The immune system plays an important role in control of cancer and in some situations adoptive transfer of immune cells can mediate tumor regression. In this review we will highlight the evidence for this effect and endeavour to explain the relative roles as well as the potential advantages and disadvantages of various forms of cellular therapy. This review will not include cancer vaccines, which are immunotherapies that are designed solely to reduce the risk of relapse by enhancing pre-existing immunity. Similarly, antibodies including antibody-drug conjugates will not be reviewed here.

saar.gill@uphs.upenn.edu, Tel: (215) 573-4015, Fax: (215) 615-5887.

Conflict of Interest

Saar Gill reports grants from Novartis Pharmaceuticals. In addition, Dr. Gill has a patent Anti-CD19 CAR T cells in combination with small molecules pending to University of Pennsylvania.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

Broadly speaking, adoptive cellular therapy can be divided into two categories: Allogeneic hematopoietic cell transplantation (HCT), cytokines that increase effector cell numbers, inhibitory receptor blockade (so-called “checkpoint blockade”) and adoptive transfer of natural killer (NK) cells or tumor-infiltrating lymphocytes (TILs) belong to the class of approaches that do not rely on *a priori* knowledge of target molecules. In contrast, chimeric antigen receptor-redirected T cells (CART cells), bispecific T cell-engaging antibodies (BiTEs) and T cell receptor (TCR) transgenic T cells require *a priori* knowledge and design that take into account possible target antigens.

While cellular immunotherapy may or may not be directed against a single molecule or antigen, in terms of effector functions it conceptually represents an opposite approach to that of “precision medicine” with small molecule pathway inhibitors. Small molecule drugs achieve a favourable therapeutic index through inhibition of a single pathway that is critical for survival of cancer cells and not of normal cells. Furthermore small molecule inhibitors are typically cytostatic and their function may be abrogated by a single mutation in a binding site or by up-regulation of compensatory signalling pathways¹⁻³. In contrast, T cells and other immune effector cells exert a variety of killing mechanisms simultaneously through production of granzymes, perforin, Fas, TRAIL, and myriad cytokines, and theoretically it would be difficult for a cancer cell to escape from all of these killing mechanisms^{4,5}. Notably, escape from potent immunotherapies has recently been described through loss of the target antigen, highlighting the fact that the most efficient way for a cancer cell to escape immunotherapy is to avoid detection^{6,7}. Data on the most important T cell function(s) mediating cancer cell death after adoptive cellular therapy are lacking.

At present, cellular immunotherapy is reserved for patients who fail traditional chemotherapy. One of the strongest adverse prognostic markers for response to cytotoxic chemotherapy is mutation or deletion of the tumor suppressor gene p53. Several clinical observations suggest that p53-mediated apoptosis is not required for susceptibility to immune effector cell-based killing: for example in chronic lymphocytic leukemia (CLL) the presence of a p53 mutation or 17p deletion does not impact the response rates to the anti-CD52 monoclonal antibody alemtuzumab or with the BTK inhibitor ibrutinib⁸⁻¹⁰. These observations raise the tantalizing possibility that traditional biological adverse prognostic factors will become less important when viewed through an immunotherapeutic prism, though intriguingly, a link between p53 and cancer immunosurveillance has been posited^{11,12}.

An additional advantage of adoptive cellular therapy is that due to properties such as expansion, persistence, memory formation patients need only receive a single dose of cells, thus abrogating the requirement for serial administration of drugs with potential logistical and financial implications¹²⁻¹⁷. Notably however, BITE molecules require continuous infusion over several cycles¹⁸. It is unknown how long adoptively transferred T cells need to persist, but evidence from long-term relapses of CML patients successfully treated with alloHCT indicate that relapses can occur 10+ years later, though it is unknown whether this due to failure of immunosurveillance which would otherwise keep at bay rare malignant “stem cells”, and whether more effective initial immune attack would be able to eradicate these rare stem cells¹⁹.

The predominant immune effector cell types used in adoptive cellular therapy include T cells (including CIK cells)²⁰ and NK cells^{21–24}. These cell types were chosen on the basis of known or purported effector functions in the endogenous immune system. There are overlapping functions between T cells and NK cells. Notably, in a healthy immune system all these cell types function together and therefore a reductionist approach that relies upon transfer of selected cell populations or cell subtypes may fail to engender an optimal immune response.

