IN THE SPOTLIGHT

Hit Parade for Adoptive Cell Transfer Therapy: The Best T Cells for Superior Clinical Responses

Daniel E. Speiser

Summary: Adoptive cell transfer (ACT) of T cells has great clinical potential, but the numerous variables of this therapy make choices difficult. A new study takes advantage of a novel technology for characterizing the T-cell responses of patients. If applied systematically, this approach may identify biomedical correlates of protection, thereby supporting treatment optimization. *Cancer Discov; 3(4); 379–81. ©2013 AACR.*

See related article by Ma et al., p. 418 (5).

T cells play a central role in immunity against infectious diseases and cancer. In the 1970s and 1980s, identification of the principles of antigen-specific T-cell reactivity led to the discovery of the T-cell receptor (TCR) and its cognate antigen, peptide presented by MHC (HLA in humans). This receptor–ligand pair guides cellular antigen specificity of the immune system. In infectious diseases, many studies have shown that specific T-cell activation correlates with improved clinical outcome. More recently, evidence has accumulated that T cells also help control many different types of tumors, at least in subpopulations of patients with cancer.

As one example, large numbers of patients with leukemia have benefited from the graft-versus-leukemia effect induced and maintained by donor T cells after allogeneic hematopoietic stem cell transplantation. Transfer of T cells for patients with solid tumors is less advanced (1); nevertheless, adoptive cell transfer (ACT) therapy may be successful in selected patients with advanced metastatic melanoma, with about 50% to 70% of patients experiencing objective clinical responses after transfer of *in vitro* expanded T cells derived from autologous tumor-infiltrating lymphocytes (TIL). Up to 20% of patients even have complete and durable regression of metastatic disease (2). ACT may also show clinical benefit in patients with synovial cell sarcomas and some leukemias (3). However, the reasons for treatment success and failure remain largely unknown. In addition, only a minority of patients qualify for ACT, in part due to the lack of TILs or the fact that patients do not tolerate the toxicities associated with preconditioning and interleukin (IL)-2 therapy after ACT (3). What else can be offered for future patients?

During the 1980s, several laboratories generated and analyzed so-called TCR transgenic mice, in which most T cells express a single TCR type. Use of these mice allowed demonstration of the basic immunologic principles of T-cell maturation and differentiation. Moreover, these animals serve as a unique source of donor T cells that can be used to study virtually any property of antigen-specific T cells relevant for immunity upon adoptive transfer in recipient mice with infections or tumors. T cells from TCR transgenic mice can often mediate disease control, suggesting that large numbers of monoclonal T cells expressing optimally selected TCRs may be of great help for patients. Clinical application has become possible thanks to the cloning of human TCRs specific for tumor antigens and technologies for the insertion of TCR-α and -β chain genes into human lymphocytes. For about 10 years, researchers have run clinical trials of ACT with genetically engineered (TCR-transfected) T cells for patients with cancer, many of whom have experienced clinical responses (4). However, despite continued progress, most patients have disease recurrence, calling for treatment improvements.

Clinical trials of ACT therapy are usually complemented with translational research studies. One can readily assess the cellular and molecular properties of the patients before and during treatment. Novel laboratory techniques provide promising tools to intensify the search for correlates of protection (Fig. 1). A new step in this direction was taken in a study published in this issue of *Cancer Discovery* by Ma and colleagues (5), who analyzed T cells from 3 representative patients selected from a trial of 14 patients with melanoma who underwent ACT therapy with lymphocytes transfected with the high-affinity Melan-A/MART-1–specific TCR "F5" (4). Three patients had initial transient tumor responses followed by disease progression within 6 months. The authors used multidimensional and multiplexed T-cell immune monitoring assays to longitudinally characterize the T cells of the patients. Ma and colleagues (5) focused primarily on functional properties, based on previous observations that phenotypic markers alone may not be sufficient. Indeed, the T cells from the 3 patients showed strong functional differences despite the observation that the transferred T cells had similar persistence and phenotypes (5). Interestingly, Ma and colleagues (5) found 2 waves of $CD8^+$ T-cell responses after ACT, a first wave with preferential granzyme B production that diminished sharply within the first 30 days after ACT and a second wave dominated by IFN-γ and TNF-α–positive

