CELL & GENE THERAPY INSIGHTS

INNOVATOR INSIGHT

Rapid CAR-T cells: accelerating manufacturing to enable fast transition of CAR-T cell therapies to the clinic

Tamara Laskowski and Kelly Purpura

CAR-T cell therapies have revolutionized the oncology landscape, leading to unprecedented successes in the clinic. Despite the remarkable progress and vast growth in the number of CAR-T cell programs transitioning into the clinic since the first approvals in 2017, the complex manufacturing processes associated with these therapies present challenges that impact patient accessibility to these potentially curative treatments. The field has begun to explore rapid CAR-T cell manufacturing approaches that enable the generation of products that possess stronger stem-like properties and exhibit robust potency and persistence when challenged *in vitro* and *in vivo*. In the clinic, first-in-human trials of rapid-CAR-T cells support these observations, reporting notable anti-tumor responses from dose-level administrations lower than those used for products manufactured under longer-term protocols. In this article, the advantages of shorter CAR-T cell manufacturing protocols and the benefits of automation will be explored. Lonza will introduce a rapid manufacturing application that consolidates, within a 72-hour automated workflow, all critical steps required for transforming T cells into potent CAR-T cell therapies. The phenotypic and functional attributes of rapid CAR-T cell products manufactured in this platform will be described, alongside a cryopreservation strategy to support the recovery of stable, viable, and functional CAR-T cells.

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Since the initial approvals of Kymriah® and Yescarta® in 2017, the global pipeline of cell therapy, specifically CAR-T cell therapies, has expanded tremendously, seeing growth in both the number of CAR-T programs in addition to expansion in different modalities

of cell therapies. The field has also seen innovation beyond autologous cell therapies.

However, most of these developments will encounter a major bottleneck in manufacturing due to their complexity. Deploying the right manufacturing strategy to enable therapies to reach patients faster is a critical component of driving a successful cell therapy program. The field has looked at alternative approaches to alleviate some of the burdens associated with delays in manufacturing and the deployment of these therapies into the clinic. These strategies include taking an 'off-the-shelf' approach, with focus on implementing allogeneic programs. This enables manufacturing in large batches in a cyclical fashion enabling cryopreservation of therapies that become available to patients when needed. A second approach is to integrate automation early on in manufacturing decisions. Automation can provide an opportunity to streamline a manufacturing process, reduce complexity, increase robustness and reproducibility, and reduce the overall cost of manufacturing. Lastly, processes may be rethought to shorten manufacturing timelines and enable therapies to reach patients faster.

AUTOMATION IN CELL THERAPY MANUFACTURING

Whether a therapy is autologous or allogeneic, the elements of the manufacturing process will have many commonalities, and automation can be a solution to alleviate many of the complexities that exist when manufacturing either type of therapy. Automation can also offer significant advantages for enabling scalable solutions in both decentralized and centralized manufacturing models.

Additionally, automation reduces the number of manual operations and end-user interaction with the manufacturing process thus reducing sources of error that can impact the final drug product. This is especially important when deploying a decentralized manufacturing strategy where multiple sites must perform the same manufacturing protocol and ensure robust and reproducible process implementation. By reducing labor complexity and ensuring better process control and reproducibility, costs in manufacturing are reduced, supporting the longevity of a cell therapy program through sustainable and efficient operations.

Logistically and operationally, automation can also add substantial advantages, ensuring better-suited commercially compliant QC and QA measures deployed at clinical manufacturing sites and helping to maintain product chain of custody and chain of identity through electronic batch records and documentation.

INTRODUCING AN AUTOMATED PLATFORM FOR CELL THERAPY MANUFACTURE

At Lonza, cell therapy manufacturing automation is achieved using the Cocoon® platform. This is an automated scalable platform for cell therapy manufacturing that has been deployed in various clinical programs specifically targeting T cell-based therapies, including CAR-T cells and TCR-T cells.

The elements of this platform include the environmental unit, which houses key components essential for manufacturing cell-based therapies, including the ability to maintain a controlled dual temperature zone and a built-in bidirectional peristaltic pump that supports fluid exchanges throughout the many steps in a given process. The Cocoon environmental unit is also equipped with pH and dissolved oxygen (DO) sensors that allow discrete assessments of the culture conditions throughout the manufacturing process.

The next element is the single-use cassette which is the consumable used for each manufacture. This tool enables functionally closed process steps that are applicable to both suspension and adherent cells within both viral and non-viral processes. It includes an integrated cold chamber for internalizing all process reagents and consumables in one form

factor. When required, media and reagents are pre-warmed prior to entering the proliferation chamber where cell culture takes place.

