CELL & GENE THERAPY INSIGHTS

CELL THERAPY UPSTREAM PROCESSING AND MATERIALS

SPOTLIGHT

INNOVATOR INSIGHT

An automated 24-hour CAR-T manufacturing process

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As the cell therapy field progresses, manufacturing challenges like patient safety, cost, automation, closed operation, and scalability must be addressed with improved capabilities and workflows. Leveraging new cell therapy technologies and tools to develop innovative end-to-end workflows can enable more cost-effective cell therapy processes and workflows. This article presents an automated and shortened lentiviral-based CAR-T workflow using the Gibco™ CTS™ Detachable Dynabeads™ CD3/CD28 magnetic beads, Gibco™ CTS™ Detachable Dynabeads™ Release Buffer, and Gibco™ CTS™ DynaCellect™ Magnetic Separation System.

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AUTOLOGOUS CAR-T WORKFLOW CHALLENGES AND SOLUTIONS

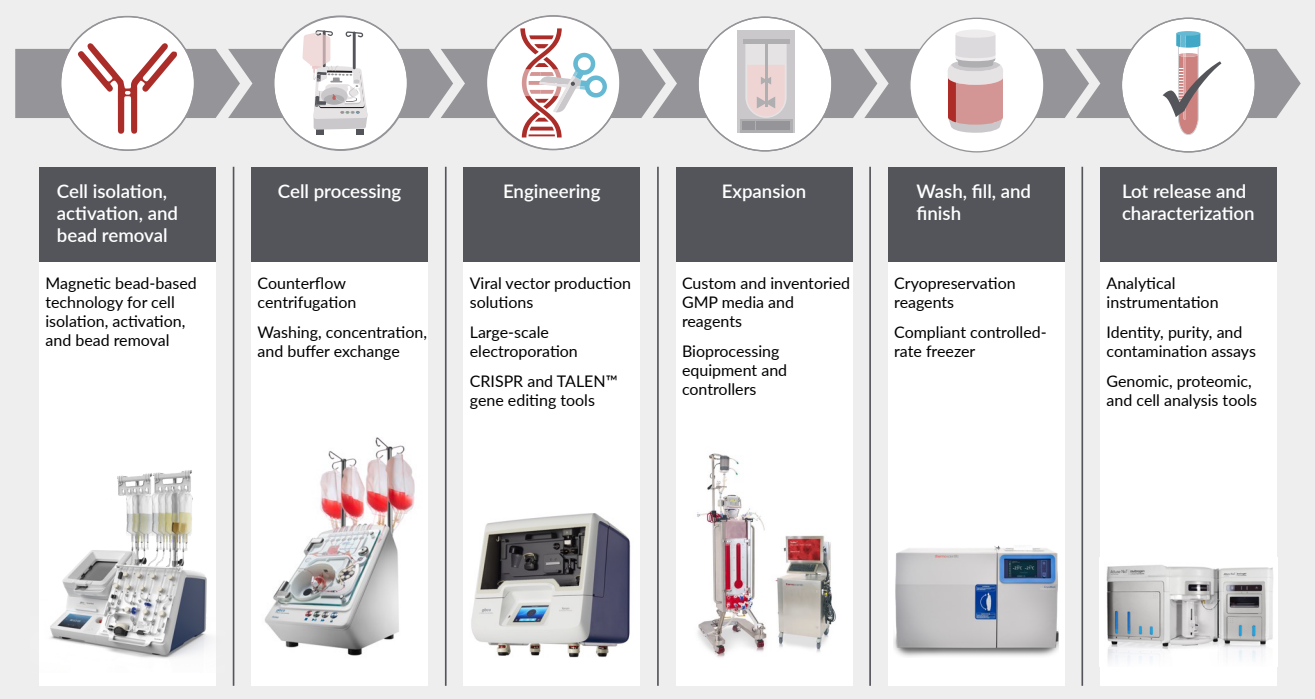
A typical autologous CAR-T workflow is a complex, multi-day GMP manufacturing process, which is often characterized by several labor-intensive and open steps that are prone to errors. These lengthy processes also require costly and extensive QC steps prior to product release. The need for CAR-T cell therapies, however, continues to grow, meaning there is a crucial need to address these challenges so that life-saving therapies can reach patients in a timely manner.