Author's Affiliations: Department of Oncology and Ludwig Center for Cancer Research, University of Lausanne, Switzerland; and Campbell Family Institute for Breast Cancer Research, Princess Margaret Hospital, Toronto, Ontario, Canada

Corresponding Author: Daniel E. Speiser, Ludwig Center for Cancer Research, Department of Oncology CHUV HO-05/1552, University of Lausanne, Ave. P-Decker 4, CH-1011 Lausanne, Switzerland. Phone: 4121-314-0182; Fax: 4121-314-7477; E-mail: doc@dspeiser.ch

doi: 10.1158/2159-8290.CD-13-0064

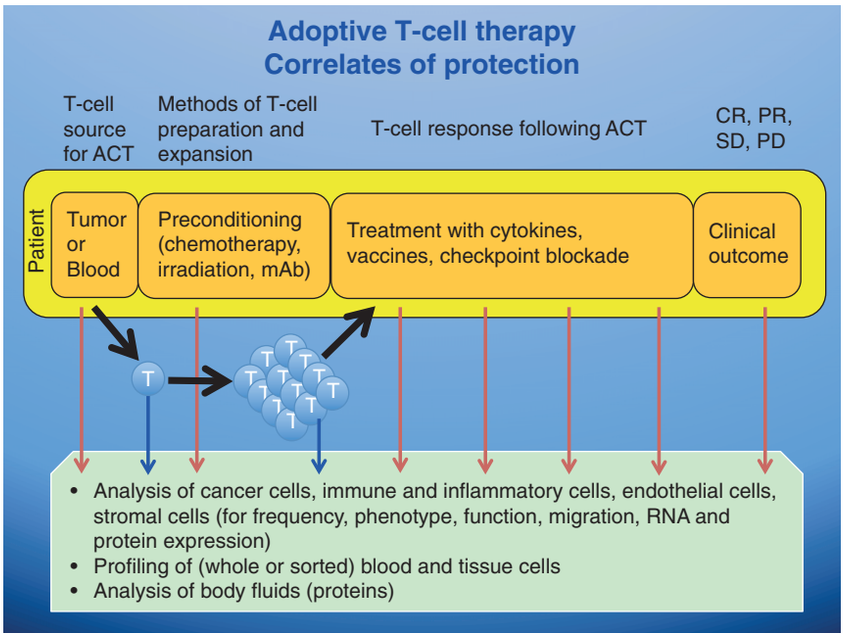

Figure 1. Identification of patient and treatment parameters correlating with clinical outcome. Comprehensive studies may include characterization of the clinical parameters of the patient, his/her biologic properties, and the T cells used for transfer, combined with systematic assessment during and after therapy. The results provide the rationales for future therapy optimization. CR, complete response; mAb, monoclonal antibody; PD, progressive disease; PR, partial response; SD, stable disease.

T cells abundant at day 60. As observed in previous studies, IL-2 production remained low. In contrast with their $CD8^+$ counterparts, the TCR transgene-expressing $CD4^+$ T cells performed poorly, as they lacked the ability to proliferate and carry out stable effector functions *in vivo*.

Together, these data raise the hypotheses that favorable clinical outcomes may be associated with strong effector functions of TCR transgene-positive and -negative $CD8^+$ T cells, with proliferative and effector functions of transgene-negative $CD4^+$ T cells, and possibly with epitope spreading. In turn, regulatory functions of transgene-negative $CD4^+$ T cells may be unfavorable. The weak functions of the transgene-expressing $CD4^+$ T cells suggest that such $CD8^-$ cells may not be helpful, making it worthwhile to consider novel strategies of limiting gene engineering of MHC class I restricted TCRs to $CD8^+$ T cells (6).

Studying 3 patients precluded firm identification of correlates of protection. Future studies may include more patients and characterize many parameters of the T cells of patients. Moreover, parallel analyses may focus on cancer cells, B cells, natural killer cells, inflammatory cells, stromal cells, and the vasculature, as well as their associations with each other and with clinical outcome (Fig. 1). Thus, patients may be studied much more comprehensively than in the past, increasing the likelihood of providing robust biomedical evidence. The results may allow optimization of treatment parameters such as the choice of autologous T-cell source and preparation, the type of patient preconditioning, and patient treatment after ACT.

