Better Cells, Better Outcomes: Improving CAR-T Manufacturing with Animal-Free

Cryopreservation and Wash Solutions

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Results

Viable Cells Retained

Expansio

Fold-

Introduction

KEY POINTS

- T cell-based therapies rely on tight control of downstream steps like washing and cryopreservation to ensure viable, high-quality final products.
- Albumin plays a key role in:
 - · Protecting T cells from shear stress during wash
 - Stabilizing cell membranes to reduce damage
 - Minimizing clumping to improve handling and dose uniformity
 - Enhancing post-thaw viability, recovery, and proliferation
 - Preserving functional phenotypes during freeze/thaw and formulation
- · However, traditional blood-derived HSA presents:
 - Safety risks
 - Regulatory barriers
 - Supply chain challenges
- · Optibumin, InVitria's recombinant human serum albumin (rHSA), offers a consistent, animal-originfree alternative.
- This study evaluates the use of Optibumin in two downstream operations:
 - Washing
 - Cryopreservation

Methods

CELL PREPARATION

- Human T cells from 2 healthy adult donors
- CD3/CD28 activation (3 days) WASH STUDY
- Washed 3x with PBS ± 1% or 5% albumin (Optibumin or HSA)
- 2-hour post-wash hold
- · Viable cell retention measured by high-content imaging

CRYOPRESERVATION

- · CryoStor CS10 (10% DMSO) or CS5 (5% DMSO)
- 0%, 5%, or 10% albumin (25% stock added, displacing CrvoStor)
- Frozen in Mr. Frosty™; stored in LN2
- · Post-thaw viability and proliferation tracked for 72 hours PHENOTYPING
- Flow cytometry at 24 & 72 hours post-wash/thaw

Leukapheresis . . <u>ه</u>و \bigcirc T Cell Isolation Formulation & & Washing R. **CAR-T** Process Harvest & Cell T Cell ITSE-A Animal Free Activatio Č. Y 000 000 Opti Leukin' 2 Cell Expansion Gene Transfer Ŏ,Ŏ

InVitria's AOF Products in Key CAR-T Manufacturing Steps

WASHING PERFORMANCE



Figure 1: T Cell Viability Post-Wash - Viable T cell retention after downstream washing. Cells washed in PBS alone retained only 15-25% of initial viable cells. Inclusion of 1% or 5% albumin (Optibumin or HSA) improved retention to ~65%. Optibumin outperformed clinical HSA in Donor 1 (p < 0.05, one way ANOVA) and matched HSA in Donor 2.

CRYOPRESERVATION

Post-Thaw Proliferation



72h Expansion Post-Thaw



CAR-T MANUFACTURING WORKFLOW

Performance. Defined.

CAR-T therapy requires precise control across a multi-step process-from leukapheresis to infusion. Among these steps, cell washing and cryopreservation are two of the most stress-sensitive and critical for maintaining final product viability.

Optibumin®, a recombinant, animal-origin-free human serum albumin (rHSA), supports these downstream operations by:

- Reducing cell loss and minimizing clumping during washing
- Stabilizing membranes to protect cells through freeze/thaw stress

• Enabling lower DMSO concentrations for safer cryopreservation Together, these benefits help manufacturers deliver scalable, regulatoryaligned CAR-T products with improved consistency and safety.



Figure 2. CD4/CD8 Ratio Post-Wash - CD4/CD8 ratios 72 hours post-wash. Donor 1 retained a 4:1 ratio; Donor 2 retained a 2:1 ratio. Albumin concentration and source had no impact, demonstrating compatibility with a range of process conditions



Figure 3. Memory Phenotype Preservation Post-Wash- T cell memory phenotype 3 days post-wash. Early memory phenotypes (Tscm, Tcm) were maintained across all conditions. Optibumin preserved phenotypes comparable to HSA and PBS controls.

Figure 4. Post-Thaw Proliferation Curves -Normalized T cell growth curves post-thaw. Optibumin enabled greater proliferation across both donors compared to HSA or PBS. Cells were monitored over 72 hours in culture.

DMSO Dilution

	CryoStor CS10	CryoStor CS5	
No albumin control	10%		5%
5% Albumin	8%		4%
10% Albumin	6%		3%

Table 1. Final DMSO Concentrations - DMSO dilution via 25% albumin stock addition. Final DMSO content was reduced from 10% to 6-8% (CS10) and from 5% to 3-4% (CS5), supporting safer formulations for clinical use.

> Figure 5. T Cell **Proliferation Post-Thaw** (72 Hours) - Viable T cell expansion following cryopreservation. T cells cryopreserved with 10% Optibumin in CryoStor CS10 expanded ~2-fold post-thaw. In CS5, 10% Optibumin abled 1 25

Conclusion

Optibumin is a high-performance, recombinant human serum albumin (rHSA) that provides a reliable, animalorigin-free alternative to plasma-derived HSA in CAR-T manufacturing.

Improves viable T cell recovery during downstream washing

- Outperforms PBS and matches or exceeds clinical-grade HSA
- Reduces shear stress, stabilizes membranes, and minimizes clumping

Preserves key phenotypes

Early memory markers (Tscm, Tcm) maintained post-wash and post-thaw



- Markers: CD45RA, CCR7
- Subsets: Tscm, Tcm, Tem, Temra
- · CD4/CD8 ratio also assessed



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CD4/CD8 Ratio Post-Thaw



Memory Phenotype Post-Thaw



expansion. Optibumin significantly outperformed HSA and no-albumin controls (p < 0.001 for CS10, p < 0.05 for CS5).

Figure 6. CD4/CD8 Ratio Post-Thaw

CD4+ and CD8+ population proportions 24 hours post-thaw. Albumin addition had no impact on helper vs. cytotoxic T cell ratio. Optibumin preserved physiological balance post-cryopreservation.

Figure 7. T Cell Memory **Phenotype Post-Thaw**

Flow cytometry analysis of T cell memory subsets 24 hours post-thaw. Tscm and Tcm populations were preserved at >80% across all albumin and control conditions. No phenotype loss was observed with Optibumin.



- Reduces final DMSO levels (from 10% to 6% in CS10; 5% to 3% in CS5)
- · Enhances compatibility with clinical delivery requirements





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