CELL & GENE THERAPY INSIGHTS

SUPPLY CHAIN: RAW & STARTING MATERIALS QUALITY, SOURCING & MANAGEMENT

CHANNEL CONTENT

INTERVIEW

Innovating in iPSC differentiation & engineering

David McCall, Commissioning Editor, BioInsights, speaks to **Emily Titus,** Senior Vice President, Technical Operations, Notch Therapeutics

EMILY TITUS obtained her PhD from the Institute of Biomaterials and Biomedical Engineering at the University of Toronto, where she used a combination of laboratory and bioinformatics approaches to define and interpret gene regulatory networks controlling embryonic stem cell fate decisions. At Notch, she oversees the Technical Operations function, which includes Process and Analytical Development, Manufacturing Sciences, Engineering and Project Management. Previously, she held the position of Vice President, Technology Advancement at CCRM, where she built cell reprogramming, genome engineering and pluripotent stem cell differentiation programs and led the company incubation program that culminated in the launch of Notch Therapeutics.

Cell & Gene Therapy Insights 2022; 8(10), 1437–1443

DOI: 10.18609/cgti.2022.209

Q What are you working on right now?

ET: Notch Therapeutics is developing induced pluripotent stem cell (iPSC)-derived immune cell therapies. At Notch, I oversee the Technical Operations function. This

includes process and analytical development, engineering, technology transfer and manufacturing sciences. I work on cell line development for the iPSC lines that will be used to create our products, as well as on scaling up our differentiation process. Our core technology is a bead-based differentiation platform, and we work in-house on the development and manufacturing of that custom bead reagent.

Q Can you tell us more about the reasons why Notch has pursued its own particular approach to developing allogeneic cellular immunotherapies, and in particular, why iPSCs were chosen as a source of starting material?

ET: While the allogeneic field has made fantastic progress in improving the number of doses per manufacturing run, we recognize that a healthy donor cell source will have a natural limitation to the eventual batch size that can be produced. With iPSCs, however, we envision an unlimited supply of consistent starting material. We can gene edit the iPSCs and create a clonal master cell bank in which every single cell has our selected edits. Then we can think about running a batch size that could support an entire clinical trial, for example. I think of iPSCs as the third generation of engineered T cell therapy starting material – we want to push the limits of how standardized and off-the-shelf we can make this kind of product.

Q You've worked in pluripotent cell programming for more than a decade at CCRM and then Notch – can you take us on the journey as you have experienced it through the rapid development of the field over this period – both in terms of the emergence of iPSCs and the application of increasingly sophisticated engineering tools?

ET: It all began during my graduate school experience, when the stem cell field first learned that we could reprogram mouse cells to a pluripotent state. Soon after we learned that we could do the same with human cells. My grad school lab quickly pivoted from working with mouse embryonic stem cells to working primarily with human iPSCs. In those early years working with embryonic cells, we mostly thought about how to use them to study developmental biology and develop drug screening platforms, but we did not think seriously about using them for human medicine and building cell therapies. This quickly changed once we were able to harness human iPSC reprogramming technology.

In the beginning, we faced significant technical challenges. It was much more difficult to perform reprogramming without the commercial products available to us now. It was hard to reproduce differentiation protocols from literature because the reagents and techniques were

DOI: 10.18609/cgti.2022.209

"...iPSC and genome editing fields have been advancing, engineered T cell therapy has progressed tremendously and taught us what applications we could pursue in the oncology setting."

not consistent from lab to lab. Over the ten years that I worked on establishing these techniques at CCRM, I saw rapid progress in standardization of research tools and techniques such as feeder-free media, defined culture matrices, improved passaging techniques, and even suspension-based culture systems. It has become much easier to think about producing iPSCs and their differentiated progeny at scale and with the consistency and quality required for clinical application.

Once joining Notch, I was fortunate to continue working closely with my former colleagues at CCRM to translate our research reprogramming protocols into a manufacturing process that was used to establish our clinical iPSC lines within their good manufacturing practice facility. Most recently, we've been able to use genome engineering tools to accomplish precision editing, including knockouts of certain genes and site-specific insertions of transgenes. With these tools in place, we can now turn our focus to thinking about the design of clonal products that we could create.

At the same time as the iPSC and genome editing fields have been advancing, engineered T cell therapy has progressed tremendously and taught us what applications we could pursue in the oncology setting. There has been a convergence of these ground-breaking technologies, and as they come together, we believe that we have a path towards treating human cancers with edited iPSC-derived immune cells. To me, the progress has been very rapid – we went from Nobel Prize-winning scientific discoveries to being on the verge of clinical application in an impressively short amount of time.

Q Coming to the present day, what do you regard as the state of the art in gene editing applied in the creation of master iPSC banks, currently? What is possible today?

ET: Companies like ours plan to create cells with multiple edits. To enable an allogeneic product, we must introduce edits to overcome both graft versus host disease (GvHD) and rejection of that product once it is infused into the patient. At Notch, we are working on ways to improve the efficiency of gene editing. If we wanted to have a triple knockout cell line with multiple transgene insertions, how could we make that as quickly as possible? If you introduce the edits sequentially, it can take a long time to build a product. We have been

working on maximizing the efficiency of any given single edit and thinking about different strategies for multiplexed engineering. This must all be done using processes and materials that will permit the use of the cell line in the clinic. In addition, in anticipation of scrutiny on the clonality of the master cell bank, we have employed high-throughput cell printing and imaging instrumentation that can document that our edited cell lines are derived from a single cell. We are focused on creating an integrated process by which we generate edited iPSCs reproducibly with high efficiency and ensure that in each campaign, given the number of clones that can be screened, we can find a clone that meets all the criteria of a clinical candidate.