Ultimately the best way to study the different approaches to cellular therapy is to examine the mechanisms of failure of each of those in published studies, in order to best inform how we might improve in the future.

A. No a priori knowledge of targets

Allogeneic hematopoietic cell transplantation (HCT)—The anti-tumor effect of HCT is mediated predominantly by T cells, as manoeuvres to deplete T cells in the stem cell graft clearly lead to an increased relapse rate²⁵. There is also evidence for a role of NK cells, since some groups have shown that mismatches in NK receptor and ligand between donor and recipient can lead to a decrease in the relapse rate^{26,27}. The targets of the so-called graft-versus-leukemia effect are generally unknown, with few exceptions, and donor T cells are generally thought to be responding to polymorphic peptides presented by HLA molecules that are shared by donor and host (so-called minor histocompatibility antigens)²⁸. Nonetheless, alloreactivity alone engenders a relatively weak anti-tumor response, perhaps because few of the transferred T cells have specificity for the relevant antigens^{28,29}. In situations where the transferred T cells can recognize the HLA molecules themselves (e.g. haploidentical transplantation), the patient is heavily immunosuppressed or T cell depletion must be performed in order to limit the development of prohibitively high rates of GVHD³⁰. Furthermore, alloreactivity by itself is clearly insufficient to mediate an efficient antitumor effect because the dose intensity of the conditioning regimen is still important: recent data published in abstract form from a multicentre clinical trial comparing MAC to RIC showed better disease control in the MAC arm³¹. Finally, recent data show that patients transplanted in morphologic complete remission but with minimal residual disease had an inferior disease control, suggesting that the GVT is quite weak indeed³². Thus, although alloHCT is the prime example and most commonly performed example of adoptive immunotherapy for the treatment of cancer, it is complex, expensive, and despite all these it mediates a relatively minor anti-tumor effect.

Cytokines to increase effector cell numbers—Given the central role of T cells and NK cells in anti-tumor immunity, efforts have been made to administer cytokines in order to increase the number or activation status of the cells. The first cytokine to be so used was IL-2³³. Exogenous administration of IL-2 leads to an increase in T cells and NK cells and has been used in the treatment of melanoma and some other malignancies, generally with response rates of 5-15%³⁴. Notably, IL-2 also increases the numbers of regulatory T cells and has been used for the treatment associated with immune over-activation such as chronic graft-versus-host disease (GVHD)³⁵. While IL-2 thus maintains T cell tolerance, the other gamma chain cytokine IL-7 supports the development and homeostasis of naïve and memory

T cells, and IL-15 supports NK cells and memory CD8 T cells in particular³⁶. Administration of recombinant human IL-7 to patients leads to a preferential expansion of naïve CD4 and CD8 T cells along with an increased TCR diversity yet without significant anti-tumor effect^{37,38}. Administration of recombinant human IL-15 leads to expansion of NK cells, gamma-delta T cells and memory CD8 T cells in patients with cancer, however preliminary reports of anti-tumor efficacy were disappointing³⁹. Both IL-7 and IL-15 are currently in clinical trials for patients with cancer.

NK cells—NK cells are innate lymphocytes that can recognize cells transformed by viruses or malignancies. Recognition is based on a complex set of rules that include absence of stimulation through inhibitory receptors (missing self) as well as activation via a restricted set of activating receptors eg NKG2D, natural cytotoxicity receptors (NCR)⁴⁰. NK cells can be shown to mediate potent cytotoxicity in vitro and do not mediate GVHD even after transfer into allogeneic recipients⁴¹. Adoptive transfer of NK cells has been investigated in several settings. Transfer of ex vivo expanded autologous NK cells into patients with advanced malignancies is well tolerated but anti-tumor responses have been minimal⁴². In the haploidentical setting a series of clinical trials in patients with active AML has demonstrated that chemotherapy conditioning, high IL-15 levels and depletion of endogenous regulatory T cells induce the best conditions for NK cell persistence and expansion, with some leukemia patients achieving a complete response and a median disease-free survival of 6 months in a subgroup of the patients⁴³. Human cytokine-induced memory-like NK cells that exhibit enhanced effector functions are produced by brief pre-activation with IL-12, IL-15 and IL-18 followed by adoptive transfer and, and a clinical trial is in progress in patients with AML⁴⁴.

