Published in final edited form as:

Nat Biotechnol. ; 30(7): 611–614. doi:10.1038/nbt.2305.

T-cell therapy at the threshold

Carl June,

Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA;

Steven A Rosenberg,

Surgery Branch, National Cancer Institute, US National Institutes of Health, Bethesda, Maryland, USA;

Michel Sadelain, and

Center for Cell Engineering, Memorial Sloan-Kettering Cancer Center, New York, New York, USA;

Jeffrey S Weber

Donald A. Adam Comprehensive Melanoma Research Center at H. Lee Moffitt Cancer Center & Research Institute, Tampa, Florida, USA.

Abstract

Despite impressive clinical activity in B-cell lymphoma and melanoma, questions remain about the immunobiology of adoptive T-cell therapies.

Adoptive T-cell therapy in advanced meta-static melanoma or B-cell leukemias is garnering increasingly encouraging clinical data. *Nature Biotechnology* approached several experts in the field to seek their insights into some of the challenges of optimizing and commercializing these experimental treatments.

What factors in the host and tumor microenvironment might compromise T-cell therapy?

Michel Sadelain: The tumor microenvironment is the battlefield where immune effectors either eradicate a tumor or fail, succumbing to various inhibitory mechanisms promoted by the tumor. The sources of such inhibition are multiple, including regulatory T cells, type 2 macrophages, myeloid suppressor cells and the tumor cells themselves. The players are multiple and differ between tumor types and individuals. Although it is not a black box anymore, a lot remains to be learned. Extratumoral factors affect adoptive T-cell therapy in many ways. Examples include medullary or splenic reservoirs of myeloid suppressor cells; dysfunctional dendritic cells in lymph nodes; and extratumoral expression of antigen or cross-reactive peptides, which are the cause of ‘on-target, off-tumor’ side effects.

Steven A. Rosenberg: There clearly are aspects of the immune system that can regulate if not suppress immune reactions, and dealing with them is important for immunotherapy. In fact, with adoptive T-cell transfer therapy, the critical aspect of getting it to work is first lymphodepleting the patient before we return to the patient either natural antitumor cells or gene-modified antitumor cells. This prior lymphodepletion, using chemotherapy and

sometimes with whole body irradiation, eliminates T-regulatory cells, myeloid-derived suppressor cells and other suppressive influences, and that's what can lead to complete durable regression in patients with melanoma who receive cell transfers. So dealing with the tumor microenvironment is critical.

Carl June: Our data show that replicative capacity of the transferred T cells may be a key factor that is required for efficacy of the procedure. In previous studies with Nan-ping Weng and Richard Hodes [at the US National Institutes of Health], we found that the replicative capacity of memory and naive T cells decreases with age. Thus, it is possible that T cells from aged patients may be less potent than those from younger patients.

Beyond lymphomas and melanomas, are there certain cancers that would be particularly challenging targets for T-cell therapy?

SAR: This whole area of genetically engineering of lymphocytes to express either conventional $\alpha\beta$ T-cell receptors or CARs [chimeric antigen receptors] is a way to expand the range of immunotherapy to other cancer types. That was first shown in our papers in *Blood* [116: 4099–4102, 2010] and the *Journal of Clinical Oncology* paper [29: 917–924, 2011]. You can transduce chimeric receptors encoding CD19 and successfully treat patients with B-cell lymphomas or traditional α - β T cell receptors and treat patients with synovial cell sarcomas.

MS: There are still very few known common, tumor-specific antigens. The cancer/testes antigens are attractive, but they are inconsistently expressed in all cases of the tumor types where they tend to appear or in all cells of positive tumors. CD19 is a great target for CAR therapy, but few other cell-surface molecules possess such a favorable profile—high expression on most tumor cells and expression in normal cells restricted to a dispensable cell type. Target identification remains a major research goal.

