Moving Toward the Ideal Autologous Adoptive T-cell Therapy for Cancer

Brian H. Ladle

Despite being one of the earliest immunotherapies to prove that the immune system can effectively recognize and eradicate cancer, autologous adoptive T-cell therapies remain largely limited to academic centers and research trials. The highly individualized protocols and the heterogeneous nature of the expanded T-cell products hinder effectiveness, commercial development, and regulatory approvals. The report by Li and colleagues details a novel method of generating cancer-specific autologous T cells from patients receiving anti-PD-1 checkpoint blockade immunotherapy. Their method achieved promising results in four initial patients treated in a pilot study. While further studies are required to characterize the autologous T-cell products generated and their effectiveness in larger cohorts of patients, the protocol they describe addresses several of the roadblocks that have prevented more widespread use of autologous adoptive T-cell therapy.

See related article by Li et al., p. 2184

In 1985, Rosenberg and colleagues reported one of the first successful adoptive cell therapies for human cancer. In their article, the authors state, "The major difficulty in the application of this approach to the treatment of human cancer has been the inability to generate sufficient numbers of autologous human cells with antitumor reactivity that could be used for systemic therapy" (1). While the field of immuno-oncology has achieved great advances in knowledge, technology, reagents, analysis, and expertise over the past 35 years, this statement still summarizes current efforts to improve adoptive T-cell therapy. While autologous adoptive cell therapy was one of the first immunotherapy approaches to put patients into complete remission, it has been passed by multiple other immuno-oncology approaches, including multiple immune checkpoint blockade antibodies, chimeric antigen receptor (CAR) engineered T cells, and the autologous cell cancer vaccine sipuleucel-T, in gaining FDA approval. The highly personalized approach required to generate large numbers of activated, polyclonal, autologous, tumor-specific T cells remains a high bar of which both the FDA and the pharmaceutical industry largely remain leery.

In this issue of *Cancer Research*, the article authored by Li and colleagues describes a novel iteration in generating an autologous adoptive T-cell product from the peripheral blood of patients with cancer (2). Their protocol lends itself to scalability and broad applicability, building on previous reports that the PD-1$^+$ fraction of T cells in peripheral blood is enriched with tumor-specific T cells (3, 4). Instead of identifying the PD-1$^+$ fraction by incubating peripheral blood cells with an anti-PD-1 antibody *in vitro*, they studied peripheral blood from patients receiving anti-PD-1 immunotherapy and identified the *in vivo*–bound PD-1$^+$ cells by labeling with a biotinylated anti-human IgG4 antibody that recognizes any of the three clinical anti-PD-1 antibodies used for treatment. Using a magnetic bead cell enrichment step, they were able to isolate a PD-1$^+$ peripheral blood population for subsequent *in vitro* expansion. The proportion of PD-1$^+$ peripheral blood cells from anti-PD-1–treated patients was both increased and more proliferative (by Ki-67 staining) when compared with paired blood samples obtained from the patients prior to anti-PD-1 treatment. PD-1$^+$ cells from 50 mL of peripheral blood were expanded using standard anti-CD3 stimulation with IL2 supplementation, and after 2 weeks yielded a sufficient number of T cells for clinical use as an adoptive cell therapy. The ingredients in this protocol of peripheral blood, a magnetic bead enrichment step, and expansion with anti-CD3/IL2 are feasible and potentially scalable for commercial production of clinical-grade products.

The crucial variable in the Li and colleagues' protocol (2) is the tumor specificity and tumor reactivity of the expanded T-cell product. Using T-cell receptor (TCR) sequencing methods, they compared paired unexpanded tumor-infiltrating lymphocytes (TIL) and the expanded PD-1$^+$ and PD-1$^-$ peripheral blood samples from 19 patients. They showed enrichment of the TIL clones in the PD-1$^+$ peripheral blood products compared with the expanded PD-1$^-$ populations. Although not unexpected, the vast majority of T cells expanded did not overlap with TIL clones. The question remains, then, as to whether TIL clones that are expanded from the peripheral blood will be of sufficient number and quality/avidity to yield a clinical response. Multiple reports delineate the variability in T-cell specificity in TIL populations from high to low avidity against tumor antigens and multiple viral-specific T cells (5, 6). Notably, Bobisse and colleagues compared neoepitope-specific T cells isolated from tumors and peripheral blood in patients with ovarian cancer and found that TILs were enriched in higher avidity clones (presumably more effective in tumor killing) compared with those isolated from peripheral blood (7). Thus, while peripheral blood does contain tumor-reactive T cells, there still may be a significant difference in the T-cell repertoire and potential antitumor potency between TILs and peripheral blood.