The final element is the software which monitors and controls process parameters and executes each step as programmed in the manufacturing protocol. The software is enabled by a protocol design component that allows protocol steps to be defined and customized according to the user's specifications. Additionally, pH/DO values can be leveraged to adjust media exchanges, recirculation, and oxygenation of culture, and thus enable process modifications to fit the therapy of choice.

Typically, the key steps involved in manufacturing of most cell therapies start with sample collection at a clinical site and transfer of the sample to the manufacturing center. In the initial phase, an optional sample preparation step may occur, followed by selection of the cell type of interest. Subsequently, an activation step is often involved, and cells are then engineered through transduction or transfection. Once the gene of interest is transferred, the cell therapy product is then expanded to achieve required dose levels. Ultimately, the culmination of the process is final formulation and patient administration.

Several of these steps are commonly carried out by operators who interact with the process throughout each stage. By implementing an automated platform such as the Lonza Cocoon, many of these unit operations can be consolidated into one instrument, facilitating and streamlining the manufacturing process, and reducing the opportunities for errors or batch failures. Moreover, through automation of these various steps, greater reproducibility can be achieved in processes performed by different operators at different locations.

INNOVATING RAPID MANUFACTURING STRATEGIES

Rapid manufacturing increases the speed at which these potentially curative drugs can reach patients. The Cocoon platform can manufacture CAR-T cells in 72 hours or less. One of the key advantages associated with rapidly manufactured products is the maintenance of stem-like properties in the final drug product. This likely leads to a product endowed with higher potency and persistence, thus reducing the need for high dose levels. By ensuring a product that has greater stemness, potency, and proliferative potential, superior persistence *in vivo* can be achieved, driving improved outcomes in patients. Moreover, by accelerating the manufacturing process, the amount of needed materials and reagents decreases, and labor requirements are reduced, thus lowering the overall cost of manufacturing. Additionally, moving from a 10-day process to a 3-day process allows more batches to be manufactured within the same timeframe, thereby producing more therapies for patients, and doing so faster.

Lonza has created a 3-day T cell manufacturing application suitable for CAR-T cells, shown in Figure 1.

In the serum-containing X-VIVO™ medium-based protocol, cells were transduced with Lonza-manufactured lentivirus vector delivering a third-generation CD19-CAR. The product was harvested and analyzed for CQAs including memory phenotype and functional response *in vitro* on day 3. Characteristics were found to be maintained without skewing production or altering the overall composition of the product. Moreover, transduction efficiencies averaging 35% of T cells were achieved in this context. $CD4^+$ and $CD8^+$ T cells showed a similar frequency when compared to the starting material, and observable levels of CAR^+ T cells were detected in both cell subsets, demonstrating successful transduction of both T cell subpopulations.

Maintaining the stem-like characteristics of the T cell product is also achievable within short manufacturing. A strategy for interrogating the CAR-T cell products for memory phenotyping was developed. Material from three separate rapid manufactures are profiled in Figure 2. Each product is driven into a fate comprising mostly stem memory and central

memory T cells, indicating a more robust phenotype, capable of stronger persistence *in vivo*.

To investigate the potency and persistence of the product further, a persistence anti-tumor cytotoxicity assay was developed, with three stages of tumor challenge. In the first challenge, tumor and CAR-T cells are co-cultured for 3 days. At the end of day 3, the culture is sampled and the performance of the CAR-T cells at eliminating the tumor cells is measured. The second and third challenges involve sequentially introducing further fresh live tumor cells to assess the persistence of

FIGURE 1

Manufacturing rapid CAR-T cells in the Cocoon® platform in serum-containing culture (X-VIVO 15™).