Several of these challenges could be addressed by scaling up the process, closing and automating the manufacturing process, and including in-line analytics. Figure 1 shows Cell Therapy Systems (CTS) solutions developed by Thermo Fisher Scientific, which help provide manufacturers with closed, automated, and modular technologies that can be combined with reagents specially formulated for cell therapy manufacturing to enable end-to-end cell therapy workflows.

The cell therapy instruments in Figure 1 are flexible, fast, and have intuitive touchscreen interfaces that enable users to easily scale their

FIGURE 1

Closed, modular CAR-T cell therapy manufacturing process workflow.



cell therapy processes from research through to clinical manufacturing. Additionally, manual touchpoints in this workflow can be reduced with the use of CTS Cellmation™ software, an off-the-shelf automation solution powered by Emerson's DeltaV™ Distributed Control System (DCS). Each of the CTS instruments comes equipped with an open platform communication unified architecture (OPC-UA) that is compatible with Emerson's DeltaV software.

CTS Cellmation software allows manufacturers and users to digitally connect these closed and modular systems, allowing for data traceability and consistency (using batch recipes), while enabling increased efficiency and scalability.

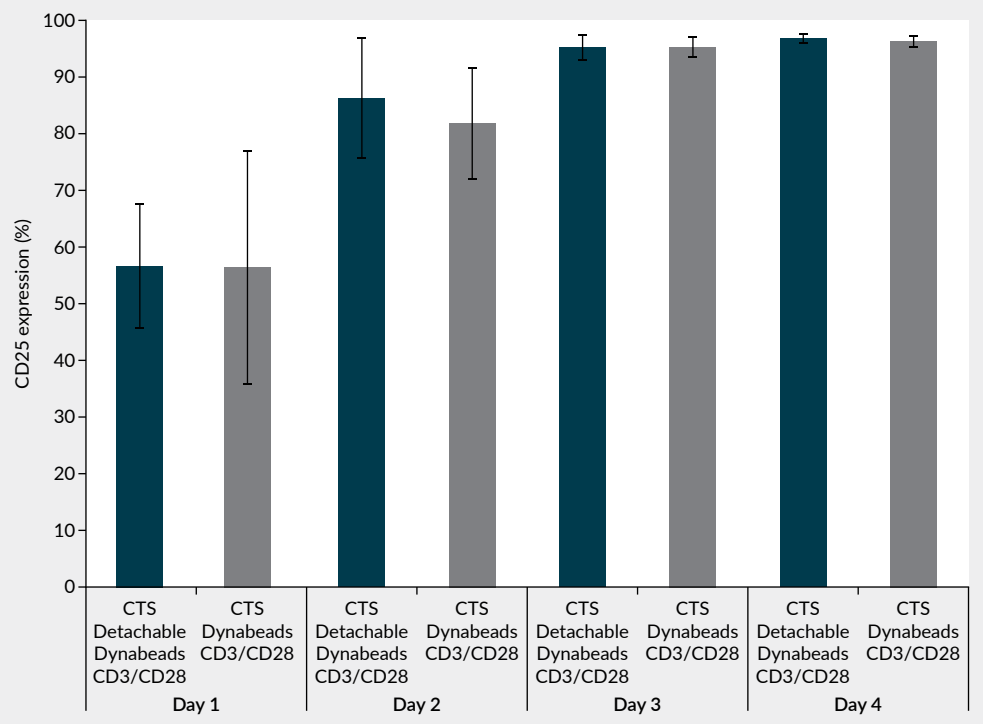
CTS DETACHABLE DYNABEADS CD3/CD28 MAGNETIC BEADS

In addition to the closed and modular instruments, significant efforts have been made to create reagents that can offer increased flexibility and that can be customized and leveraged for different cell therapy workflows. One example is the CTS Detachable Dynabeads CD3/CD28 beads, which combines CaptureSelect™ and Dynabeads™ technology. The CaptureSelect ligands are variable domain of heavy chain (VHH) fragments. The VHH antibodies are 12–15 kDa in size with tunable specificity and affinity to efficiently isolate or activate target cells of interest. These VHH antibodies are free of animal-derived components, highly stable, and suitable for use in GMP environments.

