HUMAN GENE THERAPY 25:779–784 (September 2014)

DOI: 10.1089/hum.2014.2533

Toward Synthetic Biology with Engineered T Cells: A Long Journey Just Begun

Carl H. June

Introduction: Gene Transfer Comes of Age

It is an honor to provide retrospective comments as we enter a remarkable era of gene transfer therapy that will ultimately provide cures for a number of presently incurable diseases. As in nearly all fields, the present advances have been made by extending the advances of our predecessors. Friedmann has chronicled the origins of the field for those new to this discipline (Friedmann and Roblin, 1972; Friedmann, 1992). From my own experiences, I suspect that there are three qualities in common with all of the investigators who have had a role in bringing gene transfer to the threshold of success. First, each investigator must possess a remarkable degree of stubbornness to persist in the face of repeated experimental failures; further, investigators in this field had to persist when the field was held in generally low regard by other fields of biomedical research. Second, all of the senior investigators in gene transfer technologies share the attribute of having an extraordinarily long attention span, which in most cases spans several decades of sustained effort. Finally, I suspect that all gene therapy pioneers have multidisciplinary interests, and are comfortable with multitasking, a quality that is necessary to translate basic science advances in this multidisciplinary field. Below are some of the experiences that my team has faced and lessons from the challenges that we have encountered.

In the Early Years: Learning to Embrace the Unexpected

I was raised in a family of engineers, and had always assumed that I too would one day have a career as an engineer. That all changed in 1971. I had just been accepted to attend Stanford University; however, at that time, the military draft was based on your birthdate, and my lottery number was low (50 out of 365), meaning that absent physical disqualification, I would be conscripted or ''drafted'' into military service. After considering the alternatives, an education at the Naval Academy in Annapolis seemed more desirable than the war in Vietnam. Luckily, the war had concluded by the time my collegiate studies were completed, and the Navy sent me to medical school in 1975. My first laboratory studies were in Roger D. Rossen's laboratory at Baylor College of Medicine, where I studied the immunology of rheumatoid factor, and later he arranged, along with James Woody, for me to spend a year of graduate studies in malaria immunopathology in the World Health Organization in Geneva (June *et al*., 1979a,b). By this time, I was ''hooked'' and had made the decision to become a physician scientist.

After completing residency, James Woody, a Navy Medical Officer and Head of the Navy's Experimental Transplant Unit organized my training in transplantation research, along with my colleague, Craig Thompson, at the Fred Hutchinson Research Center. There I became boarded in medical oncology and spent a year in the immunogenetics laboratory of John Hansen and Paul Martin. I learned more cellular immunology and, more importantly, John Hansen provided vision and Paul Martin taught me how to write an article. I became fascinated with T cell biology after witnessing the horrors of graft versus host disease: it was extraordinary how allogeneic T cells could destroy a patient (Thompson *et al*., 1984; June *et al*., 1985, 1986b), an unfortunately not uncommon event in the early days of bone marrow transplantation.

The Middle Years in Bethesda

At the completion of my postdoctoral studies in Seattle, I was assigned as a research medical officer to the Tissue Bank at the Naval Medical Research Institute in 1986. It was a wonderful opportunity, as I was given an independent laboratory with only 2 years of laboratory training. In 1994, I served as department head for the Immune Cell Biology Program (Fig. 1). While the Naval laboratory was small, it mattered little because the entire National Institutes of Health campus was literally just across the street. Funding was relatively easy to obtain and largely unrestricted so that long-term projects could be undertaken. I benefitted greatly from a long-term collaboration with Craig Thompson, whose laboratory was adjacent to mine at the Fred Hutchinson Cancer Research Center, and from colleagues at the National Cancer Institute.

For the first decade of my career, the laboratory studies were grounded in basic sciences, where my laboratory was among the earliest to study the biochemistry of signal transduction in human T cells. These studies were initially based on the first single-cell assay to measure calcium flux, an assay that I developed with Peter Rabinovitch at the University of

Abramson Cancer Center; Abramson Family Cancer Research Institute; and Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104-5156.

FIG. 1. Immune Cell Biology Program in 1993: Bruce Levine, top row, right; the author, Carl H. June, third row, left; other scientists were Kelvin Lee, Jeffrey N. Siegel, Ryo Abe, Thomas Davis, Steven Kessler, Peter Perrin, and David Harlan.