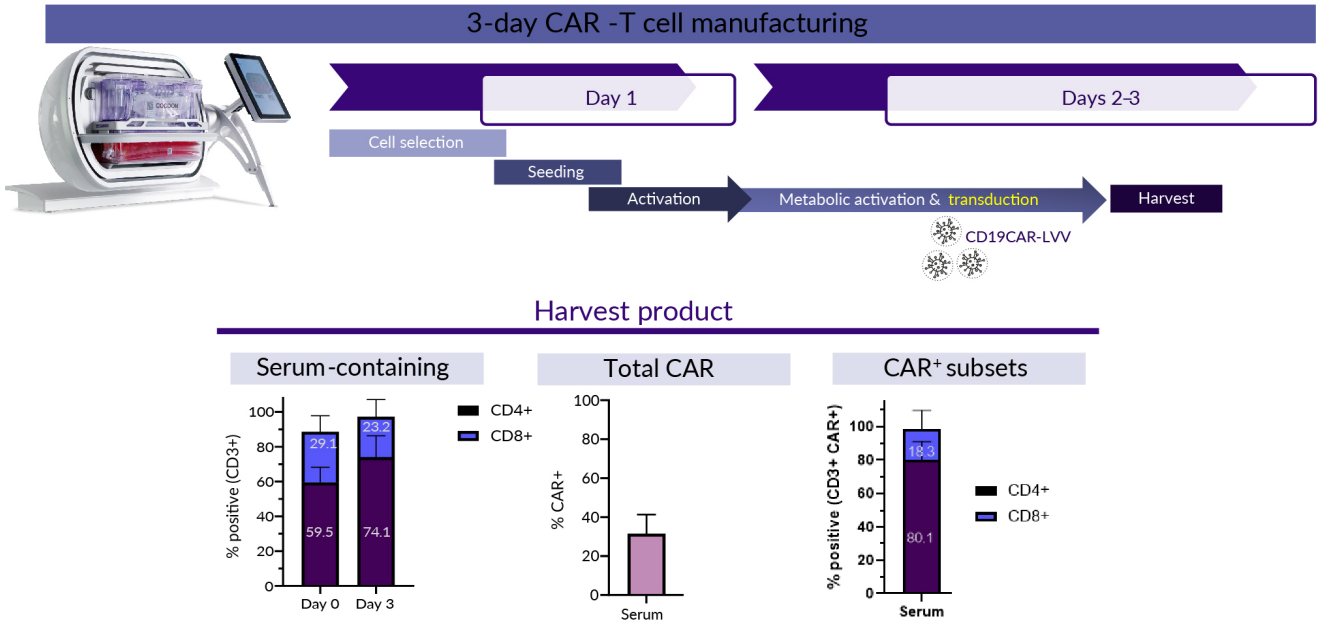
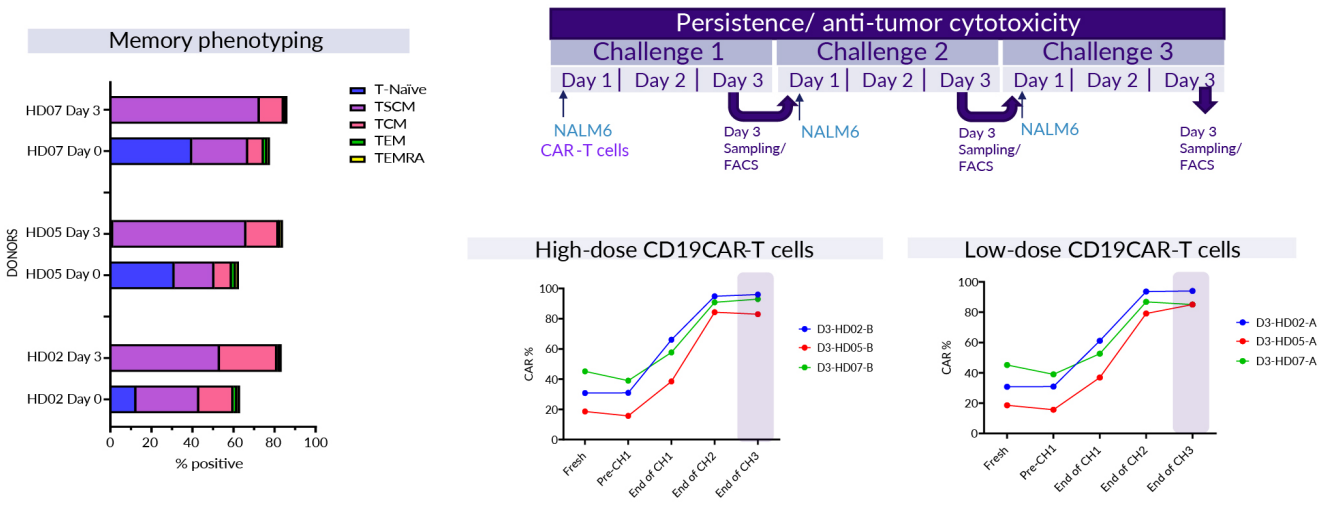


FIGURE 2

Rapid CAR-T cell products maintain memory phenotype suggesting stronger stemness and enhanced potency.



that response. This is performed with both high and low doses of CAR-T cells.

