Exploring synthetic immunity: From boutique to global

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With a father who was a research scientist and a mother who was a nurse, one would think a career in biomedical research may have been pre-ordained. However, growing up in the Apollo era, I had visions of NASA astronauts and astronomers on my mind. My early introduction to the research laboratory started in 1978 when I worked summers at the Wistar Institute while still in high school. It seemed a natural fit and I continued at Wistar while an undergraduate at the University of Pennsylvania and learned cell culture with WI-38 fibroblasts in the laboratory of Vincent Cristofalo. After graduating from Penn with a Bachelor’s in Biology, I worked as a technician in the Division of Infectious Diseases at the Children’s Hospital of Philadelphia (CHOP) with Stuart Starr and Stanley Plotkin. There, I was examining immune responses following Varicella vaccination when Merck was conducting clinical trials of the vaccine and the Measles Mumps Rubella Varicella combination vaccine. The lab was on the 8th floor and often I was in the elevator when a child in a wheelchair or a stretcher was brought onto the elevator. Although I knew I wanted to go to graduate school, the work I was doing in the lab and the environment at CHOP kindled my interest in more applied or translational medical research. I received my Ph.D. in Immunology and Infectious Diseases from the Johns Hopkins University. At Hopkins, I chose for my thesis work the laboratory of Allan Hess in the Bone Marrow Transplantation (BMT) Program of the Oncology Center. Although my thesis work examined the activation of Protein Kinase C in T lymphocytes by phorbol ester and bryostatin, I consciously chose a laboratory in the BMT program because I was fascinated by the idea of ex vivo cellular engineering.

I accepted a post-doctoral fellowship with Carl June at the Naval Medical Research Institute in Bethesda, MD having read several of his papers on T cell activation and costimulation while preparing my thesis. As a post-doctoral fellow, I studied T lymphocyte activation and growth. Based on the newly elucidated requirements of T cell signaling including a primary signal and a costimulatory signal, we developed a robust T cell culture system via anti-CD3 and anti-CD28 antibodies immobilized on beads. This vastly improved on existing methods and yielded large numbers of highly functional polyclonal T cells. I was then using this system to test the feasibility of in vitro expansion of T cells from HIV+ subjects. With anti-retroviral drug supplemented media, HIV subject T cells could be expanded. As a control, Carl June asked me to use media that was not supplemented with anti-retroviral drugs. Working with my colleagues Richard Carroll and Jim Riley, much to our surprise, no HIV replication was observed when T cells were expanded from multiple HIV+ subjects in the absence of anti-retroviral drugs. We were able to elucidate an anti-HIV effect of immobilized CD28 stimulation manifested by the downregulation of the newly discovered HIV co-receptor CCR5 and by upregulated secretion of the β chemokines, natural ligands of CCR5. This research led to two papers in Science, a paper in the Journal of Immunology, and my first U.S. Patent.

One of the outcomes of this work was that Carl June had also asked me to direct a laboratory that would support a clinical trial in adoptive immunotherapy with ex vivo expanded CD4+ T cells. Through this laboratory, which later became the Clinical Cell and Vaccine Production Facility, I was responsible for development of the large-scale methods for preparation of HIV-1 infected subject cells for an initial Phase I adoptive immunotherapy trial in HIV at the National Naval Medical Center and the Walter Reed Army Medical Center that infused the first subjects in 1996. The methods developed for this trial have become the foundation for more than 60 subsequent clinical trials in HIV and various cancers. In 1997 when we were in the midst of this first clinical trial, I read Stephen Hall’s book “A Commotion in the Blood: Life, Death, and the Immune System.” Chronicling the first century of immunotherapy, the final chapters were devoted to adoptive cellular immunotherapy. I was captivated, took notes in the margins, and set my sights on the second century of immunotherapy.