Previous TIL transfer studies have shown that the number of infused $CD27^+$ $CD8^+$ (memory-like) T cells, their telomere length, and their persistence at 1 month after transfer correlated with clinical outcome of the patients with melanoma (2). Such observations confirmed that "young" T cells have superior therapeutic potential, supporting intensive research into the properties of T cells enabling long-term protective immune responses. Naïve T cells have a high level of "stemness" and protective potential, which is not surprising as they are at the origin of all memory and effector T-cell subsets. Using inbred TCR transgenic mice, naïve T cells can be directly transferred from donor to recipient mice. In humans, however, autologous naïve antigen-specific T cells are not available in sufficient numbers. Human autologous lymphocytes need to be expanded *in vitro* for several weeks, resulting in predominantly effector cell populations, with few memory cells and no naïve cells. However, effector T cells are short-lived and can only mediate short-term protection (3). This problem is tempered by TCR gene engineering, which allows the generation of large numbers of tumor antigen-specific human T cells within a few days, preserving memory cells. Indeed, the currently applied technologies for ACT of TCR-transfected T cells permit relatively good persistence of transferred T cells in patients, at least over several months, in agreement with the notion that memory cells are superior to effector cells (4). As memory T cells are heterogeneous and include many subsets, the search for the most potent ones is challenging. The use of sorted so-called stem cell–like memory T cells has shown promising results (3). In addition, a new approach has been suggested by 2 recent studies showing the *in vitro* generation of induced pluripotent stem cell–like memory cells (7) by transfection of effector cells with the Yamanaka transcription factors Oct4, Sox2, Klf4, and c-Myc. These advances support the continued use of autologous TILs, currently the best cell source for ACT therapy.

Besides multifunctionality and "stemness" of T cells (8), several further correlates of protection may be clinically significant. TCR affinity/avidity plays a central role, as suggested more than 20 years ago by the demonstration that low TCR avidity is sufficient for *in vitro* proliferation or cytotoxicity to peptide-coated target cells but not for *in vivo* protection (9, 10). Mechanisms of immunologic tolerance

are responsible for the poor natural TCR repertoire specific for self/tumor antigens (11). Although patients may have many tumor-specific TCRs, their affinity is relatively low, eventually too low to mediate immunity. Recently, self/tumor-specific TCRs designed for high-affinity binding to cognate antigens have been used for clinical ACT, resulting in improved tumor responses but also increased toxicity (4, 12). Interestingly, high-affinity TCRs may not always be necessary, as suggested by a remarkable competence of intermediate affinity T cells (13). Even though they are less potent than high-affinity TCRs, intermediate-affinity TCRs such as those found in TILs have shown great effects in patients. With sophisticated laboratory techniques, T cells with suitable TCRs can be carefully selected by assessment of their capacity to recognize naturally low levels of antigen on tumor cells or even dendritic cells and endothelial cells *in vivo*. The experimental use of artificially high synthetic peptide concentrations or peptide analogues with enhanced MHC binding may be misleading (9, 10, 14). Another correlate of protection is the precursor frequency of antigen-specific T cells, which must be reasonably high *in vivo* (15). Finally, clonal diversity is important, as multiple different TCRs mediate superior immunity and also prevent the outgrowth of tumor cell escape mutants. Escape may further be avoided by mobilizing T cells specific for multiple different tumor antigens, presented by several different HLA alleles.

Future studies may analyze all potential key parameters (Fig. 1) in relatively large patient cohorts, broadening the knowledge base for improving immunotherapy for patients with cancer who often lack other treatment options. Such studies pose considerable challenges at multiple levels (clinical, methodical, technical, logistic, data analyses, financial, and collaborative), which can be met with dedicated disease-orientated clinical–translational programs. Results from comprehensive analyses will likely contribute to the identification of fundamental disease and treatment mechanisms.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

This work was supported in part by funds from the Ludwig Institute for Cancer Research, the Cancer Research Institute and Cancer Vaccine Collaborative, the Swiss Cancer League (02836-08-2011), and the Swiss National Science Foundation (310030_135553).