By the end of challenge one, a notable response is seen by the CAR-T cells in addition to an increase in the frequency of CAR-positive T cells. As the CAR-positive T cells increase, a decrease in the tumor cells is seen. All products achieve a high level of tumor clearance at the end of challenges two and three, with the CAR-T cells expanding throughout the sequential challenges and ultimately representing most of the cells remaining. When cells are challenged with an increased tumor burden, they do not experience a decrease in function, instead maintaining the same responsiveness to the tumor. This supports the hypothesis that more robust products lead to robust persistence in this *in vitro* setting.

DEVELOPING SERUM-FREE CAR-T CELL THERAPY MANUFACTURING

In further modifying and streamlining manufacturing, the possibility of eliminating serum was explored. Serum is a source of variability in a process and presents burdensome responsibilities on the therapy developer to validate serum lots to be utilized in the manufacturing process. The Lonza chemically defined T-VIVO® medium does not necessitate serum supplementation and is highly efficient at supporting the transduction of T cells in serum-free conditions, giving rise to products that

FIGURE 3

Manufacturing rapid CAR-T cells in the Cocoon® platform in serum-free culture (T-VIVO®).

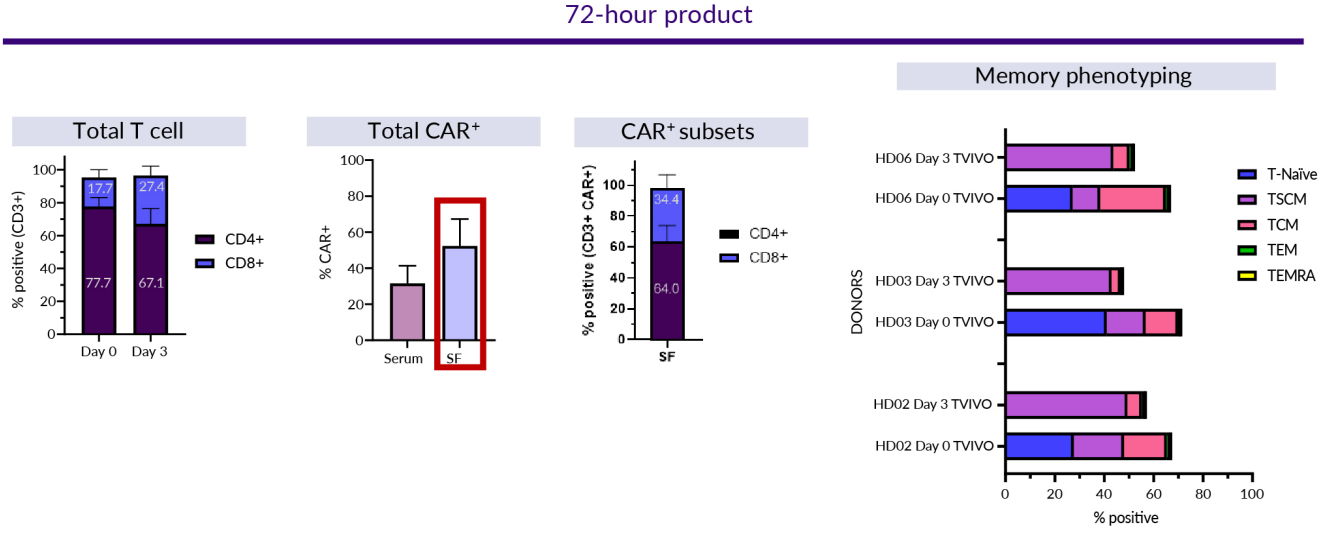
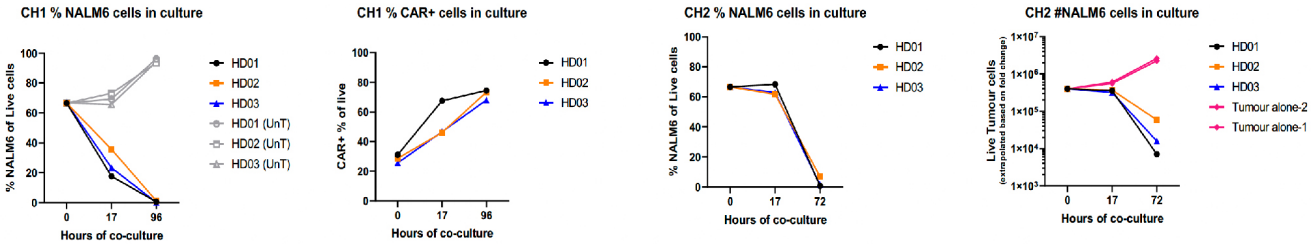


FIGURE 4

Rapid CAR-T cell products generated in serum-free conditions (three healthy donor [HD] samples) also exhibit strong cytotoxicity against NALM6 tumor cells.



possess similar memory phenotypes to those seen in products derived from serum-containing processes (Figure 3).

