Engineering a Living Drug: The Odyssey of CAR T Cells

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Introduction

I am honored to receive the 2025 Balzan Prize for Gene and Gene-Modified Cell Therapy. This prize honors the work of my team over the past thirty years and, by extension, the entire nascent field of cellular immunotherapy. This recognition, following other recent honors such as the 2024 Breakthrough Prize in Life Sciences and the 2024 VinFuture Prize, signals a global, cross-disciplinary consensus. The field has matured; what was once a high-risk experiment has now been validated as an established new pillar of medicine.

Cellular immunotherapy is centered on a revolutionary approach to cancer, a therapy that "programs patients' own immune cells to fight their cancer". The core concept, and the central paradigm for this body of research, is that of the living drug. Unlike a conventional chemical pill that is metabolized and diluted, or a biologic antibody that is cleared, a living drug is a one-time treatment. It is composed of engineered cells that can grow, adapt, and persist within the body, potentially for a lifetime, providing continuous surveillance and protection.

This honor is not a personal award, but rather a testament to the dedicated team of scientists, researchers, and clinicians and, most critically, the courageous patients who participated in the early clinical trials. Their partnership was essential in launching what has been called a lifesaving new era of medicine. In this review I will briefly review my own journey and the status of the science and technology that led to the invention and implementation of CAR T cells.

A Career in Service: My Journey from the US Naval Academy to the T Cell

The intellectual framework for this new therapeutic modality was built upon an unconventional career path, one that blended military service, transplantation biology, and immunology.

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 birth date, and my lottery number was low, 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. So, my education did not follow the traditional MD/PhD track but culminated in a BS in biology and chemistry in 1975.

The Navy paid for my postgraduate training with an MD from Baylor College of Medicine and graduate training in malaria immunopathology at the World Health Organization in Switzerland in the laboratory of Professor Paul-Henri Lambert in 1978 to 1979. It was in Geneva and Lausanne where I first began wet-lab bench research and developed a lifelong love for research. After that, I had a formative period with a post-doctoral fellowship (1983-1986) at the Fred Hutchinson Cancer Research Center in Seattle. There, I studied transplantation biology with mentors including John A. Hansen, a physician scientist who led the field of immunogenetics, and E. Donnell Thomas, a Nobel Laureate and the pioneer of bone marrow transplantation. 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, an unfortunately not uncommon event in the early days of bone marrow transplantation.

This background in transplantation – the *original* cellular therapy – instilled a deep understanding of adoptive cell transfer, the process of replacing or repairing a patient's immune system. My experience in that field was then brought into sharper focus by a subsequent career in the US Navy, which directed research efforts toward a mission-

critical problem: rebuilding the immune system, specifically in the context of T-cell collapse caused by HIV.

This unique training enabled a synthesis of two distinct fields – transplantation (the mechanics of cell transfer) and military/HIV research (a mission-oriented focus on T-cell failure and activation) – and created the necessary cross-disciplinary foundation for the translational breakthroughs to come. In 1999, I left the Navy and moved my laboratory to the University of Pennsylvania to establish a human immunology program with a clear mandate for translational research.

The Two-Signal Problem: Unlocking the T Cell

The development of a living drug required first solving a fundamental problem of immunology. The T cell is the hunter cell of the immune system. In the 1980s, it was understood that the T cell's receptor (TCR) binding to an antigen – a protein on a target cell – provided "Signal 1", the command to engage. The central mystery, however, was that Signal 1 alone was insufficient. In fact, TCR ligation by itself often led to T-cell *anergy*, or paralysis. The cell could see the enemy but refused to attack. It required a second, co-stimulatory signal to be fully activated.

During the 1980s and 1990s, my lab research focused on identifying this major control switch. The research identified the CD28 molecule as the T-cell rheostat responsible for providing this critical "Signal 2". This discovery, often referred to as CD3/28 costimulation, was the key to unlocking the T cell's full potential. Providing both Signal 1 and Signal 2 did three things:

1. It acted as an ignition switch, fully activating the T cell.
2. It instructed the cell to produce interleukin-2 (IL-2), a rocket fuel that drives massive proliferation.
3. It provided a survival signal, making the T cell resistant to apoptosis (programmed cell death) and enabling it to persist for long-term battles.

