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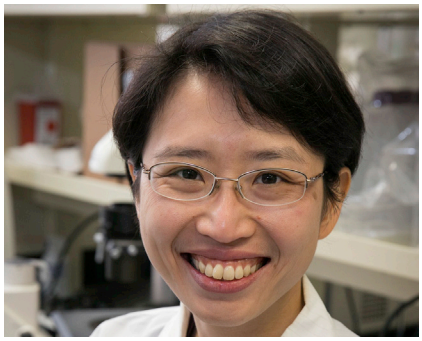
Voices

What is the Optimal Design-Build-Test Cycle for Clinically Relevant Synthetic CAR T Cell Therapies?



Yvonne Y. Chen
University of California, Los Angeles

Keep it Simple; Make it Well

Cell-based immunotherapy provides a unique opportunity for synthetic biology and biomolecular engineering to translate out-of-the-box ideas into real-world therapeutics. After more than two decades of conceptual developments in biological circuitry, engineered cells capable of Boolean logic computation have now reached the clinic. To ensure both patient safety and therapeutic efficacy, engineered cells must perform consistently and robustly in the face of dramatic patient-to-patient variability in physiology and disease presentation. As such, the pursuit for novelty and multi-layered complexity often associated with synthetic biology must be balanced with practical needs for genetic stability, functional consistency, immunological compatibility, and manufacturing feasibility.

Unsurprisingly, designs that have made it to the clinic thus far generally contain few moving parts and assiduously avoid non-human components to prevent immunogenic responses. At this time, the manufacturing of engineered cell products for clinical use remains an art that requires experienced operators with deft hands, focused minds, and a keen sense of cell biology. Automated, closed manufacturing systems could significantly improve efficiency and reduce cost, provided that such systems could clearly define, monitor, and respond to critical parameters in the biological product throughout the manufacturing process. This remains a challenging but also extremely interesting and important engineering problem to resolve.

Wendell Lim
University of California, San Francisco

The Innovator's Dilemma

First generation CAR T therapies have generated a remarkable level of excitement, investment and hype. As a result, the field has become more risk-averse and focused on solving pragmatic short-term issues – how to manufacture cells at lower cost, how to navigate higher levels of regulatory scrutiny. With the growing pains of a new industry, it makes sense to keep therapies simple. On the longer timescale, however, we need to avoid getting stuck in the classic innovator's dilemma.

It would be a shame to continue treating a CAR T cell simply like an antibody tethered to a big toxin. There is a currently an impressive trend to develop simple ways to rev up CAR T cell activity and resistance to exhaustion. But without widening the therapeutic window, such increased activity could lead to increased toxicity. Unlike other therapeutic platforms, cells are capable of sophisticated decision making. We can harness this complexity, e.g., to integrate multiple signals to control the timing and magnitude of their responses, using engineered or evolved homeostatic circuits that balance safety and efficacy.

I urge researchers to continuously push forward next-generation cell circuits, even those that seem impractical with today's constraints. A great example is deploying multiple engineered cell populations that work synergistically. Using two cell products is anathema to today's commercial and regulatory entities, but it could be extremely powerful, modular and practical – after all the immune system distributes its workload among a set of interacting cells.

The good news is that multiple entities are approaching cell engineering from different perspectives. I hope that companies, investors and regulators will ensure open paths to help drive this continued innovation.

Megan Levings
University of British Columbia; BC Children's Hospital Research Institute

Designing a Brake

Engineering regulatory T cells (Tregs) to dampen immunity has huge potential. But do chimeric antigen receptor (CAR) Tregs follow the same design-build-test rules as CAR T cells? Some aspects are similar, but others are more nuanced and require Treg-specific testing.

Design principles for both CAR Tregs and T cells include: an expansion process that balances cell yield and preserved function, testing function *in vitro* and *in vivo*, and the requirement of antigen to ensure persistence. Distinct from CAR-T cells, cell purity is a CAR Treg production bottle neck because rigorous cell isolation procedures are needed to limit potentially risky contaminating T cells. CAR Tregs also have unique responses to intracellular signaling domains – for example inclusion of 41BB, which works very well in CAR T cells, seems to destabilize CAR Tregs. CAR Treg design principles that remain unknown include the optimal affinity of CAR antigen-binding domains, ideal dosing schedules, and knowledge of which functions must be present to mediate the desired immunosuppressive effects.

CAR-Tregs are "nipping at the heels" of CAR-T cells as a transformative cell therapy, but a "plug and play" design based upon CAR-T cells is not optimal. Understanding how CAR-Treg design relates to function will accelerate the design-build-test pipeline to create a new way to put the brake on harmful immune responses.

Bruce Blazar
University of Minnesota

Efficient and Affordable CARs

There is a continued need to improve on the successes of CAR T cell therapy as applications and patients treated increase. On-target, off-tumor toxicities such as cytokine release syndrome and neurotoxicity required new diagnostic criteria and recommended interventions. Substantial efforts have been placed on altering CAR structural design to regulate CAR expression and function as a direct means of improving safety without precluding efficacy. Design modifications optimize T cell expansion, persistence, function, or migration, while reducing tumor microenvironment immune suppression and the propensity for antigen loss variants. As technology develops, incorporating new synthetic designs will become easier.