Washington (Rabinovitch *et al*., 1986; June and Rabinovitch, 1990). My initial studies were based on the clinical observation that cyclosporine was a wonderful noncytotoxic drug to block T cell activation *in vitro*, yet it was disappointing in the clinic (Storb *et al*., 1986). Paul Martin, John Hansen, and Shu Man Fu had just discovered the agonistic properties of a monoclonal antibody termed ''clone 9.3.'' The central hypothesis of my laboratory studies for the next 10 years was that signaling of T cells through the 9.3 receptor, later named CD28, explained costimulation and the cyclosporine conundrum (Martin *et al*., 1986; Ledbetter *et al*., 1987; June *et al*., 1989, 1990; Thompson *et al*., 1989).

Costimulation was the modern term for a concept originally introduced by Bretscher and Cohn that lymphocytes required two signals for full activation: the first signal was antigen specific and second signal delivered by cell–cell contact was antigen nonspecific (Bretscher and Cohn, 1970). While simplistic in retrospect, the strength of this hypothesis was that it was grounded in an important clinical observation. It soon became evident to me that many findings with transformed cell lines could not be repeated when primary cells were tested, because of differences in costimulation (June *et al*., 1986a) or contamination (Nelson-Rees *et al*., 1981). This had a profound influence over my career, with me always insisting in lab meetings that studies be done with primary cells, even though more difficult than the use of leukemic cell lines.

In retrospect, the best parts of these middle years were the early transition to independence and the stable laboratory funding, which were features of medical research in the 1980s. I am a firm believer that a combined degree is not necessary. Related to this, especially in the present era, is the conviction that many years of productive science are wasted by physician scientists who must serve long periods of time as indentured servants in the labs of senior faculty. In my experience, what is necessary is that any prospective student be exposed to laboratory and clinical medicine so that an informed decision can be made as to whether the primary focus is in the clinic or in the laboratory. I completed a chief residency and attended on the medical service for 1 month of every year until 1996, but the rest of the year was entirely devoted to projects in the laboratory. My own experience mirrors the theory espoused by Malcolm Gladwell that one needs an intensive 10,000 hr of training to enter a field (Gladwell, 2008). In those years, one could assimilate that body of knowledge in 2 years. I was fortunate to garner my 10,000 hr of training in medical oncology and in immunology while completing medical studies. Parenthetically, I was never permitted by the Navy to spend dedicated time for doctoral studies in immunology.

In contrast, the most unfortunate aspect of the years in Bethesda was that while I was trained as a specialist in leukemia, I was unable to conduct research in cancer with internal funds from the Department of Defense. Therefore, my interests in immunology were channeled into immunopathology, as the Navy could fund research in infectious diseases, and in my case, malaria and HIV/AIDS. This was a proverbial blessing in disguise, as I was forced to learn about virology, leading to the discovery of new viruses, human herpes virus 6 and 7 (Frenkel *et al*., 1990a,b), and to learn insights with HIV-1 that later aided in studies for cancer patients (Linette *et al*., 1988). The lesson from these experiences is that it is good to have laboratory experience in more than one field or, at the least, to have close collaborators from other fields, as illustrated by my later collaboration with Frederic Bushman at the University of Pennsylvania (Penn) (Levine *et al*., 2006; Wang *et al*., 2009).

The cross-cutting benefits to my laboratory having worked for a decade in infectious diseases before beginning cancer studies in earnest cannot be underestimated. A final benefit of my initial career in research within the military was that I was able to observe the large-scale vaccine efforts that the Army and Navy infrastructure had assembled, eventually leading to the first success ever with a therapeutic vaccine for HIV (Rerks-Ngarm *et al*., 2009). This experience proved essential for later studies at Penn, where my team became a virtual biotechnology company embedded in an academic institution: a process that was essential for the successful translation of gene transfer technology. Valued mentors in the Army and Navy vaccine efforts were Michael Strong, Robert Hartzman, Nelson Michael, Donald Burke, Edmund Tramont, and Stephen Hoffman.

Chimeric Antigen Receptors for HIV/AIDS

After establishing my laboratory in Bethesda, my first graduate student was Gerald Linette, a combined degree student who was interested in the immunobiology of HIV/AIDS. His initial project was to culture T cells from patients with HIV. My laboratory had been using CD28 agonistic stimulation to propagate T cells (June *et al*., 1987, 1989), and Gerry's first experiments were to extend this by growing T cells from patients with late-stage HIV infection. He found that the signals through latently infected cells were quite different from uninfected T cells (Linette *et al*., 1988).