When used with the CTS Detachable Dynabeads Release Buffer, the CTS Detachable Dynabeads CD3/CD28 magnetic beads are actively released, enabling greater control of the T cell activation signal. The same Dynabead magnetic core is currently being used in over 200 active clinical trials, as well as several approved cell therapy drugs. In combination with the CTS DynaCellect system, the Detachable Dynabeads provide a powerful tool that allows for customization,

FIGURE 2

Average CD25 expression for active- and passive-release Dynabeads CD3/CD28 magnetic beads.



which can contribute to high drug efficacy, safety, and cost efficiency downstream of cell isolation.

With an emphasis on automation, flexibility, and scalability, the CTS DynaCellect system includes fit-for-purpose, single-use consumables for cell isolation. It has a touchscreen user interface that allows for the customization of protocols for cell isolation, cell activation, depletion, and magnetic separation.

CTS DETACHABLE DYNABEADS CD3/CD28 VERSUS CTS DYNABEADS CD3/CD28 BEADS

While the passive release Gibco CTS Dynabeads CD3/CD28 (SKU: 40203D) magnetic beads are a robust product which has been used in over 200 active clinical trials and several commercialized drugs, the active release CTS Detachable Dynabeads CD3/CD28 beads help provide manufacturers with better control of the T cell activation signal. Unlike the passive release Dynabeads magnetic beads, which rely on passive dissociation of beads from target cells over time, the CTS Detachable Dynabeads magnetic beads are designed so that users can actively detach at any point, post-isolation, using the CTS Detachable Dynabeads Release Buffer. Furthermore, because inefficient bead removal may result in hyper-activated and exhausted T cells, the ability for users to actively remove the CTS Detachable Dynabeads has the potential to reduce these effects while delivering comparable or improved results compared to the passive release beads.

Figure 2 shows data from a recent study that measured the average expression of T cell activation marker, CD25, for active- and passive-release Dynabeads magnetic beads. T cell expression of CD25 was comparable for both Dynabeads magnetic beads across the time points studied.

CAR-T cells with a less differentiated T memory cell phenotype show higher effectiveness in treating patients with blood

FIGURE 3

T cell memory phenotypes for active-and passive-release CTS Dynabeads magnetic beads.