CAR expression frequently is achieved with viral vectors. Obtaining high titer virus can be the longest part of clinical translation due to relative paucity of FDA approved GMP production facilities. Advances in virus generation and nonviral transduction efficiencies will reduce the burden particularly for individual investigators. Shortening expansion cultures has lessened CAR T cell senescence and increased *in vivo* persistence.

Society will need to deal with economics of personalized autologous CAR T cell therapies. Solutions will come from use of CAR T cells given under permissive conditions for tumor targeting and persistence. Likely CAR T cell sources will include genome modified adult or umbilical cord blood allogeneic cells, expanded stem cells that have been matured into T cells *in vitro*, and induced pluripotent stem cells. With the concerted efforts and enthusiasm by the next generation, the future of the CAR T cell field is bright.

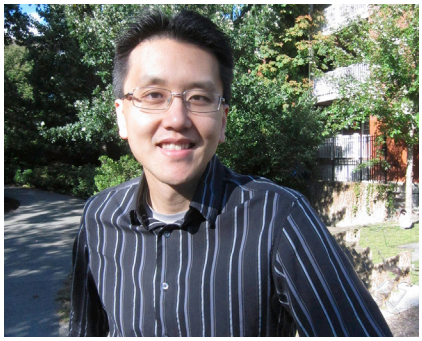
Giedre Krenciute
St. Jude Children's Research Hospital

CAR Test Cycle

Over the past decade, we have generated a substantial amount of knowledge about how CAR design affects function. Unfortunately, the process of generating both safe and effective constructs remains challenging as, when it comes to CARs, one size does not fit all. Additionally, it has become clear that a second genetic modification will be required to make CAR T cells persist long-term and produce sustained efficacy.

Based on my experience generating multiple brain tumor-specific CARs, I recommend the following cycle (which assumes a target antigen has already been chosen). First, the specificity of the antigen-binding ectodomain (scFv) must be evaluated using conventional and clinically proven CAR signaling domains e.g., CD28.z and 41BB.z. Once specificity has been confirmed using antigen-positive and -negative cell lines, the next step is to determine if new signaling domains such as CD27, mutated CD3 zeta, CD3 epsilon, MyD88, etc. can further improve effector function. These steps are straightforward and can be performed in a timely manner using standard

immunological assays. At this point, the efficacy and safety of the new CARs must be evaluated *in vivo*, ideally in an immune competent mouse model. This can then be followed with the addition of a second genetic modification (overexpression of synthetic molecules, silencing negative T cell regulators, etc.) that can make CAR T cells expand and persist longer. The additional modification then necessitates additional safety testing and likely the incorporation of a "safety switch" as well. The key to success in this cycle is having appropriate disease-relevant *in vivo* model(s).



Wilson Wong
Boston University

Rapid T Cell Engineering?

CAR T cell design iteration has proven to be very challenging. One of the most time-consuming steps is the introduction of CAR constructs into immune cells. Engineering primary T cells usually takes 2-3 weeks using lentiviral transduction, and we can only handle 6-8 samples at a time. Therefore, the throughput is very low. Optimization in cell lines (e.g., Jurkat T cells) has not been fruitful because behaviors from Jurkat T cells often cannot be recapitulated in primary T cells. Therefore, we usually check a new design in Jurkat T cells only to ensure that it is functional. All further characterization and optimization would be done in primary T cells.

To expedite the design-build-test cycle, we believe that gene delivery into T cells needs to be scaled down to enable high-throughput experimentation. Messenger RNA electroporation is probably the most suitable commercially available approach for high-throughput T cell gene delivery. However, functional assays, such as cytokine releases and cytotoxicity, would also need to be modified to use fewer cells to complement the scaled-down electroporation approach.

Manfred Lehner
St. Anna Children's Cancer Research Institute

We Shouldn't Fear Complexity

85,000 operations/sec could be performed by the computer on the Apollo spaceship. The latest chips in smartphones achieve over 1×10^{12} operations/sec. Still, the Apollo computers were groundbreaking. In the early 1960s, computers were the size of at least several refrigerators, crashed frequently, and consumed vast amounts of energy. The Apollo computer was among the first to use integrated circuits on silicon chips and it weighed "only" 70 pounds. At that time, the big challenge was to build more powerful and at the same time much smaller computers. The challenge in our field today is similarly ambivalent: We have to strongly increase the efficacy of CAR T cells and at the same time reduce their potential toxicity. This is the key step that we need to take to unfold the full potential of CAR T cell therapy.

Back then, the breakthrough lay in a completely new technology, which itself was only made possible by increasing knowledge and a number of innovations. Which innovations will lead us to the next breakthrough in our field?

Which tools will enable us to produce CAR T cells that can sufficiently differentiate tumor cells from healthy tissue? How to deal with tumor heterogeneity and antigen escape? How to boost expansion and functionality? How to get more control over our cells?

Complexity will certainly increase, and we should not be afraid of it in our attempts to find solutions. History should inspire us that something which seems impossible today might be the standard in a few years.