Manufacturing CAR-T cells in a rapid protocol without serum supplementation was also shown to yield a product with increased persistence and good cytotoxicity through a similar challenge assay as previously described, modified to include two challenges.

The number of live NALM6 tumor cells in the co-cultures decreases over time in both challenges as CAR-T cells in the culture expand in response to the tumor, a finding we observed in three products derived from three distinct donors (Figure 4).

CRYOPRESERVATION OF RAPID CAR-T CELL PRODUCTS

Next, establishing a robust strategy for the cryopreservation of rapid CAR-T cell products was explored. Various parameters associated with cryopreservation of cells were interrogated and a matrix combination of conditions was performed to refine a strategy for the successful cryopreservation and recovery of products. The parameters investigated included cell density, total volume, cryomedia used (DSMO/DSMO-free), and the type of controlled rate freezing (CRF) platform (liquid nitrogen/electric). To evaluate the various of conditions tested, readouts including total cell recovery post-thaw, recovery of CAR-T cells post-thaw, CAR-T cell expansion in culture, and CAR-T cell potency were measured.

Results for the recovery of CAR-T cells at thaw and CAR-T cell potency are shown in Figure 5. For CAR-T cell recovery, no statistical difference was found, demonstrating that the protocols developed for electric CRF and liquid-nitrogen CRF, when applied in the context of a DMSO-containing or a DMSO-free cryomedia formulation, both lead to robust product cryopreservation and recovery post-thaw. Moreover, as previously shown, all products exhibited strong anti-tumor response, able to eliminate all tumor cells within 72 hours. No statistical difference in the performance of recovered CAR-T cells was identified in this study.

The rapid CAR-T cells were also shown to expand well *in vitro* and maintain robust expression of CAR after thawing, re-activation, and expansion for 6 days in culture supplemented with IL-2 (Figure 6).

Viabilities were high for all products tested. The fold change in the amount of total T cells over 6 days was comparable across the two groups. The CAR-T cells exhibited

FIGURE 5

Results of post-recovery of cryopreserved rapid CAR-T cell products.

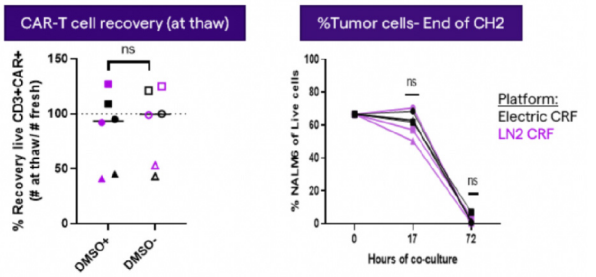
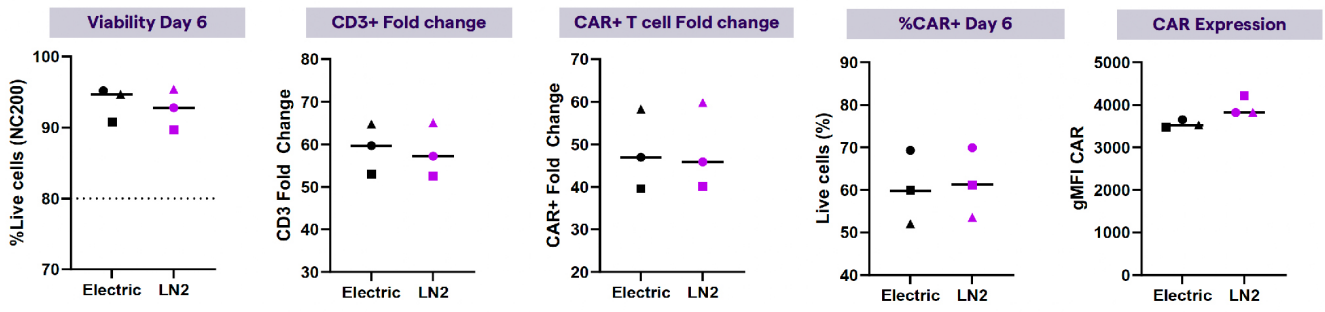


FIGURE 6

Rapid CAR-T cell expansion *in vitro*.



high viability and robust expansion in both systems upon recovery and culture. A high frequency of CAR-T cells was maintained throughout the 6 days in culture with cells showing stable, high expression of CAR on the cell surface.