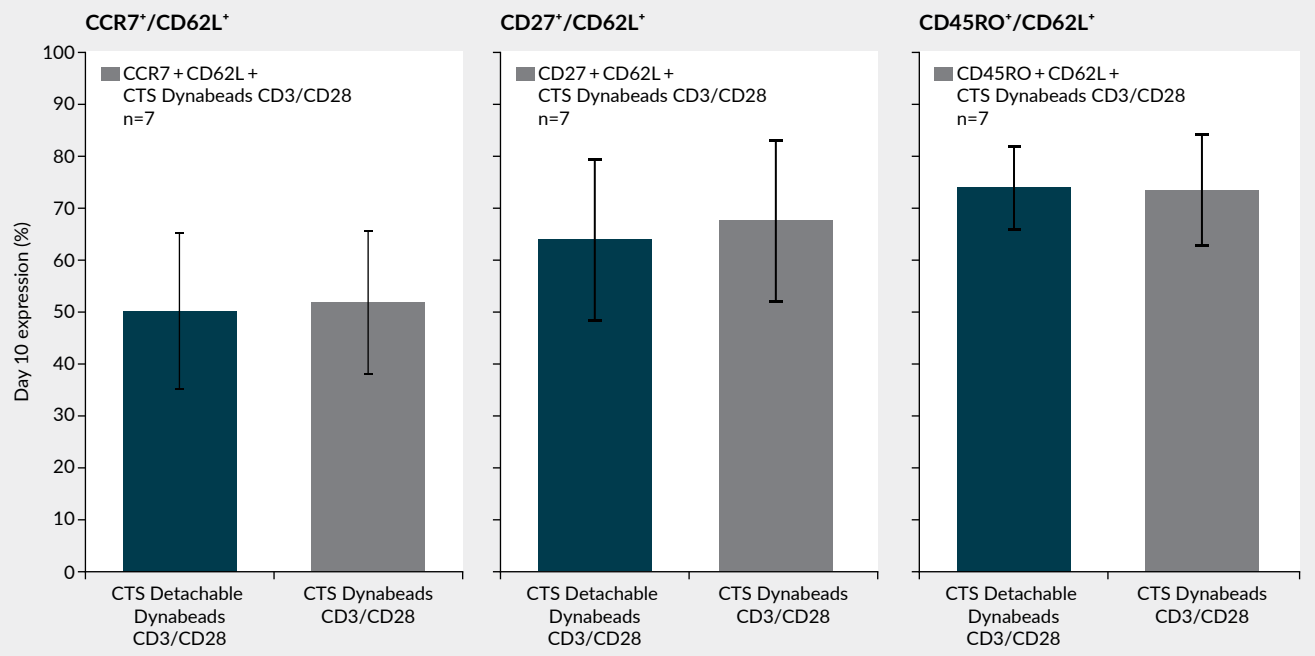
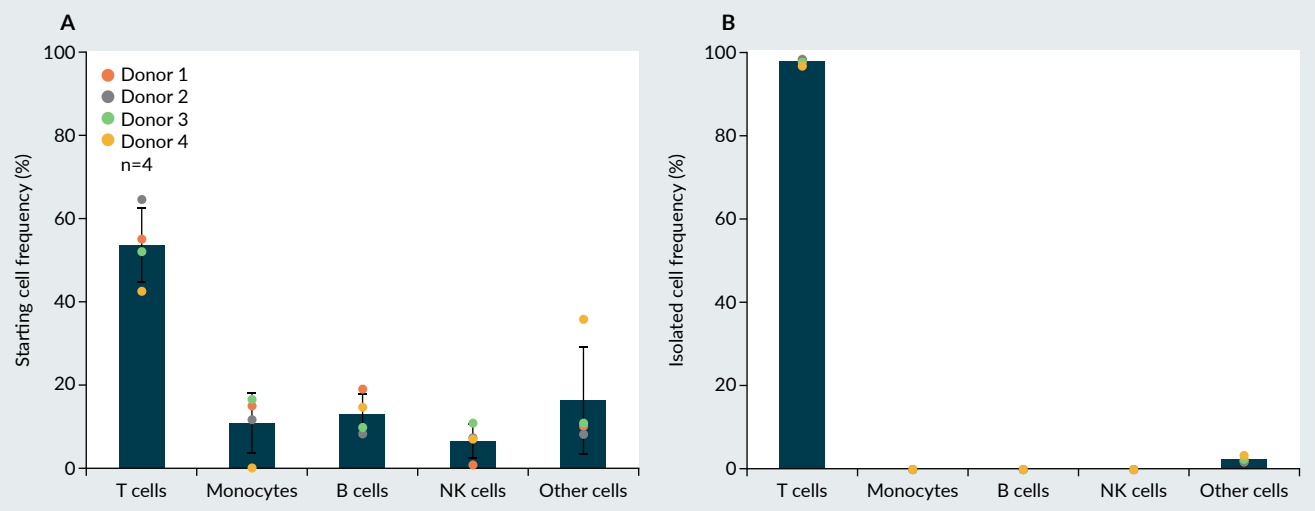


FIGURE 4

T cell purity with CTS Detachable Dynabeads CD3/CD28 beads.



malignancies. Therefore, this attribute was also investigated with both active- and passive-release Dynabeads magnetic beads. CCR7, CD62L, and CD27 were examined to assess the phenotype of early memory cells. Figure 3 shows comparable levels of naïve central memory phenotypes observed on day 10 post-isolation for both passive release and active release CTS Detachable Dynabeads.

Figure 4B shows that T cell purity levels following isolation using CTS Detachable CD3/CD28 beads of >98% was achieved with

samples from four donors. This consistent performance is necessary when starting material is characterized by great biological variability, as shown in Figure 4A. Apheresis profiles differ from one individual to another and fluctuate further based on the patient's indication and stage of disease. CTS Detachable Dynabeads CD3/CD28 magnetic beads can help overcome that challenge by consistently delivering high T cell purity post-isolation.