CJ: No! We have tested many cancers and have found that appropriately activated T cells can usually kill the tumor cells. Thus, the challenge will be whether the T cells can traffic to the tumor, which is a particular issue in some sarcomas and tumors located in other 'sanctuary sites'.

Although CD8⁺ T cells are conventionally thought of as the main effectors of antitumor responses, how much interest is there in CD4⁺ T cells?

Jeffrey S. Weber: CD4⁺ T cells should be taken quite seriously as possible effector cells, and should not be excluded from experimentation. Same for natural killer T cells [NKTs] and other effectors. Even B cells may have potential as effector cells.

MS: CD4⁺ cells can promote humoral and cell-mediated immunity, as well as mediate direct tumor eradication of [major histocompatibility complex] MHC II⁺ tumor cells. Surprisingly, however, the interplay between CD4⁺ and CD8⁺ T cells in tumor immunity is yet to be fully understood.

CJ: For CAR T-cell therapy, a balance of CD4⁺ and CD8⁺ CARs seems most effective, based on the original studies in mice by Phil Darcy [at the Peter MacCallum Cancer Centre; East Melbourne, Australia]. Results from our ongoing studies [at the University of Pennsylvania; Philadelphia] in patients with leukemia are consistent with those pre-clinical results.

Which cancers are likely to be amenable to resection and isolation of autologous tumor infiltrating lymphocytes, rather than isolation of T cells from the peripheral blood?

MS: Not very many, which is why the case of tumor-infiltrating lymphocytes [TILs] in melanoma remains a proof of principle rather than a generalizable paradigm.

JSW: Tumors that commonly present with nodal, cutaneous and soft-tissue lesions and that have large numbers of single-nucleotide variants or mutations. Melanoma is the classic. Renal-cell cancer is difficult to grow TILs from and would be more amenable to a peripheral blood approach. Prostate cancers often have mucinous tumors and large amounts of stroma and may not be the best candidates for TIL growth. Head and neck cancers often receive extensive radiation and also are not good candidates to grow TILs from.

SAR: Melanoma is the only solid cancer that readily gives rise to TILs that have antitumor activity. Other cancer types are much less productive. That is why we have had to genetically engineer lymphocytes to express TCRs [T-cell receptors] that attack antigens to treat other cancers. Our recent publication using genetically engineered T-cells to treat patients with synovial-cell sarcoma shows that it is possible to cause regression in this solid tumor. There's nothing unique about melanoma in terms of its ability to be destroyed by T cells. It's just the afferent limb that is different in patients with melanoma.

What factors go into determining the best source of T cells used in therapy?

MS: Tumor-specific T cells can be found in some patients with some tumors, but the success of such an endeavor cannot be taken for granted. The attraction of T-cell engineering is that it does not require the presence or the isolation of tumor-specific T cells from the patient as it makes use of easily retrievable peripheral blood T cells. What T-cell type or subset is best for rapid or long-term effects is still not known.

CJ: It is important for the field to integrate T-cell therapies into the standard-of-care practice for a given tumor, if this is a disruptive therapy that can become widely accepted in clinical practice. Thus, unless surgery is done routinely for a given tumor, a surgical procedure done only to obtain input T cells for manufacturing is less preferable than using peripheral blood that is readily available. With improved understanding of the cell biology of natural killer cells, NKT cells and $\gamma\delta$ T cells, there is a resurgence of interest in the use of these cells in combination with approaches that use conventional $\alpha\beta$ T cells.

In what instances would the use of CARs be preferable over TCRs, and vice versa?

MS: CARs are very attractive because they are not HLA [human leukocyte antigen]-restricted, as they directly target cell-surface antigens and therefore do not require batteries of TCRs for different HLAs. Furthermore, unlike TCRs, transduced CARs do not run the risk of mispairing with endogenous TCR chains and do not compete for the rate-limiting CD3 complex. Their limitation used to lie in the limited availability of suitable cell-surface antigens and their limited signaling. This latter problem has largely been remedied with second-generation CARs. Both TCRs and CARs are worthy of further investigation, but I believe that CARs will eventually take over.