Chimeric Antigen Receptors (CARs) are synthetic polypeptides that contain three distinct modules: an extracellular target binding module, a transmembrane module that anchors the molecule into the cell membrane, and an intracellular signaling module that transmits activation signals. CAR-based strategies provide distinct advantages in terms of redirecting effective immune effector activity. Mitch Finer, Dale Ando, Kristin Hge and colleagues at Cell Genesys, Inc. first brought CAR T cells to the clinic, in HIV. The construct “CD4zeta” was native CD4, which bound to gp120 on HIV-infected cells, coupled to the T cell signaling domain CD3zeta. Initial infusions of CAR T cells using manufacturing technology that pre-dated the system Carl June and I had developed showed that their CAR T cells rapidly
disappeared with 2–3 log declines over several weeks in patients following infusion. The company then approached us proposing a collaboration. Following a switch to our method of activating T cells, CAR T cell engraftment and persistence was detected up to a year post-infusion. The CAR T cells trafficked to lymph node and rectal mucosa, and a potential impact on HIV viral load was detected. In a long term follow up of these studies, it was observed that the CAR T cells persisted in patients for a decade, with decay half-lives >16 years. This early work set the stage for developing CAR T cells in cancer.

**Translational research and clinical trials**

I was proud to return to my alma mater the University of Pennsylvania in 1999, this time as a member of the faculty. As founding Director of the Clinical Cell and Vaccine Production Facility (CVPF), I built a highly accomplished team comprised of manufacturing and quality control technologists, Ph.D. level scientists, and quality assurance personnel. The Facility enables the translation of research, from bench to bedside, of novel cellular-based vaccines and related experimental immunotherapies administered to patients enrolled in clinical trials at Penn and collaborating institutions. First, as a small facility in a research building, we supported clinical trials of activated T cells in leukemia and lymphoma patients who had received high dose chemotherapy and stem cell transplants. The goals were to accelerate immune reconstitution of the T cell compartment so that patients would not be susceptible to opportunistic infections, and to potentially trigger tumor-specific immune responses. We showed the induction of lymphocytosis and reversal of immune defects in these trials, which in turn led to the next step, which was to combine vaccine priming and adoptive immunotherapy. In a series of myeloma adoptive immunotherapy stem cell transplant combination trials, with Carl June, Edward Stadtmauer, Kate Sullivan, and Bob Vonderheide at Penn, and Aaron Rapoport at the University of Maryland, we added vaccination with the pneumococcal vaccine Prevnar, the flu vaccine, and then putative cancer vaccines including survivin and telomerase peptides and a MAGE-A3 multi-peptide vaccine. These clinical trials demonstrated again that we could induce lymphocytosis with infusions of ex vivo activated and primed T cells and the induction of vaccine specific immune responses.

With our demonstrated ability to conduct T cell adoptive immunotherapy clinical trials, a number of companies approached us for our expertise. A small biotechnology company Virxsys, had developed HIV-derived lentiviral vectors for transduction of immune cells ex vivo. Together, we initiated pre-clinical studies to show that we could transduce T cells at clinical scale with a vector that encoded an HIV Tat- and Rev-dependent anti-sense to the HIV envelope gene. The ensuing clinical trial was the first use of a lentiviral vector in humans. In a long term follow up of these studies, it was observed that the CAR T cells persisted in patients for a decade, with decay half-lives >16 years. This early work set the stage for developing CAR T cells in cancer.

Specifically target DNA sequences like molecular scissors, enabling gene deletion or modification though non-homologous end joining. The target would be the CCR5 gene, chosen because CCR5 is an obligate co-receptor that HIV needs to enter cells. Those rare people with a naturally occurring mutation in CCR5 are highly resistant to HIV infection, with no accompanying significant immune defects. Dale proposed a collaboration to develop techniques to engineer HIV-resistant T cells using ZFN technology. Jim Riley, Richard Carroll and Elena Perez, a postdoctoral fellow, began laboratory and animal studies that formed the foundation to transition to our manufacturing group demonstrating large scale feasibility needed for the IND package to submit to the FDA. In the clinical trial of 12 subjects, we demonstrated that gene editing in humans was feasible and safe. The cells persisted in vivo and appeared to have a survival advantage when subjects temporarily discontinued their anti-viral medications.

In 2004 Michael Milone, then a fellow in Carl June’s laboratory, began pre-clinical studies to test CAR T cells in cancer. Combining an optimal chimeric antigen receptor design, delivered with a lentiviral vector to T cells stimulated with our anti-CD3/anti-CD28 bead system, this created T cells capable of antigen-specific production of cytokines and killing of targets in vitro as well as potent and persistent effects in vivo. This data and our large scale manufacturing feasibility runs, was submitted to the FDA to request permission to conduct our first study of CAR T cells targeting CD19 in B cell malignancies. The first three patients in this pilot clinical trial led by David Porter enrolled subjects with poor prognosis and refractory chronic lymphoid leukemia (CLL). What was observed following CAR T cell infusion was remarkable in-vivo proliferation and dramatic anti-tumor responses. Two of the three patients were complete responders in molecular remissions, with one partial responder. In simultaneous manuscripts published in the New England Journal of Medicine and Science Translational Medicine, detailed analyses showed that CAR T cells expanded >1000-fold, persisted for more than 6 months, and eradicated refractory CLL cells. Between 2.9 and 7.8 pounds of leukemia disappeared in these refractory patients in a few weeks after infusion. The two patients reported as complete responders in 2011 are leukemia free and healthy 7 years after their only CAR T infusions.