In 1992, Bruce Levine began postdoctoral studies in my laboratory, and his project was to pick up Gerry's project by further studying the effects of HIV on T-cell signal transduction and to develop a T-cell culture system for HIV. His first goal was to produce viral stocks from the HIV/AIDS patients so that we could then later use the virus recovered from these patients as reagents. However, when he harvested the supernatants from the T cell cultures, he was unable to find any virus in the cultures. I was incredulous and actually became angry, as how could anyone fail to

recover virus from patients with late-stage HIV/AIDS? Recall that this was long before potent antiretroviral therapy was available, and essentially all patients had high levels of viremia. So I went down to the lab and repeated Bruce's experiment—to my chagrin I also flopped by not recovering virus from the patient-derived T cell cultures. We later learned that this so-called CD28 antiviral effect was because of the downregulation of the CCR5, the HIV-1 coreceptor on CD4 cells (Riley *et al*., 1997). This happy accident eventually resulted in two more articles in *Science* (Levine *et al*., 1996; Carroll *et al*., 1997), and launched our initial studies toward adoptive transfer of T cells for patients with HIV/AIDS (June *et al*., 1993). The lessons learned from this experiment were that the most interesting results are those that are unexpected and to not resist changing the course of research as new opportunities arise.

To culture T cells from patients with HIV infection, Levine developed beads with immobilized antibodies to the T cell receptor and CD28 to develop a system that for the first time provided robust growth of T cells from patients with HIV or cancer, and it also was scale independent: he could culture T cells in volumes that varied by a million-fold from 96-well microplates to liter-sized culture flasks (Levine *et al*., 1998). Bruce used this new culture system to conduct fundamental studies on the growth potential of human T cells (Weng *et al*., 1995, 1996; Levine *et al*., 1997; Palmer *et al*., 1997; Liu *et al*., 1999). Levine developed a good manufacturing practice (GMP)-compliant cell culture system that enabled the first trials with the adoptive transfer of CD4 cells in patients with late-stage HIV/AIDS. We found that CD4 counts improved, as did immune function in the patients (Levine *et al*., 2002; Bernstein *et al*., 2004).

As a result of our initial success with adoptive T cell transfers in AIDS patients, Dale Ando and Kristen Hege at Cell Genesys contacted us with a proposition to collaborate with a chimeric antigen receptor (CAR) for HIV/AIDS patients. This was intriguing, as I'd never heard of the concept of CARs that had first been made independently in three laboratories in 1991 (Irving and Weiss, 1991; Letourneur and Klausner, 1991; Romeo and Seed, 1991). Bryan Irving and Arthur Weiss at the University of California had constructed CD8:zeta and CD4:zeta CARs to study signal transduction, and they realized that the CD4:zeta CAR might have a practical application to retarget T cells to HIV-infected T cells. Zelig Eshhar and colleagues later improved the utility of CARs by grafting antibody domains to the signaling domains, extending the library of targets for CAR T cells (Eshhar and others 1993). In 1997 we used Bruce Levine's cell culture system to conduct phase I trials with the CD3:zeta ''first-generation'' CAR for HIV (Mitsuyasu *et al*., 2000; Walker *et al*., 2000). Happily, the trials were successful in that they demonstrated safety and feasibility and improved immune function in the patients with HIV.

With Stephen Deeks and Cell Genesys, we conducted the first randomized phase 2 trial with gene-modified T cells in patients with HIV, and while this trial again demonstrated safety, there were only modest antiviral effects with CD4:zeta CAR T cells (Deeks *et al*., 2002). However, when we analyzed the cells from these patients a decade later, we were surprised to discover that the vast majority of patients had retained engraftment for a decade or more following infusion of these gene-modified CAR T cells. The CAR T cells were present at high frequencies with stable levels, and had a projected half-life that exceeded 17 years in these patients (Scholler *et al*., 2012).

In addition to our collaboration with Cell Genesys, we also established successful interactions with VIRxSYS, Sangamo, and Adaptimmune that enabled additional first-in-human trials with gene-modified T cells. With VIRxSYS, we conducted the first trial using lentiviral-engineered T cells testing the VRX496 antisense envelope construct, showing safety and some immunogenicity directed to VSV-G that was used to pseudotype the lentiviral vector (Levine *et al*., 2006). With Sangamo, we used zinc finger nucleases to disrupt CCR5, rendering the cells resistant to HIV infection in a mouse model (Perez *et al*., 2008) and later in a phase I trial in humans testing gene-edited T cells (Tebas *et al*., 2014). These collaborations were noted by Don Kohn to be a model for academic–biotechnology partnerships (Kohn, 2007), and ultimately led to the alliance between Penn and Novartis in 2012. In addition, these interactions were beneficial in that they fostered the development of productive interactions with the FDA in the area of gene-modified T cells. This proved beneficial, as one of our trials with Adaptimmune uncovered an off-target effect with T cells modified to express a T cell receptor for MAGE A3 that resulted in serious toxicity (Cameron *et al*., 2013; Linette *et al*., 2013).