CJ: For practical purposes, CARs have the ‘off the shelf’ advantage over TCRs in that matching to HLA is not required. From considerations of tumor biology, some tumors, such as neuroblastoma and carcinomas, are frequently found to have low or absent expression of HLA class I molecules, and thus they are resistant to CTLs [cytotoxic T lymphocytes]. TCRs have evolved an extremely efficient signal-amplification system capable of detecting very low levels of tumor-derived peptides, and for this reason they may be inherently safer than CARs. On the other hand, CARs may confer an advantage over TCRs in that the synthetic CAR signals are distinct from TCR signals. CARs may signal T cells in a fundamentally different manner from natural TCRs and thereby confer resistance to some of the immunosuppressive aspects of the tumor microenvironment. One final advantage of CARs over TCRs is that the preclinical testing to predict off-tumor but on-target toxicity is more straightforward than for TCRs. This is an important consideration for testing new TCRs because it is very difficult to ascertain the absence of expression of antigens in non-tumor tissue, given the exquisite sensitivity of the TCR to peptides with low abundance.

SAR: CARs and TCRs recognize very different structures. CARs recognize the three-dimensional structure of a cell-surface protein. In contrast, conventional TCRs recognize peptides from internal cellular proteins that are presented on surface MHC molecules. So it depends on what you are targeting. If you are targeting a cell-surface protein, like CD19 in lymphomas, then CARs are used. If you are targeting cancer testis antigens, which are internal cellular proteins, then you have to use a conventional $\alpha\beta$ TCR.

What are the main issues in antigen selection for engineered T cells?

MS: Now that we have found out how to generate more potent and longer-lasting T cells, we are coming back to the original question of targeting. There are still very few good targets, such as WT1 and NY-ESO-1 for TCRs and CD19 for CARs. New antigens are needed to target tumors. Novel tandem approaches to spread the response beyond the targeted antigen are needed.

CJ: With cancer vaccines, the natural T cells elicited by vaccination have generally not caused significant toxicity when targeted to tumor-associated antigens. This is a good toxicity profile, but the efficacy is <5% according to the many studies summarized by Steven [Rosenberg] and Nicholas Restifo at the NIH [US National Institutes of Health]. However,

this is very different from the situation in which engineered T cells endowed with high-affinity TCRs or CARs are infused, where significant toxicity occurs when tumor-associated antigens are targeted but not when tumor-specific antigens are targeted. This was first shown by Cor Lamers and the group at Erasmus in Rotterdam, who tested a CAR with specificity to carbonic anhydrase IX. Thus, the major issue with antigen selection for engineered T cells is specificity rather than abundance.

Is there a preferred vector for engineering T cells?

MS: Retrovirus and lentivirus both have their advantages and disadvantages. Overall, they both work well. The gammaretroviruses are cheaper to manufacture. The safety concerns of gammaretroviruses are real in the context of stem and progenitor cell therapies, but their safety profile in T cells is so far excellent. There is therefore no safety or cost rationale in favor of the lentiviral vectors. The long-term expression of lentiviral vectors may be superior to that of gammaretroviral vectors, but this has not been established. The safety and efficacy of transposons is presently unknown. It will be difficult to unseed the retroviral vectors based on expression and safety characteristics. The cost may ultimately favor nonviral approaches, provided they are equally effective and safe.

CJ: It is fortunate that the toolbox of approaches to engineer T cells is overflowing with clinically feasible approaches. From a cost-of-goods standpoint, transposons may be preferable to live viral vectors. At the University of Pennsylvania, we are beginning clinical studies with Renier Brentjens and Michel Sadelain at Memorial Sloan-Kettering Cancer Center [New York] to compare CARs that are introduced with either a retroviral or lentiviral vector. This is a major issue facing the field.