CASE STUDY: ADDRESSING THE CURRENT CHALLENGES IN CELL THERAPY MANUFACTURING USING A SHORTENED LENTIVIRAL-BASED CAR-T CELL WORKFLOW

The high cost of commercially available CAR-T cell products creates a major access barrier and limits its broad application for

FIGURE 5

Workflow for a 24-hour CAR-T process.

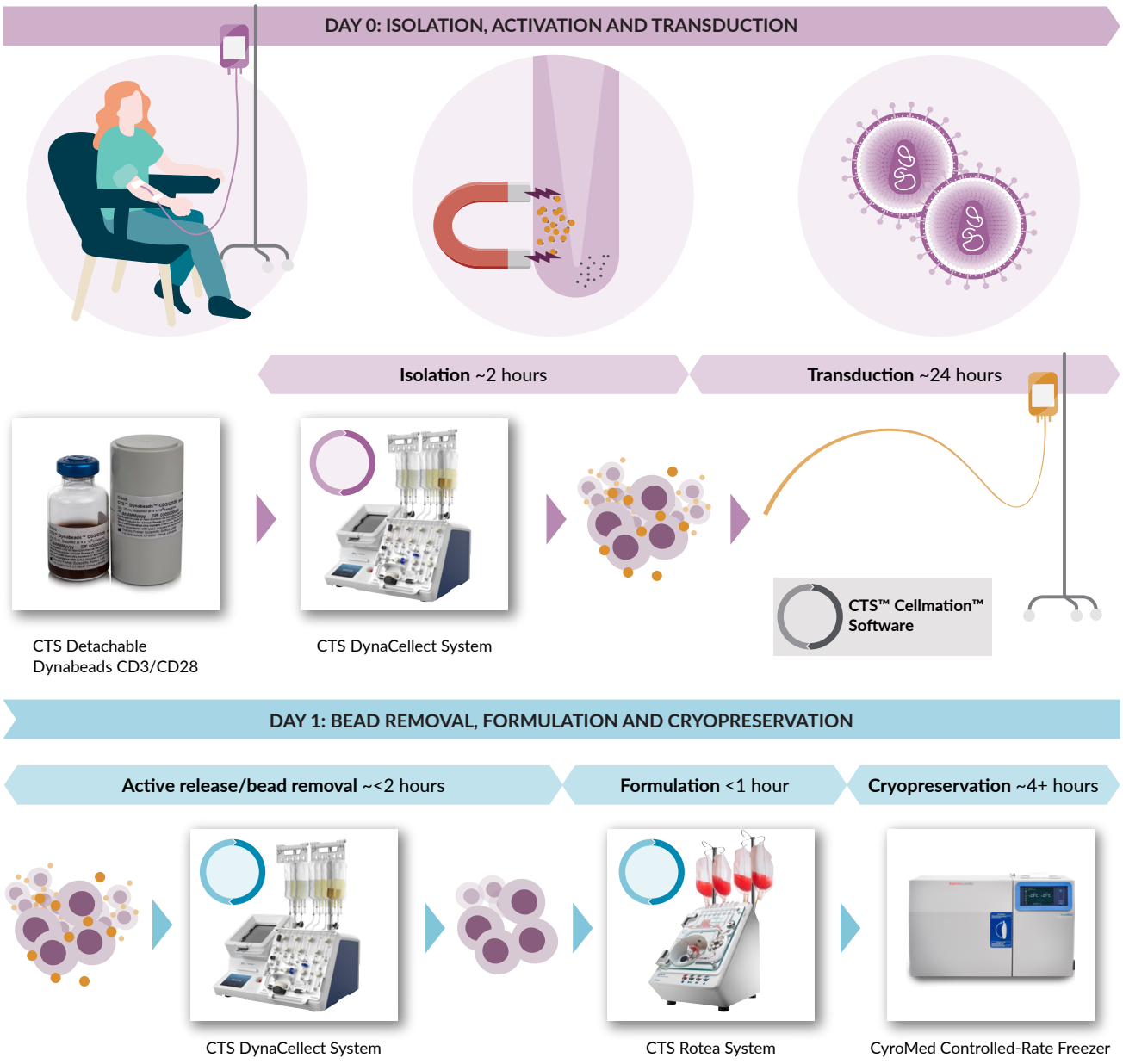


FIGURE 6

LV-CAR expression and total anti-CD19 CAR positive T cells.