This fundamental understanding of the basic science of T-cell activation from the 1990s would, more than a decade later, become

the essential engineering component of all successful CAR T Cell therapies.

An Unexpected Teacher: Lessons from HIV

Upon arriving at the University of Pennsylvania, the lab's translational focus was on engineering T cells for both cancer and human immunodeficiency virus (HIV). Using the newly understood second-generation designs which included the costimulatory signal, our team was among the first to conduct clinical trials of engineered T cells designed to attack HIV-infected cells. As a treatment for HIV, the trials were a disappointment. The therapy was only mildly effective, and, fortunately, the parallel development of highly active antiretroviral therapy (HAART) rendered this complex cellular approach unnecessary.

These failed trials, however, provided the single most important, counter-intuitive insight of the entire journey to CAR T cells for cancer therapy. In following these patients with HIV, we discovered that the genetically modified T cells could *persist* in their bodies for *years* – in some cases, more than a decade. This finding was revolutionary. The prevailing dogma had been that such highly manipulated, ex vivo cultured cells would be quickly rejected by the patient's own immune system or would simply die off. Indeed, we have unpublished data that some of our HIV/AIDS patients still have engineered T cells in circulation more than twenty years after a single infusion.

This HIV trial was the essential bridge from basic science to viable cancer therapy. It was the first *human proof-of-concept* that a living drug was possible. The CD28 costimulation, which theory predicted would grant persistence, had now been proven to do so *in vivo*. This discovery de-risked the entire concept. If the cell could persist for years, in principle, it could provide long-term surveillance against cancer. The engineering platform was stable; all that needed to be done was to change the target.

Engineering the Serial Killer: The Birth of the Chimeric Antigen Receptor (CAR) T Cell

The Chimeric Antigen Receptor (CAR) T Cell is a product of synthetic biology, fusing parts that do not exist in nature to create a cell with a new and specific purpose. The process of creating this living drug for a patient was an ultimate feat of personalized medicine, moving from bench-to-bedside and back again. The process can be broken down into five simple steps:

1. **Collection:** The process begins with a blood draw. Using a procedure called leukapheresis, the patient's T cells are separated from the rest of their blood components.

2. **Reprogramming:** In a specialized, sterile manufacturing facility, a vector, typically a disarmed lentivirus (a virus from the same family as HIV), is used as a delivery vehicle. It inserts a new gene into the T cell's own DNA.
3. **The CAR Construct:** This new gene instructs the T cell to build a synthetic Chimeric

   Antigen Receptor on its surface. This receptor is chimeric because it combines:

   - **The Tracker (Outside):** An antibody fragment (scFv) designed to recognize a specific lock or antigen on the surface of cancer cells. For B-cell leukemias and lymphomas, this target is a protein called CD19.

   - **Ignition (Inside):** The internal signaling domains. This includes "Signal 1" (from CD3-zeta) to give the kill command, and critically, "Signal 2" (a co-stimulatory domain like CD28 or 4-1BB) to give the proliferate and persist command.

4. **The Army (Expansion):** These newly engineered CAR T cells are activated and grown in the lab, expanding from a small sample into an army of hundreds of millions.
5. **The Living Drug (Infusion):** This army is infused back into the patient's bloodstream. The cells now circulate, acting as serial

killers that will hunt and destroy any CD19 target- bearing cell in the body.

The key innovation that enabled clinical success is visualized in the table below. Early first-generation CARs, which only had Signal 1, failed in the human body. It was the addition of the costimulatory Signal 2 domain – the direct application of the fundamental science of CD28 – that transformed the CAR T cell into a persistent, living drug.

Table 1: The Evolution of CAR T cell Engineering

Generation	Design Components	Clinical Result
First Generation (e.g., Kurosawa, 1987 Eshhar, 1989)	scFv (Targeting) + CD3-zeta (Signal 1)	Poor T-cell proliferation and persistence. No durable remissions.
Second Generation (e.g., June lab and Sadelain lab, 1990s-2000s)	scFv + **Costimulatory Domain (e.g., CD28, 4-1BB)** + CD3-zeta	Robust proliferation, long-term persistence, durable remissions. **(The clinical breakthrough)**.
Third Generation and Beyond (Global development in multiple laboratories)	scFv + Multiple Co-stimulatory Domains (e.g., CD28+4-1BB)	Preclinical data suggests improved function; clinical benefit over second generation is still under study.