SAR: We use gammaretroviruses to introduce genes into these lymphocytes but you can also use lentiviruses. Those are the two main ways. There are other possibilities. Cells can be electroporated, and there are ways to use transposons to insert genes. So there are a variety of techniques. But the overwhelming majority of studies use retroviruses.

What will be the importance of engineering T-cell proliferative potential or trafficking?

CJ: Realistically, the only way to eradicate large tumors with adoptive cellular therapy is for the T cells to proliferate sufficiently to achieve an appropriate effector-to-target ratio *in vivo*. Thus, approaches to safely increase the proliferative potential of T cells are attractive. Fortunately, our studies indicate that T cells appear to be inherently resistant to transformation, but this is always a risk that must be addressed with these ‘souped-up’ T cells. In terms of trafficking, several laboratories have impressive preclinical data showing that it is possible to enhance trafficking to tumors. Patrick Hwu at the MD Anderson Cancer Center [Houston] is planning such a trial in melanoma patients.

SAR: One of the exciting opportunities using genetic manipulation of lymphocytes is that these genes can be inserted with very high efficiency, so there is an enormous panoply of genes that are possibilities. You can insert genes that encode costimulating molecules. You

can put in telomerase genes to keep telomeres from shortening, you can knock out regulatory elements, you can put in genes like *Bcl-X* [*BCL2L1*] or *BCL2* that can counteract apoptosis. So one has enormous opportunities to create cells in the laboratory that normally do not exist.

MS: It is not clear how often trafficking is actually limiting. Since only a few genes can be simultaneously transduced with current gene transfer methodologies, it seems unlikely that the chemokine receptors will often take up one of only two or three vector slots, although they may be helpful in some cases.

How important is non-myeloablative lymphodepletion for the success of T-cell therapy?

MS: Host conditioning and modulation of the tumor microenvironment are integral to adoptive T-cell therapy and absolutely essential. It needs to be better understood so that it can be accomplished with selective, nontoxic regimens rather than aggressive chemotherapies and total-body irradiation.

CJ: A large body of data indicates that lymphodepletion enhances the engraftment, proliferation and effector functions of transferred T cells in preclinical models and in clinical trials. However, I don't think that lymphodepletion is always necessary. For example, we have found that CAR T cells can survive for at least a decade at high levels in immune-reconstituted patients with HIV infection who were given no chemotherapy. We have also observed potent effects of CAR T cells in leukemia patients who were not given chemotherapy. So it is likely that the need for chemotherapy and lymphodepletion are context dependent, depending on the specific issues of the tumor and the host.

SAR: All of the adoptive T-cell therapies depend, for a variety of reasons, on prior lymphodepletion to get rid of regulatory elements. The second [reason] for lymphodepletion is to eliminate cells that compete for homeostatic cytokines like IL-7 and IL-13, which are responsible for sustaining lymphocyte survival; we want to eliminate endogenous lymphocytes so that those cytokines act only on the cells that are administered.

What are the best ways of assessing clinical response in peripheral blood or the tumor?

MS: In addition to the classic immune monitoring tools, next generation multiplexed single cell assays to monitor T cell function and epitope spreading and exome sequencing, the use of genetically modified cells uniquely enables clonal tracking of genetically tagged (bar-coded) T cells and the use of non-invasive imaging to ascertain T cell biodistribution. At Memorial Sloan-Kettering Cancer Center, we are currently tracking targeted T cells in prostate cancer patients treated with CAR-modified T cells utilizing positron emission tomography.

CJ: Various molecular assays as well as conventional FACS [fluorescence-activated cell sorting] can easily track and document the persistence and function of adoptively transferred

cells. I think that the major need in the field is a clinically compliant imaging approach to better characterize trafficking of infused cells.

