Fully Automated and Closed Cell Processing System for Clinical-Scale Final Formulation, Fill, and Finish of Engineered T-Cells

Kyra Sur[1], Elisaveta Todorova[1], Savannah Szemethy[1], Tyler Sinacola[1], Brent Mcavoy[1], Casey Kuenn[2], Vandana Keskar[1]
[1]TScan Therapeutics, Waltham, MA, USA; [2]ScaleReady, Saint Paul, MN, USA

INTRODUCTION

- The final formulation, fill, and finish (F/F/F) step in the manufacturing of Cell and Gene therapies is essential to harvest, wash, and prepare engineered cells for cryopreservation.
- Current methods of F/F/F either require manual processing, which has the potential to result in operator error, or utilize automated systems with limited system capabilities. Manual methods or semi-automated systems may present the risk of increased exposure time of cells to the cryoprotectant, causing a reduction in post-thaw viability and low recovery.
- The Cue® Cell Processing System (Fresenius Kabi) provides an opportunity to fully automate a functionally closed F/F/F unit operation using enhanced system features for consistent and robust processing.

Fig. 1. Cue® Cell Processing System

Table 1. Cue® Cell Processing System for fully automated and closed F/F/F

Desired Instrument Criteria	Cue®	Competitor(s)
GMP Status	GMP Certified	GMP Certified
Closed/Automated	✓ / Fully automated	✓ / Semi-automated
Sampling In-Process	✓	×
Mixing Capabilities	✓	×
Temperature Controlled	✓	×
Aliquoting Flexibility	✓	×
Air Removal	✓	×
Output Format	Bags, Closed system vials, QC sample	Bags, Vials
Fill Accuracy	Max ± 10% or 1mL[1]	Max ± 25%
Additional Unit Ops	✓	✓
Processing Time	< 30 min per cycle	1-2 hrs[2]
Maximum Cell Input	N/A	Max 5e10 total cells[3]
Integrated Interface	✓	✓
Footprint (sqft)	4.6	Max 4.8[3]

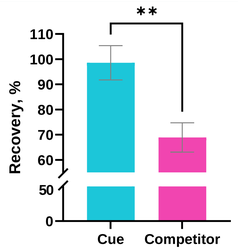
[1]Whichever is greater; [2]Dependent on input volume; [3]Device dependent

METHODS

- T cells were isolated from healthy donor samples (n=2), engineered, and subsequently expanded in culture.
- On harvest day, cells were split (1 billion total engineered T cells for n=3 runs) and run on both the Cue®, using a fully automated protocol generated by ScaleReady, and the Competitor, using a semi-automated protocol.
- The Cue® system performed a washout of spent culture media and reformulated cells into complete cryopreservation media at the desired final cell concentration. The Competitor performed a washout of spent culture media and suspended cells into formulation buffer, cryopreservation medium was then manually added to achieve the desired final cell concentration.
- Cell count and viability (CCV) samples were taken following harvest from bioreactor, suspension in buffer, and final formulation with cryoprotectant. Flow cytometry was performed on harvest samples.
- Final formulated cell suspension for both the Cue® and the Competitor was manually aliquoted into cryovials, all cryovials were cryopreserved using a controlled rate freezer.
- Samples for all runs (n=3 per F/F/F method) were thawed and recovered in culture media, post-thaw samples were analyzed for CCVs and via flow cytometry.

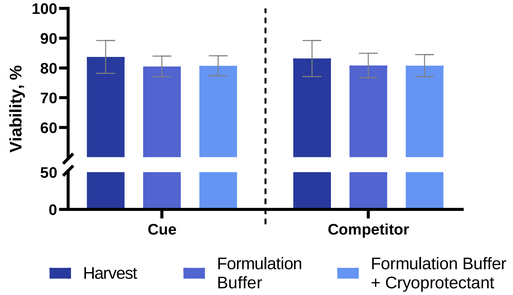
PROCESSING RESULTS

Fig. 2. Total engineered T cell output recovery



- Fig. 2. displays average output recovery and standard deviation of total engineered T cells. Recoveries were calculated following automated processing for each F/F/F method.
- Post-processing recovery was significantly higher (p=0.0046) for the Cue® compared to the Competitor (98.6% ± 7.9% vs. 68.9% ± 6.2% respectively).