Q Can you expand on the some of the successes and the remaining obstacles in successfully differentiating iPSCs for specific immune cell therapy modalities?

ET: I have always considered T cells to be one of the most challenging cell types to differentiate. Early on in the iPSC field, we saw evidence that we could differentiate into many useful cell types, including cardiomyocytes, neurons, retinal cells, and pancreatic cells to name a few. But it has been a long time coming to see T cells being differentiated, to be able to specify which type of T cell is produced and to ensure it has therapeutically useful functional properties. Adding to this challenge, the field has also struggled to produce hematopoietic precursor cells with lymphoid potential. Without sufficient and consistent sources of the precursor cell, downstream exploration of the right conditions for T cell maturation was naturally hampered.

To solve this, Notch first focused on developing high efficiency and scalable hematopoietic precursor manufacturing. These precursor cells are cryopreserved to establish a consistent input material for the subsequent stage of differentiation. Next, the Notch bead technology is used to drive rapid expansion and differentiation to progenitor T cells in scalable suspension bioreactors. Entering into the final stage of differentiation, we have ample material to compare various media and processing steps to optimize the final maturation to CD8 single positive αβ T cells. The final step is to iterate and compare the iPSC-derived T cells to primary T cells to achieve the functionality and potency desired in a clinical product.

Q How is the analytical side of things keeping up with innovation in this field? Are there any shortfalls that require attention?

ET: We are heavily editing iPSCs and we need to develop assays that can monitor for any mistakes introduced during that process. Innovation, standardization, feedback from regulators will all be helpful in developing approaches to survey for off-target edits.

DOI: 10.18609/cgti.2022.209

Secondly, iPSCs can acquire culture-associated abnormalities and as such, iPSCs are always going to be scrutinized for their genomic stability throughout reprogramming, *in vitro* expansion, and differentiation. At Notch we think about how to best apply a genomic integrity tracking and monitoring program across our research and development work to determine whether any of our processing steps have introduced a genomic instability. No single genomic integrity assay tells the whole story. Whether deploying karyotyping, array comparative genomic hybridization (aCGH), or analysis of specific regions that are likely impacted by abnormalities, the resolution or coverage of any one particular assay will have limitations.

Q What are the remaining steps to successful commercialization in the area of iPSC-derived cellular immunotherapies - what will they involve and require?

ET: Many future challenges remain to be solved for iPSC therapies to progress from clinical to commercial success. Many of those challenges will be addressed in a stage-appropriate manner. That said, when we began this work at Notch, we recognized that iPSC therapies have a unique catch. The iPSC line that you use for clinical development will ideally also be used long-term in the commercial product. You do not want to have to go back to the beginning and start with a new cell line because you did not keep commercial use in mind when sourcing and testing the original donor material or you did not have adequate documentation and traceability of materials during the initial derivation and editing of the line. Furthermore, your development team would ideally love to be working on the clinical cell line as early as possible while they test the function of edits and optimize the differentiation processes. There is enough information existing now to support companies to generate their iPSC starting material so that it can be used in the long-term.

Another key aspect of getting ready for commercialization is scaling up your manufacturing process. In my team, our approach is to work on scalable systems that can be translated to larger volumes without completely changing the culture format. In particular, the transition from adherent to suspension-based culture is a challenging step. We do not want to have to re-discover our differentiation process down the road. Although not widely used for iPSC expansion and differentiation protocols, I am a big proponent of working in stirred-tank bioreactors, and we have now demonstrated each stage of our process in a platform that spans volumes from 200 mL to 50 L. Now I have confidence that I can stop scaling at 1, 3 or 10 L, as dictated by our stage of

"Many future challenges remain to be solved for iPSC therapies to progress from clinical to commercial success. Many of those challenges will be addressed in a stage-appropriate manner."

development, but I know that the equipment is available to scale-up further when required. Investment in early translation to a scalable platform will pay dividends in the future.

Q Finally, can you sum up some key goals and priorities, both for yourself in your own role and for Notch Therapeutics as a whole, over the coming 12–24 months?

ET: At Notch, we have made good progress on our cell differentiation process and its scalability. A key priority now is to define a product profile that we want to invest in moving into the clinic. We want to be able to iterate on a product design. One thing that is notable about building iPSC products is that the design is locked in once you introduce the edits into the cell line. If you do not have the throughput to generate and test multiple designs, you are not going to be able to quickly solve challenges you might see in preclinical development that would prevent you from moving a product forward. From an operational perspective, my priority is to think about how to design our workflows at Notch to enable that rapid iteration of product design, to ensure we make efficient progress towards the clinic.

AFFILIATION

Emily Titus, PhD
Senior Vice President, Technical Operations,
Notch Therapeutics.

DOI: 10.18609/cgti.2022.209

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *The named author takes responsibility for the integrity of the work as a whole, and has given her approval for this version to be published.*

Acknowledgements: *None.*

Disclosure and potential conflicts of interest: *The author has no conflicts of interest.*

Funding declaration: *The author received financial support for the research, authorship and/or publication of this article from Notch Therapeutics.*

Article source: Invited; externally peer reviewed.

Submitted for peer review: Oct 27 2022; **Revised manuscript received:** Nov 22 2022; **Publication date:** Nov 28 2022