Intense global media coverage of these results spurred thousands of patients to inquire about the possibility of enrolling in the leukemia clinical trial, or treatment for another type of cancer. It also allowed the building of a bridge to span the developmental “valley of death.” Biotechnology and pharmaceutical companies began contacting the University of Pennsylvania with interest in licensing the technology for commercial development. Novartis was considered the fastest path to later phase clinical trials and, ultimately, wider patient access. The Novartis-Penn Alliance was formed to develop and test new CAR targets, and to take CAR T cells targeting CD19 (CTL019) to later phase clinical trials and regulatory approval.

With striking results in CLL, the potential application in additional CD19+ malignancies became evident. Patients with relapsed and chemotherapy-refractory pre-B-cell ALL have a poor prognosis despite the use of aggressive therapies such as allogeneic hematopoietic stem-cell transplantation. With David
to prevent relapse in the CNS and supported the testing of chimeric antigen receptor T-cell–directed therapies for CNS lymphomas and primary CNS cancers. We showed that ALL, a much more rapidly growing leukemia than CLL, could be successfully treated with CAR T cells, even in relapsed/refractory patients. The emergence of CD19 negative leukemia has pointed to the need for a CAR targeting CD22 (or CD20) in clinical development. The extension of this experience in CLL and ALL also led to opening of a CAR T cell trial led by Stephen Schuster in Non Hodgkin Lymphoma, including Follicular Lymphoma and Diffuse Large B Cell Lymphoma. What has further distinguished these results in ALL and NHL is the durable clinical effect, and the long persistence of the CAR T cells.

As a major goal of the alliance with Novartis to develop CAR T cells, we began to transfer the methods for manufacture and testing to their personnel. We conducted comparability studies demonstrating the effectiveness of taking a hugely complex process from academia to industry. Novartis submitted the results of these studies to the FDA, which allowed Novartis to proceed with manufacturing CAR T cells for their own clinical trials in leukemia (ALL) and lymphoma (NHL). With striking clinical results demonstrated in our trials at Penn and the Children’s Hospital of Philadelphia, Novartis began 25 center 4 continent global registration trials in each indication. In the pediatric ALL registration trial, 83% of patients infused with CAR-T cells achieved complete remission or complete remission with incomplete blood count recovery at three months post infusion. Interim analysis in the relapsed/refractory diffuse large B cell lymphoma NHL trial, showed overall response rate was 45%.

A highlight of my career was witnessing a U.S. Food and Drug Administration advisory committee unanimously (10-0) recommend approval of Novartis Pharmaceutical’s T-cell therapy for acute lymphocytic leukemia. The voting question was “Considering the efficacy and safety results of [the pivotal study], is the benefit-risk profile of tisagenlecleucel favorable for treatment of pediatric and young adult patients (age 3–25 years) with relapsed (second or later relapse) or refractory (failed to achieve remission to initial induction or reinduction chemotherapy) B-cell precursor acute lymphoblastic leukemia (ALL)?” This represents a huge milestone in cell therapy: on August 30, 2017 the product Kymriah™, was the first ever gene therapy to be approved by the FDA. Novartis expects to submit an application for market authorization with the European Medicines Agency in late 2017 in pediatric ALL and file with the FDA for Non-Hodgkin Lymphoma in the same time frame.

There are now 40 companies worldwide developing CAR T and NK or T cell receptor redirected immune cells, representing multiple billions of dollars of investment in this newly evolving personalized cell therapy field. Making these therapies more widely available to patients in medical need will require continued investment in new methods of gene delivery, cell activation and culture, and automation. I think of patients enrolling on our first cell therapy trials as our astronauts. They are testing new technology, not sure of whether they might return safely from the journey. Without their assistance, we would not make progress in new technology, in this case a new type of medicine that is a living drug, that we engineer to fight disease.