Published online April 11, 2013.

REFERENCES

1. Ho WY, Blattman JN, Dossett ML, Yee C, Greenberg PD. Adoptive immunotherapy: engineering T cell responses as biologic weapons for tumor mass destruction. Cancer Cell 2003;3:431–7.
2. Rosenberg SA, Yang JC, Sherry RM, Kammula US, Hughes MS, Phan GQ, et al. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. Clin Cancer Res 2011;17:4550–7.
3. Restifo NP, Dudley ME, Rosenberg SA. Adoptive immunotherapy for cancer: harnessing the T cell response. Nat Rev Immunol Nature Publishing Group 2012;12:269–81.
4. Johnson LA, Morgan RA, Dudley ME, Cassard L, Yang JC, Hughes MS, et al. Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen. Blood 2009;114:535–46.
5. Ma C, Cheung AF, Chodon T, Koya RC, Wu Z, Ng C, et al. Multifunctional T-cell analyses to study response and progression in adoptive cell transfer immunotherapy. Cancer Discov 2013;3:418–29.
6. Zhou Q, Schneider IC, Edes I, Honegger A, Bach P, Schonfeld K, et al. T-cell receptor gene transfer exclusively to human CD8+ cells enhances tumor cell killing. Blood 2012;120:4334–42.
7. Nishimura T, Kaneko S, Kawana-Tachikawa A, Tajima Y, Goto H, Zhu D, et al. Generation of rejuvenated antigen-specific T cells by reprogramming to pluripotency and redifferentiation. Stem Cell 2013;12:114–26.
8. Lugli E, Dominguez MH, Gattinoni L, Chattopadhyay PK, Bolton DL, Song K, et al. Superior T memory stem cell persistence supports long-lived T cell memory. J Clin Invest 2013 Jan 2. [Epub ahead of print].
9. Speiser DE, Kyburz D, Stübi U, Hengartner H, Zinkernagel RM. Discrepancy between *in vitro* measurable and *in vivo* virus neutralizing cytotoxic T cell reactivities. Low T cell receptor specificity and avidity sufficient for *in vitro* proliferation or cytotoxicity to peptide-coated target cells but not for *in vivo* protection. J Immunol 1992;149:972–80.
10. Alexander-Miller MA, Leggatt GR, Berzofsky JA. Selective expansion of high- or low-avidity cytotoxic T lymphocytes and efficacy for adoptive immunotherapy. Proc Natl Acad Sci U S A 1996;93:4102–7.
11. Baitsch L, Fuertes Marraco SA, Legat A, Meyer C, Speiser DE. The three main stumbling blocks for anticancer T cells. Trends Immunol 2012;33:364–72.
12. Morgan RA, Chinnasamy N, Abate-Daga D, Gros A, Robbins PF, Zheng Z, et al. Cancer regression and neurological toxicity following anti-MAGE-A3 TCR gene therapy. J Immunother 2013;36:133–51.
13. Hebeisen M, Rufer N, Oberle S, Speiser DE, Zehn D. Signaling mechanisms that balance antiviral, auto-reactive, and anti-tumor potential of low affinity T cells. J Clin Cell Immunol 2012;S12:003.
14. Speiser DE, Baumgaertner P, Voelter V, Devêvre E, Barbey C, Rufer N, et al. Unmodified self antigen triggers human CD8 T cells with stronger tumor reactivity than altered antigen. Proc Natl Acad Sci U S A 2008;105:3849–54.
15. Rizzuto GA, Merghoub T, Hirschhorn-Cymerman D, Liu C, Lesokhin AM, Sahawneh D, et al. Self-antigen-specific CD8+ T cell precursor frequency determines the quality of the antitumor immune response. J Exp Med 2009;206:849–66.

Hit Parade for Adoptive Cell Transfer Therapy: The Best T Cells for Superior Clinical Responses

Daniel E. Speiser

Cancer Discovery 2013;3:379-381.