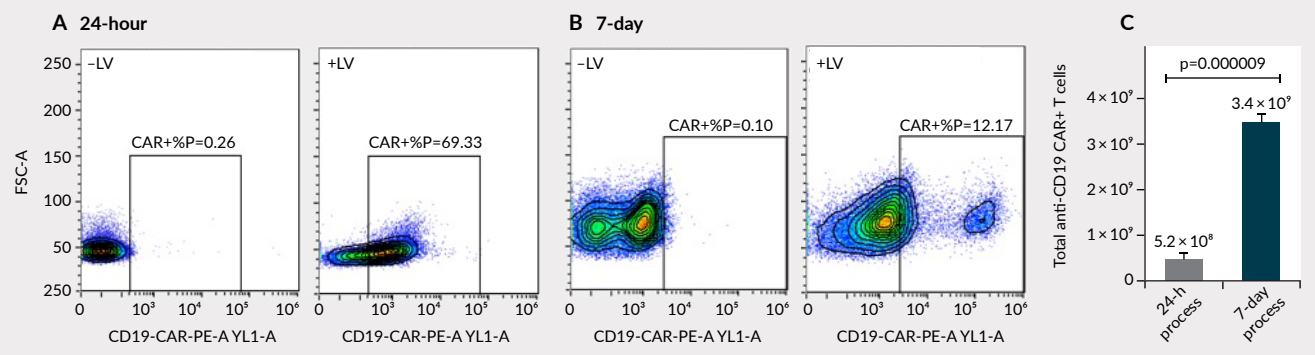
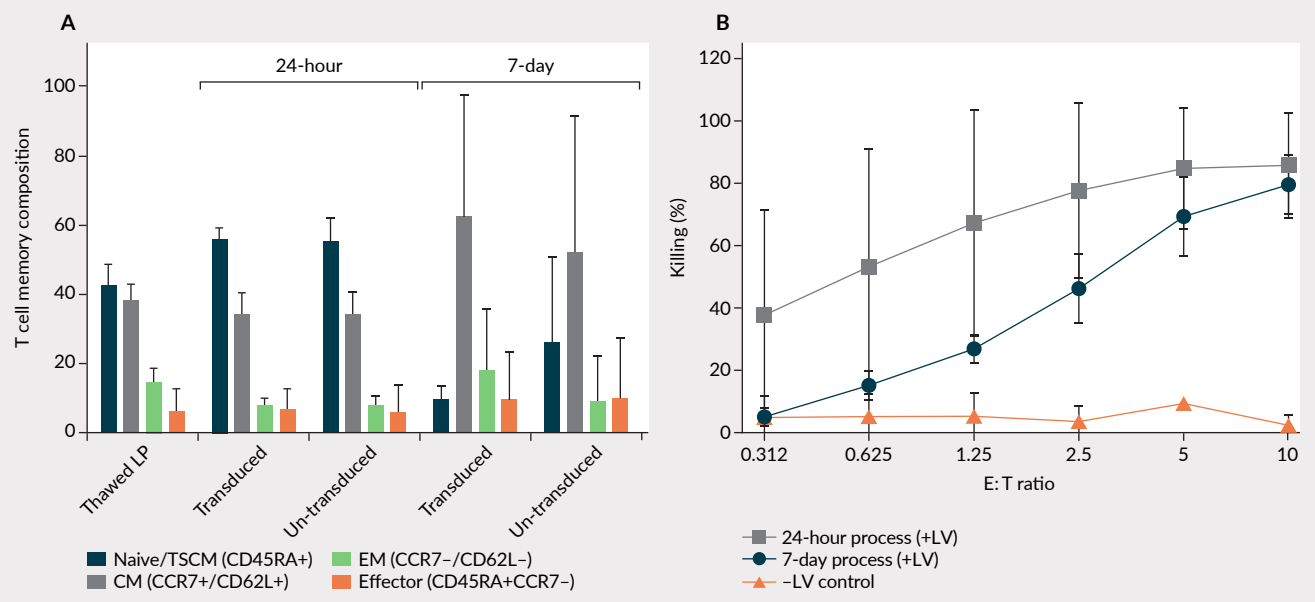