SAR: There are classic criteria for measuring responses, called the RECIST [Response Evaluation Criteria In Solid Tumors] criteria. These involve a 30% decrease in the sum of the largest diameters of target lesions. One of the confusions in oncology and immunotherapy is that people tend to use nonvalidated criteria for response, like tumor necrosis, or infiltration with lymphocytes or patients living longer than expected. These are not valid endpoints.

How do you see T-cell therapy combining synergistically with other regimens?

MS: There will likely be an explosion of therapeutic combinations of T cells with antibodies, drugs, small molecules and other agents designed to augment T cell-mediated tumor eradication. As said above, the promise of T cells is that of achieving targeted tumor eradication with minimal toxicity, which is what a good immune response should do. Combining specific T cells with less specific and toxic agents is not where I hope these therapies will end.

SAR: We haven't seen a relationship between tumor bulk and the likelihood of having a complete tumor regression. So I don't think there is a role for tumor reduction by combining T cells with surgery [for example].

What are some of the major issues concerning commercialization of these therapies?

JSW: My safety concerns are the same as the US Food and Drug Administration [FDA]: cross-reactivity of high-affinity and potent TCRs with normal tissues would lead to profound side effects and death, as we have seen with the recent [US National Cancer Institute; NCI] MAGE-3 trial in metastatic melanoma. Some safety molecules should be included in single TCR or chimeric constructs. Once there is FDA approval of a TIL-type approach, technology will need to be developed to allow continuous [rather than batch] approaches, which are more commercially viable. Clinical trials beyond proof of concept will likely be done at commercial facilities that have the experience to expand cells to large numbers in bulk. Small phase 1 or proof-of-concept trials will continue to be done at selected centers with cell growth facilities.

MS: The complexity of preparing T cells should not be overstated. Some robust technologies are already in place for TILs (NCI) and for CARs [e.g., *J. Immunother.* **32**, 169–180, 2009]. The biology and process development are complex, but these protocols are quite streamlined and can be implemented outside of expert centers. T-cell purification steps add complexity, but it remains to be established whether subset purification will benefit the cost or effectiveness of T-cell therapies.

CJ: One of the main challenges is that the field needs to conduct trials according to pharmaceutical industry standards and then analyze them on an intent-to-treat basis. For too many years, adoptive transfer therapy has largely been done as pilot trials conducted in boutique settings at highly specialized cancer centers. It is now time for adoptive T-cell therapy to move beyond this. This is really no different from standard drug development.

SAR: If you don't use the patients' own cells, the cells you administer will get rejected because they contain allo-antigens. One of the issues regarding widespread implementation of cell transfer therapies is that some of the cell therapies are quite complex. They are the ultimate in personalized medicine because a new drug is created for every patient; you are using a patient's own cells to treat them. So this doesn't fit into the paradigm of big pharmaceutical companies that favor drugs in a vial off the shelf that they can use. Drug companies can spend half a billion dollars to make the first vial of a new drug, so long as the second vial can be produced for a few dollars. These cell therapies that are highly personal require a different approach to treatment. When people realize how effective some of these treatments are, ways will be found to make them more available. [Immunotherapy is] ready for commercial development; hospital blood banks could readily prepare antitumor cells for therapy as could commercial companies. [However,] they are complex. T cell-transfer therapies for metastatic melanoma provide the patient with the best chance for cure of their disease. So I am confident that commercial development of these therapies will occur.

Biographies

Michel Sadelain is Director, Center for Cell Engineering & Gene Transfer and Gene Expression Laboratory, and Stephen and Barbara Friedman Chair, Sloan-Kettering Institute, Memorial Sloan-Kettering Cancer Center, New York.

Steven A. Rosenberg is Chief of Surgery at the US National Cancer Institute, Bethesda, MD.

Carl June is Richard W. Vague Professor in Immunotherapy, Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania.

Jeffrey S. Weber is Director of the Donald A. Adam Comprehensive Melanoma Research Center at H. Lee Moffitt Cancer Center, Tampa, Florida.