SUMMARY

Adopting automation early into manufacturing and building an integrated solution is key to success in the manufacturing of cell therapies. Moreover, shortening the manufacturing of CAR-T cells can provide opportunities for reducing costs and accelerating production. The Lonza Cocoon® Platform enables T cells to be seeded, activated, transduced, and cultured in a streamlined and automated protocol that requires minimal manual touchpoints. Cryopreservation of CAR-T cell products should be robust, reproducible, and enable the recovery of a product that maintains its critical quality attributes associated with function and persistence.

Q&A

Tamara Laskowski (left) and Kelly Purpura (right)

Q Is your rapid manufacturing application flexible to use with various media and reagents?

KP: Yes—the Cocoon is agnostic to media and reagents. We can use various media including X-VIVO 15 with serum and T-VIVO, in addition to those from other vendors and products. These can be fit into both the rapid process as well as the standard process.

Q Have you tested your rapid manufacturing application in the context of different vectors and CAR constructs?

TL: Yes, we have tested second-generation CARs and different lentiviral vectors, and have seen equally robust results. We are confident that your vector of choice can work well in this manufacturing process in the context of the Cocoon Platform.

Q Are there any specific changes that you recommend when transitioning your CAR-T cell manufacturing process from serum-containing to serum-free culture conditions?

KP: We can run similar protocols for both serum and serum-free media. However, we have found that an increase in recirculation for oxygenation in media exchange may be beneficial. This is due to different serum protein levels or how chemically defined media has been shown previously to influence oxygenation. Often, people feed more aggressively for longer processes. However, for a rapid process, aggressive feeding may not be as impactful as we do not typically expand the cells during that short time window. There are minor changes to be made when tailoring to a specific process.

Q What are the key differences in the process when transitioning from a standard 9-day to a 3-day CAR-T cell manufacturing?

TL: Firstly, a 3-day process is much faster. The expansion phase seen in a longer process will not occur in a 3-day process, so dose levels should be addressed differently. In terms of manufacturing, the critical components, such as transduction and activation, required for transforming T cells into T cell therapeutics will remain the same.

At Lonza Personalized Medicine, our team can help to facilitate adjusting processes and dose levels for those looking to convert a 9-day process to a 3-day process.

Q How soon in the development process would you look at manufacturing systems?

TL: By designing your process and manufacturing strategy early on, and implementing automation, you can ensure the longevity of the program as it evolves. Today, your need may only be for a small number of patients in the early clinical phase of your program, but the hope is that, as you progress, you will be treating hundreds of patients. Planning a strategy to support the evolution of your program should be considered early on, as these can be critical decisions.

Q Have you observed any key differences between CAR-T cell products that are generated in serum-free conditions compared to serum-containing conditions?

KP: We do often see enhanced transduction in the CAR-T products that are generated in serum-free conditions for lentiviral vectors. There is also strong recovery and maintenance of the T cell expression. Phenotypically, the product is also high in stem and central

"...some of the same QC assays can be applied to rapid products. Others may have to be modified."

memory expression. That being said, we do not typically see many differences between the products.

Q Can you outline any common regulatory and technical challenges for rapid CAR-T cells?

TL: It is a relatively novel concept for the field, which is still adjusting to what the regulatory requirements for the release of a rapid product will entail. Fortunately, there are some rapid CAR-T cell products already reaching clinical trials, so, in some respects, that pathway has already been defined for us. At Lonza, we have partnered with professionals in the field who have established robust CAR-T cell programs to leverage that understanding.

With respect to product release, some of the same QC assays can be applied to rapid products. Others may have to be modified. We have seen that even at the lower starting cell input in rapid manufacturing, we have sufficient material to assess CAR expression, profile product viability, and perform some of the critical testing that is typically required for the release of these drugs. We await regulatory guidance to further define what the required QC and release assays for rapid CAR-T cell products will consist of.

Q Do you have *in vivo* data comparing rapid versus standard expansion times for pK and efficacy?

TL: Yes, we do. We have infused these rapidly manufactured products in animals with aggressive leukemias and have seen *in vivo* anti-tumor responses. We hope to soon release these data. We have seen encouraging results compared to standard 9-day manufactured products.

Q In rapid CAR-T manufacturing, the final doses are low. The Cocoon has an optimal seeding of between 50 and 100 million cells. What is the benefit of using the Cocoon here?