FIGURE 7

CAR-T cell memory phenotypes and cytotoxic activity.



patients who could benefit from these modalities. Recent reports indicate that a significant portion (20%–30%) of patients with B-cell acute lymphoblastic leukemia (B-ALL) who were scheduled to receive CAR-T cell therapy did not end up receiving it due to either rapid disease progression or manufacturing failure [1,2]. Therefore, speeding up the time between apheresis and CAR-T cell therapy infusion is crucial for patients with relapsed refractory B-ALL, as well as other fast-progressing cancers. Additionally, streamlining and automating these processes can help reduce the risk of treatment delays.

Furthermore, traditional CAR-T cell manufacturing often involves a cell expansion step, which can lead to a more differentiated CAR-T cell profile. These highly differentiated cells may exhibit lower cytotoxic activity and cytokine activity compared to less differentiated T cell phenotypes, such as naïve central memory cells or stem cell-like memory T cells.

Shortening these manufacturing workflows not only results in a cost-effective process that helps provide patients with quicker access to CAR-T cell therapies, but importantly, it could result in a product with higher potency [1,2].

Recently, CTS Detachable Dynabeads CD3/CD28 beads were used with the CTS DynaCellect system for one-step isolation and activation of T cells in a 24-hour lentiviral (LV)-based CAR-T workflow. The goal of this study was to eliminate the expansion step in regular autologous CAR-T workflows and shorten the process from a standard 7- to 14-day process to a 24-hour process. Figure 5 highlights a general overview of this workflow.

Under the control of CTS Cellmation software, T cells were isolated with the CTS Detachable Dynabeads CD3/CD28 and the CTS DynaCellect system from quarter Leukopaks. Post-isolation, cells were transduced with a lentiviral vector encoding a CD19-targeted CAR gene with a multiplicity of infection (MOI) of 2. 24 hours later, the CAR-T cells were separated from the CTS Detachable Dynabeads using the CTS DynaCellect system and the CTS Detachable Dynabeads Release Buffer. These CAR-T cells were then washed and concentrated using the Gibco CTS Rotea™ Counterflow Centrifugation System, while the separated CTS Detachable Dynabeads CD3/CD28 beads were captured by the CTS DynaCellect system.

One portion of the washed and concentrated CAR-T cells was cryopreserved using the Thermo Scientific CryoMed™ Controlled-Rate Freezer while a second was cultured for an additional seven days for comparison.

This method resulted in CAR-expressing T cells just 24 hours after lentiviral transduction, as shown in Figure 6.

Figures 6A and 6B illustrate anti-CD19-CAR expression levels on transduced cells at 24 hours and following culture for 7 days.

While a high CAR expression 24 hours post-transduction was observed, there was a drop in CAR-expressing T cell percentage by day 7 post-transduction. This is likely due to high levels of pseudotransduction at earlier time points post-transduction. This observation is not surprising, as it is well known that membrane proteins expressed in packaging cells could be incorporated into an HIV envelope, which can then passively transfer to both activated and naïve T cell membranes.

The lower-than-expected levels of transduction efficiency on day 7 may also be a result of using an MOI of 2 for these experiments, which is on the lower end of what has been routinely used for these types of processes. The percentage of CAR-expressing T cells remained above 40% on day 7 when higher MOIs, (e.g. MOI=5) were used in small scale experiments (data not shown).

Figure 6C shows that the total number of CAR-expressing T cells increased from day 1 to day 7. Although there was a lower percentage of CAR-expressing T cells on day 7 (Figure 6B), there was still effective expansion of CAR T cells resulting in approximately 3.4 billion CAR-T cells by day 7 post-transduction.

As mentioned previously, a key factor in determining the ability of CAR-T cells to engraft following adoptive transfer is their state of differentiation. Preclinical studies show that less differentiated and naïve stem cell-like memory T cell (TSCM) populations show greater potency [1–2].