Fig. 3. Cell viability throughout F/F/F unit operation



- Fig. 3. exhibits average viability and standard deviation of engineered T cell population throughout F/F/F.
- Viability throughout processing was maintained between the Cue® system in comparison to the semi-automated competitor (Harvest: 83.7% ± 6.4% vs. 83.2% ± 6.9, Formulation buffer: 80.5% ± 4% vs. 80.8% ± 4.6%, Formulation buffer and cryoprotectant 80.7% ± 3.9% vs. 80.7% ± 3.8%).

Sources
Fig. 1. Copyright of ScaleReady. Image used with permission. Source: ScaleReady.com/Cue

Property
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Acknowledgements
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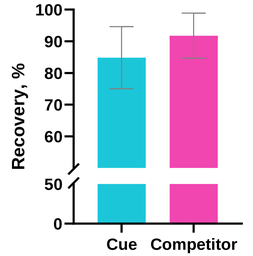
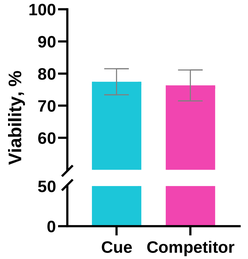
POST-THAW RESULTS

Fig. 4. Post-thaw sample recovery



- Samples for all runs (n=3 per F/F/F method) were thawed and recovered in pre-warmed culture media, then CCVs were taken.
- Fig. 4. displays average sample recovery and standard deviation post-thaw.
- Post-thaw recovery on the Cue® was comparable to the Competitor (84.8% ± 11.1% vs. 91.8% ± 7.7% respectively).

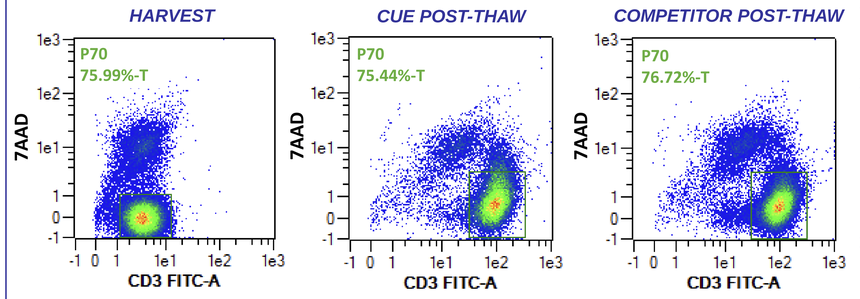
Fig. 5. Post-thaw sample viability



- Samples for all runs (n=3 per F/F/F method) were thawed and recovered in pre-warmed culture media, then CCVs were taken.
- Fig. 5. shows average viability and standard deviation post-thaw.
- Viability was preserved across both the Cue® and the Competitor (77.5% ± 4.6% vs. 76.3% ± 5.4% respectively).

FLOW CYTOMETRY RESULTS

Fig. 6. Live CD3+ flow cytometry plots for bioreactor harvest and post-thaw samples



- Flow cytometry was performed on all samples (n=9) from each run (three representative plots depicted).
- Fig.6. depicts live, cluster of differentiation 3 positive (CD3+) cell. Plots shown are samples from bioreactor harvest (left), post-thaw formulated on the Cue® (center), and post-thaw formulated with the Competitor (right).
- CD3+ population was maintained throughout F/F/F and cryopreservation between the Cue® and the Competitor.

CONCLUSION

- The Cue® Cell Processing System (Fresenius Kabi) enables a fully automated and functionally-closed final formulation, fill, and finish of engineered T-cells for streamlined clinical-scale manufacturing.
- Results display comparable recovery and viability of samples to pre-existing instrumentation and methods.
- Integration of the system into a manufacturing workflow can provide a more reliable and robust final formulation, fill, and finish unit operation in the generation of Cell and Gene therapies.