Tumor Infiltrating Lymphocytes—This concept was advanced by the NCI group. The principle is that T cells with tumor specificity are enriched within the tumor microenvironment (e.g. melanoma deposits, or marrow in some hematologic malignancies). There is a complex manufacturing protocol that over several weeks results in extensive expansion of terminally differentiated cells for infusion. Experience obtained from these studies was very important in advancing the field of adoptive cell therapy, demonstrating the importance of lymphodepleting chemotherapy and highlighting that autoimmune complications such as vitiligo or uveitis can occur from on-target specificity of the transferred cells, correlating with an anti-tumor response^{45–47}. This modality has been particularly successful in melanoma, resulting in a complete response rate of approximately 25%, likely due to the fact that melanoma carries a relatively high rate of mutations that can be recognized by T cell receptors. Marrow-infiltrating lymphocytes (MILs that were expanded ex vivo with anti-CD3/CD28 beads and IL-2) from patients with myeloma have been infused into 25 patients with some evidence that an anti-myeloma immune response correlated with responses post-high dose chemotherapy⁴⁸.

Recently, T cells with tumor specificity have been shown to circulate in the peripheral blood and to express the activation/exhaustion marker PD-1⁴⁹. Advances in TIL approaches have finessed the concept of antigen specificity and require *a priori* knowledge of tumor targets but have not yet been used in hematologic malignancies. These approaches require whole

exome sequencing for individual mutations, followed by gene transfer of minigenes that encode the mutations into autologous antigen presenting cells (APC). Only those T cell clones that show an immunological response to the APC are then expanded ex vivo. This approach has been shown to work in a very limited number of patients to date, and is extremely resource and time intensive⁵⁰.

Checkpoint blockade—The use of inhibitory receptor blocking antibodies has been recently reviewed extensively⁵¹. A recent meta-analysis of randomized clinical trials showed an overall survival advantage to receiving checkpoint inhibitors with a hazard ratio of approximately 0.75⁵². Despite the marked enthusiasm for these agents, it is notable that the minority of patients with solid tumors respond to single agent therapy. In Hodgkin lymphoma, due to overexpression of PD-L1, there appears to be a high response rate although follow up to date is quite short⁵³. The challenges in this field include predicting which patients will respond, defining the optimal combination with other checkpoint inhibitors or with other agents such as chemotherapy or radiation, how to further enhance response rates, and dealing with the emergence of previously unseen autoimmune complications that arise from removing the brakes in a potentially indiscriminate manner on T cells without known specificity^{54–56}.

B. A prior knowledge of targets is required

Viral-specific T cells—Malignancies that arise from viral reactivation can be treated with viral antigen-specific adoptive T cell therapy. In this instance, patient or donor T cells are expanded ex vivo in the presence of antigen presenting cells pulsed with viral peptides. Culture lasts several weeks, as the precursor frequency of viral-specific T cells is quite low. Furthermore, this product cannot easily be made from naïve donors who have not previously been exposed to the target virus. An advantage is that by definition these cells have restricted T cell receptor specificity to the virus and hence are not capable of causing GVHD. Viral specific T cells have been produced against EBV and used in the setting of patients with Hodgkin or non-Hodgkin EBV-associated lymphoma, leading to a complete response rates of approximately 50% in the settings of active disease⁵⁷. However, the major disadvantage here is that relatively few viruses cause cancers that express viral antigens. Viral-specific T cells have also been used in the setting of allogeneic CAR T cell therapy in order to limit the possibility of GVHD⁵⁸.