CARs Move to Philadelphia

After retiring from the Navy in 1996, at the conclusion of my 12-year service obligation incurred from the training support that I had received in college and medical school, I considered several academic positions. However, I was extremely fortunate to be recruited to Penn by Jim Wilson and Craig Thompson. In Philadelphia, as opposed to other institutions such as free-standing cancer centers, I was able to continue research in immunotherapy for HIV, while beginning adoptive immunotherapy experiments in cancer patients. I was fortunate to recruit many of the members from the original immune reconstitution team in Bethesda to move to Penn (Fig. 2). Jim Riley, Bruce Levine, Katia Schlienger, and Richard Carroll were appointed as assistant professors in the research track at Penn. Bruce Levine, along with Julio Cotte, who also relocated to Philadelphia, established the first GMP-compliant cell-manufacturing facility at Penn. Jim Riley

FIG. 2. Immune Cell Biology Group in 1997. This was the team for HIV studies: Richard Carroll and Jim Riley to the author's right in the back row, and to the author's left, Bruce Levine and Katia Schlienger. Other scientists were Dan St. Louis, Owen Wieslow, Wendy Bernstein, and Sumesh Kaushal.

established a Human Immunology Core. Bruce and Jim have since become independent faculty and have risen to the rank of full professor, while Katia has had a successful career in the pharmaceutical industry. Unfortunately, Richard Carroll, who became my laboratory manager, died from pancreatic cancer in 2010 at the age 52.

We first tested adoptively transferred T cells in cancer patients with chronic myelogenous leukemia, with Aaron Rapoport at the University of Maryland. We also evaluated this approach in patients with advanced lymphoma, in collaboration with David Liebowitz at the University of Chicago and with Gina Laport at Penn (Laport *et al*., 2003) using the same cell-manufacturing approach that Bruce had developed for HIV. The initial patients with treated chronic myelogenous leukemia were successful, in that their T cell counts increased; one patient of the four initially reported remains disease-free in a molecular remission now more than 15 years after infusion of her T cells (Rapoport *et al*., 2004).

At Penn the basic science part of my lab continued working on signal transduction. My first graduate student at Penn was Marcela Maus, who studied the role of artificial antigen presenting cells and the role of the 4-1BB costimulatory molecule in addition to CD28 (Maus *et al*., 2002, 2004). Marcela and, later, Megan Suhoski, a graduate student, found that 4-1BB promoted human CD8 cell growth and could augment CD8 T cell growth and function beyond that provided by the CD28 signal (Suhoski *et al*., 2007). Carmine Carpenito and Michael Milone were postdocs in the lab working on CARs based on our initial work with CD4:zeta cars for HIV. Their projects were to develop CARs for leukemia, and for this we selected CD19 as did others in the field (Roessig *et al*., 2002; Brentjens *et al*., 2003; Cooper *et al*., 2003), and for solid tumors we tested mesothelin as a target. Carpenito and Milone, along with Jim Riley, adapted the CD4:zeta CAR into a ''second-generation'' CAR. In the process, they changed from retroviral vector to a third-generation lentiviral vector developed by Dull and colleagues at Cell Genesys (Dull *et al*., 1998). They further demonstrated that EF-1alpha was a superior internal promoter for constitutive expression in human T cells, and chose 4-1BB as an improved costimulatory domain compared with CD28 (Carpenito *et al*., 2009; Milone *et al*., 2009).

Milone and Carpenito had the second-generation CD19:4-1BB:zeta CAR working in preclinical studies in 2004. David Porter, the principal investigator for our trials in leukemia, dubbed the CAR ''CART19'' for chimeric antigen receptor in T cells redirected to CD19. However, it was difficult to get funding for clinical trials, as we were not successful in obtaining funding from the NIH for a pilot clinical trial with CART19. Fortunately, Edward and Barbara Netter, along with Savio Woo, had formed the Alliance for Cancer Gene Therapy, and they, along with the Leukemia and Lymphoma Society, provided funding for our pilot trials for CART19 (clinicaltrials.gov NCT01029366). When we finally infused our first patients in 2010, the results were breathtaking. Two of three patients with end-stage advanced leukemia achieved a complete remission that is lasting more than 4 years since infusion; the third patient had a partial response (Kalos *et al*., 2011; Porter *et al*., 2011). We have now treated more than a hundred patients with CART19 and continue to have striking responses in both acute and chronic leukemia (Grupp *et al*., 2013; Maude *et al*., 2014). We were all enormously proud when the FDA granted this therapy ''Breakthrough Designation'' status in July 2014. To our knowledge, this is the first time any academic center has received Breakthrough Designation by the U.S. FDA. The field of CAR therapies has grown enormously and numerous academic centers have now shown potent activity with CAR T cells for leukemia (Maus *et al*., 2014).