As shown in Figure 7A, a higher naïve TSCM memory phenotype (CD45RA+/CCR7+) was observed in the 24-hour CAR-T cells, while the 7-day CAR-T cells had an increased number of the more differentiated central memory phenotype (CD45RA-/CCR7+). Both CAR-T cell products had lower numbers of the more differentiated T cell phenotypes-effector memory cells and effector T cells.

The CAR-T cell potency for each process was measured by exposing the two different CAR-T cell products to CD19-expressing NALM6 cells. 24-hour CAR-T cells exhibited higher cytotoxicity, especially at the lower effector-to-target (E:T) ratios (Figure 7B).

Overall, this data suggests that the 24-hour process results in a highly functional CAR-T product with an enrichment of early T memory phenotype cells.

SUMMARY AND FUTURE DIRECTIONS

In this study, a simplified and automated 24-hour LV-based CAR-T cell manufacturing workflow produced CAR-T cells with improved cytotoxicity compared to a 7-day process.

Future efforts will be made to expand optimization for these shorter workflows. It is also key that the potency of such products is tested with animal models. There are important ongoing efforts to implement proper assays to better characterize cell therapy products generated through shorter workflows such as the 24-hour LV-based workflow. For example, one concern regarding these shorter LV-based workflows is the pseudotransduction that appears to happen at earlier time points post-transduction.

Besides using flow cytometry to check the CAR expression on the cell surface, a more direct approach must be implemented to allow more input regarding CAR integration at earlier time points. This can help the users of such workflows to better determine the dosage of the final drug product.

Q&A

MINA AHMADI AND FABIO FACHIN

Q Have you observed any difference in CD4 versus CD8 transduction efficiency in the shorter process?

MA: We did not see any differences between the transduction efficiency in our CD4 and CD8 populations. The transduction efficiency was similar in both early time points as well as later time points.

Q Does this shorter CAR-T workflow fit a centralized or a decentralized model?

FF: Both of these models can and will coexist. This is an area that will generate a lot of excitement in terms of the future and some of these new approaches. When it comes to start-ups, biotechs, and academic centers, I do feel that the decentralized approach represents a novel manufacturing space where these players can now demonstrate their drugs.

That approach was not available at the beginning of the industry. The ability to be able to manufacture close to the clinical centers and be able to leverage this broader network, especially with some of these 'GMP on demand' and movable GMP pods that are starting to become available, is quite exciting. It also fits nicely into this shorter manufacturing span where there is more turnaround of the plots.

At the same time though, as I said, these models will have to coexist. It is naïve to think that manufacturing infrastructure will be available everywhere. That balance will need to play out, especially as products move towards the commercial side.

That is perhaps where the more centralized approach can be leveraged. There are benefits there in terms of economies of scale, which is something to consider. I am very curious to see how it pans out.

So, to answer the question: I do believe that this rapid process does lend itself more to a decentralized approach than the more traditional CAR-T cell therapy manufacturing processes.

Q What are the pros and cons of all-in-one versus modular automation?

FF: It depends on the maturity of your process and product. Simplistically, the more you move towards, for example, Phase II clinical trials or commercialization, the more your process and product become concrete.

In that regard, there are many benefits of an all-in-one solution because you have a locked-in process to pursue. The downside of having an all-in-one device is that you are allocating that instrument to your lot for the entire duration of the manufacturing. That is where I see, again, the rapid manufacturing approaches being a little more amenable to the solution.

They provide a shorter timeframe, so you are not utilizing an instrument for 12 days, but rather you are dedicating only 3 days or so to that instrument. However, there are also the downsides of being locked in to one partner as opposed to having the ability to derisk your supply chain. To rectify this, there are solutions in progress with a little bit more flexibility. On the other side, the modular approach is more dynamic. It gives you the ability to mix and match, to some extent.

New instrumentation is coming all the time, so another downside to an all-in-one approach is that if a great technology comes out, you would need to essentially get out of your all-in-one and do a process in this other modular component and then go back, which could introduce error. The modular approach is easier to use, especially earlier in your development, but this approach also has more potential failure points. There are different connections, different disposables, and different data systems that need to be considered. There are also more vendors that you need to manage and support.

The balance between both approaches will continue to evolve, and it is important that each continues to be implemented and pursued.

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BIOGRAPHIES

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AUTHORSHIP & CONFLICT OF INTEREST

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