Chimeric Antigen Receptor redirected T cells (CAR T Cells)—If a tumor cell surface antigen is known, single chain variable fragments (the antigen recognition domain) of an antibody can be genetically encoded into a novel polypeptide that also incorporates signalling and co-stimulatory domain, typically derived from molecules that are normally found in T cells. This polypeptide is a chimeric antigen receptor that upon binding its ligand transduces a T cell activation signal that leads to proliferation, cytokine production, and degranulation of effector molecules. Results using CAR T cells against CD19 are most advanced and have been reviewed elsewhere in this edition. In brief, however, the last few years have illustrated the potential power of CAR T cells and their potential drawbacks. Anti-CD19 CAR T cells can lead to complete response rates of up to 90% in heavily-pretreated patients with acute lymphoblastic leukemia (ALL), in per-protocol

analyses^{13,15,16}. These high response rates are tempered by the requirement for individual product manufacturing for each patient, the high costs of gene transfer technology (usually viral transduction with retrovirus or lentivirus), and emerging problems such as limited persistence in some patients and the observation of antigen-loss relapses^{6,13,15,16}. Preliminary results indicate that molecules other than CD19 can also be effectively targeted^{59,60}. Future developments in the field will include suicide-gene approaches, combinatorial logic-gated designs and multi-antigen specificity.

T Cell Receptor transgenic T cells—Most tumor-associated antigens (TAA) are intracellular molecules and must be targeted using TCR that are specific for TAA peptides that are presented in the context of the MHC class I or II groove on APC and tumor cells. There was evidence of an anti-tumor response in 2 of 11 AML patients receiving healthy donor-derived T cell clones specific for the tumor-associated antigen WT1 post allogeneic HCT⁶¹. However, most TCR with specificity for self antigens have relatively low affinity (unlike T cells with specificity for viral antigens)^{62,63}. Therefore high affinity TCR may be engineered into otherwise polyclonal T cells. This can be done either by cloning “natural” antigen-specific TCR from donors who happen to have high affinity TCR, or by “affinity enhancement” of the TCR by mutating residues in the antigen recognition domain^{64–66}. While this approach augments anti-tumor responses, affinity enhancement can lead to unanticipated novel cross-reactivity with self antigens that leads to potentially catastrophic consequences^{67,68}. Additional potential issues include mis-pairing between the endogenous and the introduced TCR alpha or beta chains, also leading to novel and unanticipated specificity⁶⁹. Nonetheless, adoptive transfer of T cells with specificity for MAGEA3, NY-ESO1, CEA and MART1, have been reported in clinical trials^{68,70–73}. Of 20 patients with MM treated with NYESO1-LAGE1-specific T cells, 16 achieved encouraging clinical responses⁷³. Of 17 patients with melanoma treated with MART1-specific TCR transgenic T cells, 2 achieved partial responses along with T cell persistence⁷². The reasons for failure have not been comprehensively reported but may include HLA molecule downregulation, lack of presentation of the intracellular antigens, antigen loss or mutation, and failure of T cell persistence.

T cell engaging antibodies—An alternative way to redirect T cells to a selected cell-surface target antigen is by infusing bispecific T cell engaging antibodies that are specific for the CD3 molecule on the T cell and an antigen of choice such as CD19 on the target cells. Similar constructs have been that engage CD16 on NK cells²³. The leading example of bispecific T engaging antibodies is blinatumomab, a compound that has recently gained FDA approval for the treatment of Philadelphia chromosome-negative B-ALL. Approximately 40% of patients treated active disease achieve a complete response, and 80% of patients treated in a minimal residual disease achieve a MRD negative setting^{18,74}. Administration must be via continuous intravenous infusion for four weeks on and two weeks off, and continues for up to 5 cycles – although whether a longer duration of administration would enhance disease-free survival without additional therapy is at present unknown. This point would be best informed by long-term clinical trials evaluating the main reasons for treatment failure and whether this occurs during or after therapy. Other formats

for combining effector cell with target specificity, and other targets such as CD33 and CD123 are currently under evaluation.

The above discussion is summarized in **Table 1**, where we also speculate on ways to advance the field by combining the available therapeutic modalities.

CONCLUSIONS

We have entered a golden age of cellular immunotherapy. Rather than replacing other anti-cancer modalities such as surgery, chemotherapy, radiotherapy or small molecule pathway inhibitors, it is likely that the bountiful harvest of new technology will be deployed in a complementary fashion to further individual and population-based outcomes from cancer.

Nonetheless, based on extant results several conclusions can be drawn.