Lessons: Follow Your Passions

Over the years we've had a number of lessons from our experiences in this journey to develop cell-based therapies for HIV and cancer. First, your best publications probably won't end up in top-tier journals. If they're too far ahead of their time, they won't be appreciated until later. In Bruce Levine's case, his observation of the self-renewing stem cell-like properties of T cells discovered in the 1990s (Levine *et al*., 1997) was not widely appreciated until recently (Gattinoni *et al*., 2011). Second, accidents can be good: embrace the unexpected results and follow up on these as they are often times more scientifically interesting than predictable responses from less imaginative experiments. Third, cross-cutting fields have low-hanging fruits. By working in HIV and cancer immunotherapy in parallel, we had many benefits, such as the first use of lentivirus in HIV that was then applied to engineered cancer T cells, leading to improved persistence and expression. Fourth, a nonstandard genealogy in training can be an advantage; my initial education in the Navy was far from the usual pathway to academia, yet provided me with certain advantages when I entered academia. It is notable that others trained in the Navy later found advantages in academia and biotechnology, such as Craig Thompson, Judah Folkman, and Craig Venter.

Acknowledgments

I would like to think my many colleagues and mentors for inspiration over the years: during training, Roger D. Rossen and Robert R. Rich at Baylor College of Medicine, Luc Perrin at the World Health Association, John Hansen and Paul Martin at the Fred Hutchinson Cancer Center, and Al Singer and Richard Hodes at the National Cancer Institute. At the Naval Medical Research Institute, many thanks to mentors Jim Woody, Kenneth Sell, Robert Hartzman, and D. Michael Strong and many academic colleagues, including Kelvin Lee, Thomas Davis, Steven Kessler, Ryo Abe, and David Harlan, and especially to the team that moved me to Penn, including Jim Riley, Richard Carroll, Katia Schlienger, and Bruce Levine. My long-term clinical colleagues have been invaluable in clinical translation, including Wendy Bernstein, Naomi Aronson, Pablo Tebas, David Porter, Stephen Schuster, and Stephan Grupp. At Penn I have had numerous interactions with colleagues in HIV who have helped provide insights into our cancer therapies, including Frederic Bushman, Robert Doms, and James Hoxie. I am indebted to Anne Chew, Gabriela Plesa, Dana Hammill, Jennifer Leight, and others in the Translational Research Program at Penn and to Jim Wilson and Bill Kelley, who are true pioneers in the science and the field of gene transfer and who provided the infrastructure that has allowed my group to be successful. Finally, I thank my wife, Lisa, and our five children for their support.

This work is dedicated to the memory of Cynthia June, Chris Broeren, and Richard Carroll, who had untimely deaths from cancer.

Author Disclosure Statement

C.H.J. is an advisor to Novartis, Celldex Therapeutics, Globimmune, and Immune Design. In addition, he is an inventor on patents licensed to Novartis and receives grant support from Novartis.

References

Bernstein, W.B., Cox, J.H., Aronson, N.E., *et al.* (2004). Immune reconstitution following autologous transfers of CD3/CD28 stimulated CD4(+) T cells to HIV-infected persons. Clin. Immunol. 111, 262–274.

Brentjens, R.J., Latouche, J.B., Santos, E., *et al.* (2003). Eradication of systemic B-cell tumors by genetically targeted human T lymphocytes co-stimulated by CD80 and interleukin-15. Nat. Med. 9, 279–286.

Bretscher, P., and Cohn, M. (1970). A theory of self-nonself discrimination. Science 169, 1042–1049.

Cameron, B.J., Gerry, A.B., Dukes, J., *et al.* (2013). Identification of a titin-derived HLA-A1–presented peptide as a cross-reactive target for engineered MAGE A3–directed T cells. Sci. Transl. Med. 5, 197ra103.

Carpenito, C., Milone, M.C., Hassan, R., *et al.* (2009). Control of large, established tumor xenografts with genetically retargeted human T cells containing CD28 and CD137 domains. Proc. Natl. Acad. Sci. USA 106, 3360–3365.

Carroll, R.G., Riley, J.L., Levine, B.L., *et al.* (1997). Differential regulation of HIV-1 fusion cofactor expression by CD28 costimulation of CD4+ T cells. Science 276, 273–276.

Cooper, L.J., Topp, M.S., Serrano, L.M., *et al.* (2003). T-cell clones can be rendered specific for CD19: toward the selective augmentation of the graft-versus-B-lineage leukemia effect. Blood 101, 1637–1644.