TL: There are various benefits. The data shown here were generated based on a seeding number of 200 million T cells. We have higher flexibility and can seed more than 200 million in the rapid manufacturing application. We have seen that we essentially recover the number of cells that we put in, or even a few more, as the cells experience subtle proliferation in the 3 days of manufacture. We have made the same observation when

running patient samples through this application. If you seed 500 million, you likely get 500 or 550 million cells in the output. There is a degree of flexibility in the input to achieve the output you wish.

The expectation for a rapid CAR-T cell product is that you can do more with fewer cells. In our *in vivo* models, we have been able to use dose levels as low as 10-fold or even 100-fold lower than a standard dose for a typical 9-day CAR-T cell product.

Q Did you use CAR-T concentration within clinical levels to limit the adverse events related to CAR-T cell therapies?

TL: We performed a cytokine analysis of these products to identify any of the culprits associated with toxicities. We did not see these in the assays that we have run for these products. *In vitro*, we selected doses based on standard settings for *in vitro* assays. Some of those determinations have translated well to *in vivo* studies (in which we did not see evidence of toxicities), but it can be a bigger leap to infer in-human outcomes based on *in vitro* data.

Q For rapid CAR-T, how do you demonstrate that there is no vector in the final product when levels of free virus quantified by qPCR concern the time of culture? Is there a specific product for washing the cells before harvest?

TL: This is something we considered when we first thought about shortening manufacturing and driving cell engineering of any cell type via viral vector in a rapid context. There are a few approaches we are taking to address this. We have looked at how early the expression of CAR can be seen, which in some cases can be as early as day 2. We have also kept side cultures on a small scale and kept the actual rapid manufacture in the Cocoon for the longer term to observe the progression of those T cells. In these experiments, we have carefully monitored the kinetics of the expression of CAR as a result of transduction.

We have also performed vector copy number (VCN) studies on these products. Combined, the data show that day three is a snapshot in CAR expression, and normally by day 5, we have stable expression of CAR. Typically, that number changes ~10 to 20% from day 3–5. We have seen that samples on day 3 can give slightly higher VCN numbers. We are continually designing studies to understand expression kinetics and its correlation with VCN.

Q Were the target cells irradiated in the challenge experiment?

TL: No, they are not irradiated. They are live, fully competent cells in their rapid growth phase. We make the tumor challenge very challenging for the CAR-T cells.

"We have frozen product in both vials and bags and we have not seen substantial differences between these vessels."

Q What is the maximum manufacturing scale for the Cocoon system?

TL: Currently, the 9-day process we have described leads to about 4 billion total cells. We have new iterations of the Cocoon cassette that will enable yields of up to 10 billion cells.

Q What are some of the common issues expected in the use of the Cocoon system?

TL: This will vary depending on the application of choice. As with any other automated manufacturing platform, you need to adjust and design your protocol according to the therapeutic you are developing. In the Cocoon, we can gain insight into how the culture is progressing through pH and DO measurements. This allows us to change process parameters, and optimize key steps in the manufacturing process.

The process development team at Lonza can adjust and refine the Cocoon manufacturing process to meet the needs of your program and prevent problems from happening in the manufacture of your specific product. There is no one-size-fits-all approach, but rather a customizable strategy that allows us to minimize hindrances and obstacles that may impact production.

Q Is your final T cell product frozen in vials or bags and have you seen a difference between these configurations?

KP: We have frozen product in both vials and bags and we have not seen substantial differences between these vessels.

TL: The study we have shown here is a snapshot of that. We hope to release an application for cryopreservation in which we delineate the differences between vials and bags, data obtained from those studies, and the protocols we have generated for the CRF-based cryopreservation methods.

Q What is the data collection interface of the Cocoon to connect to an upper system?

TL: At Lonza, we have a central digitization tool that can serve as an upper system. Directly in the Cocoon, the software captures every step in the process in electronic batch

records that can be connected to each specific manufacture. The interface of the Cocoon can be connected to the broader chain of custody and chain of identity tools to enhance data retrieval from sample collection to product infusion.

Q Any ideas on applying this methodology for natural killer (NK) cells?

TL: This is something that we are looking into. The field of NK cells is continually gaining traction, and we are beginning to see much lower dose levels of NK cells now in clinical trials. This could bring together the idea of memory NK cells with increased potency, reflecting some of the same features that are highly sought after in T cells. We are already working on NK protocols that may be suitable.

Q Have you checked the viability and efficacy of the rapidly manufactured CAR-T cells *in vivo*?