The First Victories: Terror, Triumph, and a New Era in Medicine

In 2010, this living drug was administered to the first patients with late-stage, refractory cancers. The results, published in 2011 in *The New England Journal of Medicine* and *Science Translational Medicine*, were terrifying, triumphant, and revealed a new biology.

Patient 1: Bill Ludwig (2010)

Bill Ludwig, a 64-year-old retired corrections officer, was the

first patient treated for refractory chronic lymphocytic leukemia (CLL). He had run out of all other options. Days after the infusion, he became critically ill. His clinical team, led by Dr. David Porter, was convinced he had a fatal infection.

Lab tests, however, revealed the truth. His infused T cells had expanded over 1,000-fold. What was initially a small infusion of a billion engineered cells had grown into an army of a *trillion* cells. The sickness was, in fact, the *war*. The CAR T cells were eradicating *pounds* of cancer from his body. In less than a month, he was in complete remission. That remission held for over a decade. Mr. Ludwig ultimately passed away in 2021 from COVID-19 pneumonia.

Patient 2: Emily Whitehead (2012)

Emily Whitehead was the first pediatric patient, treated for relapsed acute lymphoblastic leukemia (ALL) at the Children's Hospital of Philadelphia (CHOP). She, too, became critically ill – far sicker than Bill. In a coma and on a ventilator, she was suffering from multi-organ failure.

This case led to the second eureka moment. Her clinical team, led by Dr. Stephan Grupp, recognized that her symptoms were not from an infection, but from the T cells working too well. They were releasing a massive, fatal flood of inflammatory proteins known as a cytokine storm. We and others termed this dangerous side effect Cytokine Release Syndrome (CRS) or Cytokine Storm.

This revealed a complete paradigm shift in oncology. The severe side effect was not a case of *off-target* toxicity like chemotherapy; it was a *direct, on-target* consequence of the therapy's profound efficacy. The sickness turned out to be a direct correlate of the cure.

Racing against time, our team, led by Michel Kalos at the time, analyzed Emily's blood, found an astronomical level of a specific cytokine (IL-6), and made an intuitive leap. We administered tocilizumab, an off-label arthritis drug designed to block that exact cytokine. The result was one of the most dramatic in modern medicine. Emily woke from her coma on her seventh birthday. Her cancer was gone, and she remains cancer-free to this day.

This discovery was as important as the discovery of the CAR T cell itself. Without an antidote for CRS, the therapy would be unacceptably toxic for widespread use. This is reflected in the US Food and Drug Administration's (FDA) historic decision on 30 August 2017. On the same day, it approved the CAR T cell therapy *and* approved tocilizumab for the management of CAR-T-induced CRS.

From the Bench to the World: Building a New Form of Therapy

The journey from the first three patients in 2011 to a globally approved medicine required building an entirely new *kind* of therapeutic infrastructure. This work was not done in a single lab but required an academic and industrial ecosystem.

At the University of Pennsylvania, the Center for Cellular Immunotherapies (CCI) serves as the umbrella organization, bridging basic science with clinical trials. The initial trials were supported by the university's Clinical Cell and Vaccine Production Facility (CVPF) which was directed by Professor Bruce Levine, who began his studies in my laboratory as a postdoctoral student in 1992.

However, after the dramatic results in 2011, our program was struggling to find funding. The pharmaceutical industry was deeply skeptical of this totally different paradigm. This first cellular therapy for cancer was not a pill in a bottle, but required a complex, patient-specific ("autologous") manufacturing process more akin to surgery or bone marrow transplantation.

The turning point came in 2012, when Penn's Center for Innovation forged an exclusive global partnership with Novartis. This alliance created a new, symbiotic model for developing high-risk therapeutics. Novartis invested $30 million to build the Center for Advanced Cellular Therapeutics (CACT) at Penn, a state-of-the-art manufacturing facility dedicated to scaling up the process for multi-center global trials.