Deeks, S.G., Wagner, B., Anton, P.A., *et al.* (2002). A phase II randomized study of HIV-specific T-cell gene therapy in subjects with undetectable plasma viremia on combination anti-retroviral therapy. Mol. Ther. 5, 788–797.

Dull, T., Zufferey, R., Kelly, M., *et al.* (1998). A third-generation lentivirus vector with a conditional packaging system. J. Virol. 72, 8463–8471.

Eshhar Z, Waks T, Gross G, Schindler DG. (1993). Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors. Proc. Natl. Acad. Sci. U S A 90, 720–724.

Frenkel, N., Schirmer, E.C., Katsafanas, G., and June, C.H. (1990a). T-cell activation is required for efficient replication of human herpesvirus 6. J. Virol. 64, 4598–4602.

Frenkel, N., Schirmer, E.C., Wyatt, L.S., *et al.* (1990b). Isolation of a new herpesvirus from human CD4+ T cells. Proc. Natl. Acad. Sci. USA 87, 748–752.

Friedmann, T. (1992). A brief history of gene therapy. Nat. Genet. 2, 93–98.

Friedmann, T., and Roblin, R. (1972). Gene therapy for human genetic disease? Science 175, 949–955.

Gattinoni, L., Lugli, E., Ji, Y., *et al.* (2011). A human memory T cell subset with stem cell-like properties. Nat. Med. 17, 1290–1297.

Gladwell, M. (2008). *Outliers: The Story of Success*. (Little, Brown and Company, New York, NY).

Grupp, S.A., Kalos, M., Barrett, D., *et al.* (2013). Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. N. Engl. J. Med. 368, 1509–1518.

Irving, B.A., and Weiss, A. (1991). The cytoplasmic domain of the T cell receptor zeta chain is sufficient to couple to receptor-associated signal transduction pathways. Cell 64, 891–901.

June, C.H., and Rabinovitch, P.S. (1990). Flow cytometric measurement of intracellular ionized calcium in single cells with indo-1 and fluo-3. In *Methods in Cell Biology*. L. Wilson, ed. (Academic Press, Inc., San Diego, CA) *pp.* 37–58.

June, C.H., Contreras, C.E., Perrin, L.H., and Lambert, P.H. (1979a). Improved detection of immune complexes in human and mouse serum using a microassay adaptation of the C1q binding test. J. Immunol. Methods 31, 23–29.

June, C.H., Contreras, C.E., Perrin, L.H., *et al.* (1979b). Circulating and tissue-bound immune complex formation in murine malaria. J. Immunol. 122, 2154–2161.

June, C.H., Thompson, C.B., Kennedy, M.S., *et al.* (1985). Profound hypomagnesemia and renal magnesium wasting associated with the use of cyclosporine for marrow transplantation. Transplantation 39, 620–624.

June, C.H., Ledbetter, J.A., Rabinovitch, P.S., *et al.* (1986a). Distinct patterns of transmembrane calcium flux and intracellular calcium mobilization after differentiation antigen cluster 2 (E rosette receptor) or 3 (T3) stimulation of human lymphocytes. J. Clin. Invest. 77, 1224–1232.

June, C.H., Thompson, C.B., Kennedy, M.S., *et al.* (1986b). Correlation of hypomagnesemia with the onset of cyclosporine- associated hypertension in marrow transplant patients. Transplantation 41, 47–51.

June, C.H., Ledbetter, J.A., Gillespie, M.M., *et al.* (1987). T-cell proliferation involving the CD28 pathway is associated with cyclosporine-resistant interleukin 2 gene expression. Mol. Cell Biol. 7, 4472–4481.

June, C.H., Ledbetter, J.A., Lindsten, T., and Thompson, C.B. (1989). Evidence for the involvement of three distinct signals in the induction of IL-2 gene expression in human T lymphocytes. J. Immunol. 143, 153–161.

June, C.H., Ledbetter, J.A., Linsley, P.S., and Thompson, C.B. (1990). Role of the CD28 receptor in T-cell activation. Immunol. Today 11, 211–216.

June, C.H., Linette, G.P., Pierce, P.F., *et al.* (1993). Potential clinical applications of signal transduction measurements in marrow transplantation and HIV-1 infection. Ann. N.Y. Acad. Sci. 677, 225–232.

Kalos, M., Levine, B.L., Porter, D.L., *et al.* (2011). T cells expressing chimeric receptors establish memory and potent antitumor effects in patients with advanced leukemia. Sci. Transl. Med. 3, 95ra73.

Kohn, D.B. (2007). Lentiviral vectors ready for prime-time. Nat. Biotechnol. 25, 65–66.