In this report, Li and colleagues (2) describe four patients (three with melanoma and one with clear-cell renal carcinoma) treated with expanded PD-1$^+$ peripheral blood products using their protocol. These patients had all progressed during treatment with anti-PD-1–blocking antibodies. Four cell doses (given every 3 weeks) were combined with continued anti-PD-1 antibody treatment and resulted in one complete response and two partial responses. Detailed TCR analysis of the T-cell products used in these patients to ascertain the number of TIL clones

Department of Oncology, The Johns Hopkins University School of Medicine, The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland.

Corresponding Author: Brian H. Ladle, Department of Oncology, The Johns Hopkins University School of Medicine, The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, 1650 Orleans Street, CRB1 Rm 209, Baltimore, MD 21287. Phone: 443-287-3534; E-mail: bladle@jhmi.edu

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expanded was not included. It would certainly be of great interest to determine whether the composition of the expanded T-cell product correlates with clinical responses. A clinical trial using this approach is ongoing by the investigators with a goal of accruing 30 patients.

The most novel aspect of the protocol described by Li and colleagues (2) is the use of peripheral blood from patients currently receiving anti-PD-1 antibody therapy and using a secondary antibody to label and enrich for cells bound to anti-PD-1 *in vivo*. This technique may add another level of enrichment for potent tumor-reactive clones to be present in the expanded population. Several reports highlight the expansion effect of anti-PD-1 therapy on tumor-reactive T cells, particularly on $CD8^+$ T cells (8). Importantly, the T cells that proliferate in response to anti-PD-1 have been shown to have more stem cell–like properties (e.g., TCF1 expression) in murine models and human samples (9, 10). The TCF1-expressing T cells have a less exhausted phenotype and proliferate, generating both potent $TCF1^-$ effector T cells and $TCF1^+$ memory precursor T cells. While not explored in the Li and colleagues' report, it could be hypothesized that anti-PD-1 therapy increases the number of this key $TCF1^+$ T-cell population in the $PD\text{-}1^+$ fraction in the peripheral blood, which is further expanded by the *ex vivo* stimulation. Interestingly, the expanded product with the Li and colleagues' protocol was predominantly $CD8^+$, with about 90% of these $CD8^+$ T cells expressing CD62L and CD45RA; markers were also reported to be expressed on $CD8^+PD\text{-}1^+TCF1^+$ T cells in melanoma-specific T cells (10).

In the current era of engineered T cells receiving FDA approval and pharmaceutical industry support, with multiple new targets being pursued, the future role of nonengineered autologous T-cell products may be questioned. CAR T-cell approaches yield potent, at times even life-threatening, immune activation against specific antigens and have provided a life-saving therapy to some patients with relapsed and refractory cancers. However, limits to this technology exist that could be overcome with effective nonengineered, autologous T-cell products. While approaches are being developed to target multiple tumor antigens, the immune pressure of CAR products targeting single antigens allows for the possibility of immune escape by antigen-negative disease. In contrast, expanded adoptive T-cell therapy can concurrently target a multitude of tumor antigens based on the immune repertoire present in the patient. With polyclonal T-cell populations targeting multiple antigens, the chance of any tumor clone losing expression of multiple antigens is diminished. Moreover, the gene engineering required to produce CAR products introduces significant expense, accompanying regulatory burden, and potential long-term risks. While gene editing technologies are constantly evolving, this step is eliminated when using nonengineered autologous adoptive T-cell therapy, thus bringing down costs and potentially making it available to more patients. Early CAR approaches have been most appealing to the pharmaceutical industry because of their clinical potency against an immune target shared by several cancers (e.g., CD19), thus increasing the potential indications for its use. One of the strengths of the autologous adoptive T-cell approach described by Li and colleagues is the potential generalizability to treat multiple cancers with the same platform.

In addition to the culture method used by Li and colleagues, other methods of improving expanded autologous T-cell products have been described and continue to be studied. Examples include different methods of *in vitro* stimulation/culture, selection of T cells with different cell surface markers to better define tumor-specific populations (e.g., CD39, CD103, and CD137), and manipulating populations of T cells to be transferred back to the patient (e.g., controlling the $CD4^+$ to $CD8^+$ T-cell ratio). All of these advances continue to push the field forward and closer to developing ideal autologous adoptive T-cell therapies for cancer.

Authors' Disclosures

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