1. Of the modalities that do not require *a priori* knowledge of targets, checkpoint inhibitors have the unique ability to mediate convincing regressions of large volumes of disease in selected types of cancer with heavy mutational burden (or with exceptional levels of PDL1/PD1 interaction). The main side effects include high rates of autoimmune reactions, particularly when two different pathways are blocked simultaneously.
2. Numerical increase or non-specific activation of immune effector cells, such as transfer of NK cells, CIK cells, or infusion of cytokines with important roles in T cell homeostasis and development, leads to relatively minor responses in patients with cancer.
3. Antigen-specific T cells, redirected genetically or by means of bi-specific engaging peptides, have the most potent single agent activity in patients with advanced hematologic malignancies. Of these, CAR T cells appear the most active. However, CAR T cells and bi-specific T cell engagers require a cell-surface target that is either unique to the tumor, or if not unique to the tumor then the normal cell counterpart that bears the targeted antigen must be at least temporarily dispensable (e.g. B cells)
4. T cells bearing a TCR specific for tumor-associated antigens (TAA) have the potential to mediate tumor-specific immunity. However, some TAA are at least in part shared by normal tissues. T cells that are specific for tumor-associated mutations have been generated from a handful of patients with solid tumors at great effort and expense and represent the purest example of tumor-specific adoptive cellular immunotherapy, but it remains to be seen whether the herculean efforts required to generate such T cell products can be generalized beyond single highly-specialized centers.

Planes, trains and automobiles each fulfil a unique non-redundant and complimentary role in a well-designed transportation system. Having a range of options facilitates customization and enables us to find the right solution to each given problem, be it how to get from geographical A to B, or how to fight cancer.

It is likely that in the coming years we will learn which of the immunotherapeutic modalities is best suited for each role, and where immunotherapy can be enhanced with or support more traditional methods of anti-cancer therapy. Given the relatively early stage of development of most of these modalities, some important questions should be addressed in the coming years:

1. Head to head comparisons of various cellular immunotherapy approaches (e.g. CART versus BiTE)
2. Comparisons of cellular immunotherapy with approaches (e.g. BiTE versus antibody-drug conjugates, CART versus small molecule B cell receptor pathway inhibitors)
3. Combinations of immunotherapy with other approaches (e.g. checkpoint inhibitors in combination with radiotherapy⁷⁵, CART in combination with cytokines or with checkpoint inhibitors)

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Table 1

available therapeutic modalities

Modality	Mechanism of anti-tumor activity	How could be improved
Type A: No a priori knowledge of targets is required		
Allogeneic HCT ³⁰⁻³²	Pre-existing specificity for mismatched HLA or minor histocompatibility antigens	Separation of GVHD from GVL (management of toxicity)
Exogenous cytokines ^{37,39}	Increased CD4, CD8 or NK proliferation, activation, +/- role in maintaining a memory phenotype	Increase antigen recognition?
NK cells ^{43,44}	Missing self recognition (haploidentical setting), stimulation through activating receptors	Increase antigen recognition?
TILs ^{46,50}	Pre-existing specificity towards tumor-specific mutations	Simplify and streamline manufacturing; combine with checkpoint inhibitors?
Checkpoint inhibitors ^{53,54}	Blockade of inhibitory signals from tumor or micro-environment via inhibitory receptors on T cells	Combine with chemotherapy or radiation
Type B: A priori knowledge of targets is required		
Virus-specific T cells ⁵⁷	Pre-existing specificity towards viral antigens	Combine with checkpoint inhibitors?
CAR T cells ^{13,15,16}	Engineered recognition of cell surface antigen on tumor cell by a chimeric immunoreceptor, along with in-built co-stimulation	Management of toxicity; New targets other than on B cells; Combine with novel agents?
TCR transgenic T cells ^{72,73}	Engineered recognition of intracellular tumor-associated antigen in the context of MHC class I or II	Combine with checkpoint inhibitors? Combine with exogenous cytokines?
Bi-specific T cell engaging antibodies ^{18,74}	"non-specific" T cell activation via CD3 cross-linking is redirected to a specific cell surface target using a peptide linker.	Mitigate toxicity so dose can be increased? Prolonged administration schedule? Engineering re-design to simplify administration schedule? Combine with checkpoint molecules or cytokines?