Laport, G.G., Levine, B.L., Stadtmauer, E.A., *et al.* (2003). Adoptive transfer of costimulated T cells induces lymphocytosis in patients with relapsed/refractory non-Hodgkin lymphoma following CD34+-selected hematopoietic cell transplantation. Blood 102, 2004–2013.

Ledbetter, J.A., Gentry, L.E., June, C.H., *et al.* (1987). Stimulation of T cells through the CD3/T-cell receptor complex: role of cytoplasmic calcium, protein kinase C translocation, and phosphorylation of pp60 c-src in the activation pathway. Mol. Cell Biol. 7, 650–656.

Letourneur, F., and Klausner, R.D. (1991). T-cell and basophil activation through the cytoplasmic tail of T-cell-receptor zeta family proteins. Proc. Natl. Acad. Sci. USA 88, 8905–8909.

Levine, B.L., Mosca, J., Riley, J.L., *et al.* (1996). Antiviral effect and ex vivo CD4+ T cell proliferation in HIV-positive patients as a result of CD28 costimulation. Science 272, 1939–1943.

Levine, B.L., Bernstein, W., Craighead, N., *et al.* (1997). Effects of CD28 costimulation on long term proliferation of

CD4+ T cells in the absence of exogenous feeder cells. J. Immunol. 159, 5921–5930.
Levine, B.L., Cotte, J., Small, C.C., *et al.* (1998). Large scale production of CD4+ T cells from HIV-infected donors following CD3/CD28 stimulation. J. Hematother. 7, 437–448.
Levine, B.L., Bernstein, W.B., Aronson, N.E., *et al.* (2002). Adoptive transfer of costimulated CD4+ T cells induces expansion of peripheral T cells and decreased CCR5 expression in HIV infection. Nat. Med. 8, 47–53.
Levine, B.L., Humeau, L.M., Boyer, J., *et al.* (2006). Gene transfer in humans using a conditionally replicating lentiviral vector. Proc. Natl. Acad. Sci. USA 103, 17372–17377.
Linette, G.P., Hartzman, R.J., Ledbetter, J.A., and June, C.H. (1988). HIV-1-infected T cells show a selective signaling defect after perturbation of CD3/antigen receptor. Science 241, 573–576.
Linette, G.P., Stadtmauer, E.A., Maus, M.V., *et al.* (2013). Cardiovascular toxicity and titin cross-reactivity of affinity enhanced T cells in myeloma and melanoma. Blood 122, 863–871.
Liu, K., Schoonmaker, M.M., Levine, B.L., *et al.* (1999). Constitutive and regulated expression of telomerase reverse transcriptase (hTERT) in human lymphocytes. Proc. Natl. Acad. Sci. USA 96, 5147–5152.
Martin, P.J., Ledbetter, J.A., Morishita, Y., *et al.* (1986). A 44 kilodalton cell surface homodimer regulates interleukin 2 production by activated human T lymphocytes. J. Immunol. 136, 3282–3287.
Maude, S., Frey, N., Shaw, P., *et al.* (2014). Sustained remissions with chimeric antigen receptor T cells for leukemia. N. Engl. J. Med. In press.
Maus, M.V., Thomas, A.K., Leonard, D.G., *et al.* (2002). *Ex vivo* expansion of polyclonal and antigen-specific cytotoxic T lymphocytes by artificial APCs expressing ligands for the T-cell receptor, CD28 and 4-1BB. Nat. Biotechnol. 20, 143–148.
Maus, M.V., Kovacs, B., Kwok, W.W., *et al.* (2004). Extensive replicative capacity of human central memory T cells. J. Immunol. 172, 6675–6683.
Maus, M.V., Grupp, S.A., Porter, D.L., and June, C.H. (2014). Antibody modified T cells: CARs take the front seat for hematologic malignancies. Blood 123, 2625–2635.
Milone, M.C., Fish, J.D., Carpenito, C., *et al.* (2009). Chimeric receptors containing CD137 signal transduction domains mediate enhanced survival of T cells and increased antileukemic efficacy in vivo. Mol. Ther. 17, 1453–1464.
Mitsuyasu, R.T., Anton, P., Deeks, S.G., *et al.* (2000). Prolonged survival and tissue trafficking following adoptive transfer of CD4 z gene-modified autologous CD4+ and CD8+ T cells in HIV-infected subjects. Blood 96, 785–793.
Nelson-Rees, W.A., Daniels, D.W., and Flandermeyer, R.R. (1981). Cross-contamination of cells in culture. Science 212, 446–452.