TL: We have conducted studies in hematological patient-derived xenograft mouse models, and we hope to release the data soon in 2024.

Q What has been the highest number of cells that this process has been tried with?

TL: We have input 200 million cells to keep the processes manageable and consume less of our viral vector. That input material is flexible–you would require more viral vector particles to transduce a higher number of cells, but the platform can accommodate higher numbers of T cells at the input.

Q It has been shown that in rapid manufacturing, high transduction efficiency in the early days may be observed due to pseudotransduction. Have you checked the transduction efficiency of the rapidly manufactured cells after more days in culture?

TL: Pseudotransduction has been reported in some cases, and we have methods for identifying if pseudotransduction is occurring or if we have stably transduced T cells. We have kept these cells in culture for 9 days and interrogated the product downstream. We have performed these assessments in products generated in alternative platforms and in the Cocoon. We have completed extensive interrogations on the metabolic profile of these cells and we observed that the product is stably transduced. We have not seen any indication of pseudotransduction.

Q Is there an option to select CD4/CD8 cells with the Cocoon?

TL: The Cocoon has an integrated magnetic selection capability, and in 2024, we hope to release a CD4/CD8 solution applicable to the Cocoon, which will be compatible with rapid or standard T cell therapy manufacturing.

BIOGRAPHIES

TAMARA LASKOWSKI is the Senior Director and Head of Clinical Development at Lonza Personalized Medicine. She supports the transition of novel adoptive cell therapies from pre-clinical stage into clinical manufacturing. Laskowski received a dual doctorate degree in the fields of human molecular genetics and immunology from the University of Texas Health Science Center at Houston, where her work focused on genetic engineering of immune cells and stem cells. As a post-doctoral fellow at MD Anderson, she developed platforms for off-the-shelf production of genetically-modified NK and T cells. Subsequently, Tamara transitioned to a Senior Scientist position at the Immunotherapy Platform led by Dr James Allison where her work focused on characterization of immune response to solid tumors in the clinical setting. For her contributions to the development of innovative analysis platforms, Laskowski was awarded a fellowship to the National Science Foundation Innovation Corps and received a prize for outstanding performance.

KELLY PURPURA has a decade of experience in process optimization for stem cell and immunological cell cultures. She has specialized in process translation to the Cocoon® Platform at Lonza for various applications and for immunotherapy. Kelly obtained her MASc and PhD at the University of Toronto through the departments of Chemical Engineering and Applied Chemistry and the Institute of Biomaterials and Biomedical Engineering with work focused on the phenotypic and functional analysis of osteoprogenitor hierarchy and on controlling the emergence of hematopoietic progenitor cells from pluripotent stem cells.

AFFILIATIONS

Tamara Laskowski
Global Head,
Clinical and Process Development,
Lonza Personalized Medicine

Kelly Purpura
Process Development Manager,
Clinical Development,
Lonza

Lonza
Cell & Gene

AUTHORSHIP & CONFLICT OF INTEREST

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

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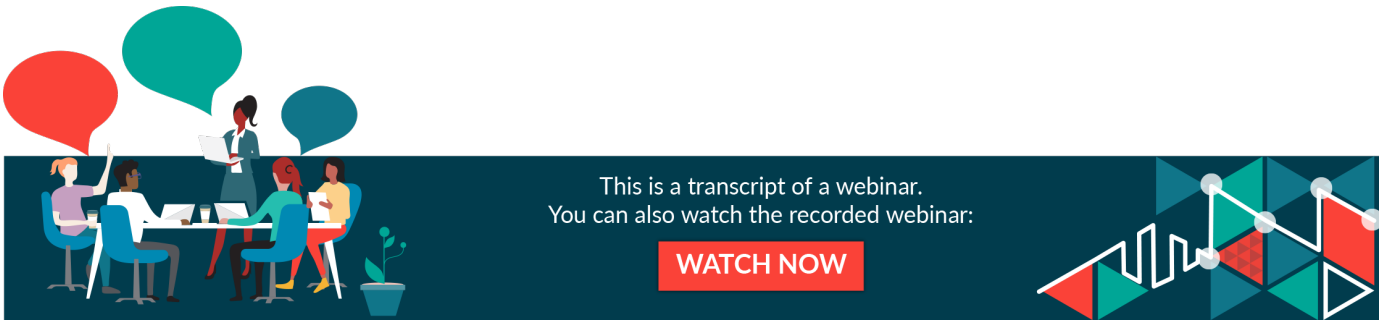

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