This hybrid model – academic discovery (June Lab) coupled with industrial capital and scale (Novartis) – provided the logistics and resources necessary for regulatory approval. On 30 August 2017, the FDA approved this therapy, now known as Kymriah™

(tisagenlecleucel), for pediatric and young adult B-cell ALL. This was a watershed moment: the first-ever gene therapy approved in the United States. The approval was later expanded to include adult relapsed/refractory (r/r) Large B-cell Lymphoma (DLBCL) and r/r Follicular Lymphoma (FL).

The Future Horizons of Cellular Engineering

The Balzan Foundation's award recognizes future developments, and it is clear that the breakthrough in blood cancer is just the first chapter. The engineering platform of the CAR T cell is now being aimed at a vast array of human diseases.

1. **The Next Frontier: Solid Tumors**

   CAR T cell therapy has been transformative for *hematological* (blood) cancers. However, the vast majority of cancer patients suffer from *solid tumors* (e.g., breast, lung, brain), which have proven to be much more difficult to treat. We are now seeing the first promising outcomes in challenging cancers like neuroblastoma and glioblastoma. It is a widely shared belief that in the next ten years, we will overcome the obstacles to treating solid tumors.

2. **Beyond Cancer: The Autoimmune Revolution**

   Perhaps the most exciting new horizon is the application of this technology *beyond* cancer. Many chronic autoimmune diseases, such as systemic lupus erythematosus (SLE), are driven by the very same B cells that Kymriah was designed to target.

   Recent pioneering studies from researchers in Germany have shown that a *single infusion* of anti-CD19 CAR T cells can induce deep, drug-free remissions in patients with severe refractory lupus. This suggests that the therapy can reboot the immune system, representing a complete paradigm shift in how we treat autoimmunity.

   Cellular immunotherapy has the potential for more widespread use with an even safer profile for autoimmune and other disorders. The B-cell targets in lupus are far less numerous than in leukemia, meaning an effective treatment may require a *much*

lower dose. A lower dose, in turn, would significantly reduce the risk of severe toxicity and lower the manufacturing cost. This opens the door to treating tens of millions of patients with chronic diseases. The potential is boundless. Other teams at Penn are already exploring CAR T cells to treat heart failure by engineering them to remove fibrotic scar tissue.

3. The Imperative of Access (Cost & Manufacturing)

The extraordinary power of this therapy is matched by its extraordinary cost. The list price for a single dose of CAR T therapy is over $475,000, with total procedural costs often exceeding $1 million. This creates a serious ethical crisis, limiting access to the wealthy and to well-resourced nations.

The solution to this problem is not just policy, but *innovation*. The current autologous model is a one-off bespoke procedure for every patient. The path to global access lies in two key areas:

1. Streamlining manufacturing: Researchers are already shortening the complex ex vivo manufacturing process from nine to fourteen days down to three days or even less, which will significantly lower costs.

2. Off-the-shelf allogeneic cells: The ultimate goal is to move from *autologous* (patient-specific) to *allogeneic* ("off-the-shelf") cells and in vivo delivery of CAR T by viral vectors and lipid nanoparticles. This involves using T cells from healthy donors, gene-editing them (e.g., using CRISPR) to remove the receptors that cause graft-versus-host-disease (GVHD), and creating large, standardized, cryopreserved batches. This would make the therapy immediately available for any patient and decrease the cost dramatically, changing the economic model from a bespoke suit to mass production.

Conclusion: A Shared Journey

The 2025 Balzan Prize is a profound honor. It serves as powerful recognition of a new form of medicine. But this breakthrough was not the effort of one individual, as I stated previously, but the culmination of years of hard work by a dedicated team of scientists, researchers, and clinicians.

This work is built on the shoulders of colleagues at Penn Medicine, the Children's Hospital of Philadelphia, Novartis, and scientific collaborators around the world. Sincerest thanks must be extended to the patients who participated in our trials and their families. It was their courage in the face of uncertainty that turned a radical idea into a clinical reality. The stories of Bill Ludwig, Emily Whitehead, and now many others are not anecdotes; they are the foundation of this new field.

CAR T cell therapy is now a living drug that has saved the lives of tens of thousands. But this is just the first chapter in an inspiring, lifesaving new era of medicine. The work is only just beginning.

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