Palmer, L.D., Weng, N.P., Levine, B.L., *et al.* (1997). Telomere length, telomerase activity, and replicative potential in HIV infection: analysis of CD4+ and CD8+ T cells from HIV-discordant monozygotic twins. J. Exp. Med. 185, 1381–1386.
Perez, E.E., Wang, J., Miller, J.C., *et al.* (2008). Establishment of HIV-1 resistance in CD4+ T cells by genome editing using zinc-finger nucleases. Nat. Biotechnol. 26, 808–816.
Porter, D.L., Levine, B.L., Kalos, M., *et al.* (2011). Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. N. Engl. J. Med. 365, 725–733.
Rabinovitch, P.S., June, C.H., Grossmann, A., and Ledbetter, J.A. (1986). Heterogeneity among T cells in intracellular free calcium responses after mitogen stimulation with PHA or anti-CD3. Simultaneous use of indo-1 and immunofluorescence with flow cytometry. J. Immunol. 137, 952–961.
Rapoport, A.P., Levine, B.L., Badros, A., *et al.* (2004). Molecular remission of CML after autotransplantation followed by adoptive transfer of costimulated autologous T cells. Bone Marrow Transplant. 33, 53–60.
Rerks-Ngarm, S., Pitisuttithum, P., Nitayaphan, S., *et al.* (2009). Vaccination with ALVAC and AIDSVAX to Prevent HIV-1 Infection in Thailand. N. Engl. J. Med. 361, 2209–2220.
Riley, J.L., Carroll, R.G., Levine, B.L., *et al.* (1997). Intrinsic resistance to T cell infection with HIV type 1 induced by CD28 costimulation. J. Immunol. 158, 5545–5553.
Roessig, C., Scherer, S.P., Baer, A., *et al.* (2002). Targeting CD19 with genetically modified EBV-specific human T lymphocytes. Ann. Hematol. 81 Suppl 2, S42–S43.
Romeo, C., and Seed, B. (1991). Cellular immunity to HIV activated by CD4 fused to T cell or Fc receptor polypeptides. Cell 64, 1037–1046.
Scholler, J., Brady, T., Binder-Scholl, G., *et al.* (2012). Decade-long safety and function of retroviral-modified chimeric antigen receptor T cells. Sci. Transl. Med. 4, 132Ra53.
Storb, R., Deeg, H.J., Whitehead, J., *et al.* (1986). Methotrexate and cyclosporine compared with cyclosporine alone for prophylaxis of acute graft versus host disease after marrow transplantation for leukemia. N. Engl. J. Med. 314, 729–735.
Suhoski, M.M., Golovina, T.N., Aqui, N.A., *et al.* (2007). Engineering artificial antigen-presenting cells to express a diverse array of co-stimulatory molecules. Mol. Ther. 15, 981–988.
Tebas, P., Stein, D., Tang, W., *et al.* (2014). Gene editing of *CCR5* in autologous CD4 T-cells of persons infected with HIV. N. Engl. J. Med. 370, 901–910.
Thompson, C.B., June, C.H., Sullivan, K.M., and Thomas, E.D. (1984). Association between cyclosporin neurotoxicity and hypomagnesaemia. Lancet 2, 1116–1120.
Thompson, C.B., Lindsten, T., Ledbetter, J.A., *et al.* (1989). CD28 activation pathway regulates the production of multiple T- cell-derived lymphokines/cytokines. Proc. Natl. Acad. Sci. USA 86, 1333–1337.
Walker, R.E., Bechtel, C.M., Natarajan, V., *et al.* (2000). Long-term *in vivo* survival of receptor-modified syngeneic T cells in patients with human immunodeficiency virus infection. Blood 96, 467–474.
Wang, G.P., Levine, B.L., Binder, G.K., *et al.* (2009). Analysis of lentiviral vector integration in HIV+ study subjects receiving autologous infusions of gene modified CD4+ T cells. Mol. Ther. 17, 844–850.
Weng, N.P., Levine, B.L., June, C.H., and Hodes, R.J. (1995). Human naive and memory T lymphocytes differ in telomeric length and replicative potential. Proc. Natl. Acad. Sci. USA 92, 11091–11094.
Weng, N.P., Levine, B.L., June, C.H., and Hodes, R.J. (1996). Regulated expression of telomerase activity in human T lymphocyte development and activation. J. Exp. Med. 183, 2471–2480.

Address correspondence to:
Dr. Carl H. June
Smilow Center for Translational Research
3400 Civic Center Boulevard, Building 421
8th Floor, Room 123
Philadelphia, PA 19104-5156

E-mail: cjune@exchange.upenn.edu

Received for publication September 2, 2014;
accepted after revision September 3, 2014.