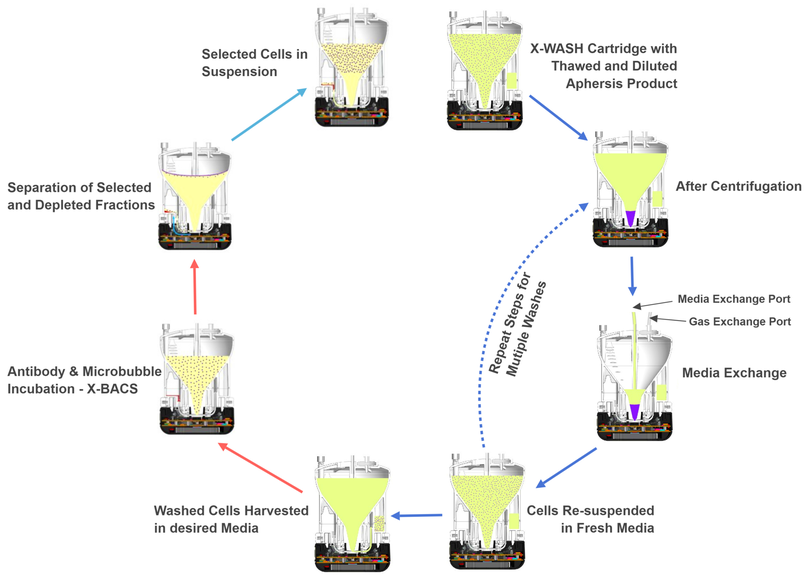
DMSO Removal & CD3⁺ Cell Selection from Cryopreserved Apheresis Products Using Novel BACS Technology

Dalip Sethi[1*], Jillian Miller[1], Zelenia Contreras[2], John Perea[2] and Stephen Truong[2]
[1]Cesca Therapeutics, Inc., Rancho Cordova, CA; [2]ThermoGenesis Corp., Rancho Cordova, CA

Introduction

Cancer therapy is a complex and growing field of medicine. With the advent of adoptive immunotherapy, coupled with excellent clinical outcomes in CD19⁺ hematologic malignancies, CAR-T cell therapy has created intense interest within the medical and scientific communities. Although genetic-modification of immune cells is one of the most advanced therapies, the manufacturing process still relies on traditional cell processing techniques. We have previously developed a quick and easy method to remove DMSO from thawed apheresis products and have now coupled this technology with the selection of CD3⁺ T-cells using ThermoGenesis' technology, Buoyancy-Activated Cell Sorting (X-BACS™) process. DMSO-removal was successfully achieved using the X-WASH™ System.

Process Flow



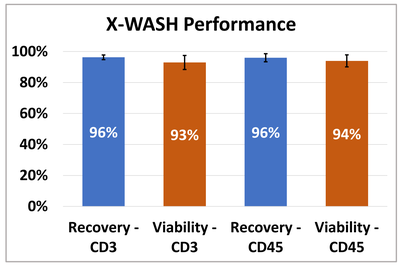
*Corresponding author: dsethi@cescatherapeutics.com

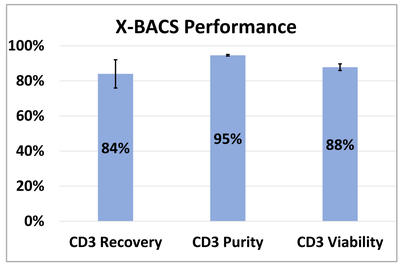
Materials and Methods

Healthy adult cryopreserved apheresis samples were thawed at 37°C and diluted with thaw-wash media (2.5% HSA, 5% Dextran, and DNase I in saline). The sample was transferred to an X-WASH Disposable Cartridge (DC). Post-centrifugation, waste media was removed using positive pressure and new media was added using controlled negative pressure. Washed cells were transferred to a new DC and incubated with CD3 antibody followed by incubation with microbubble reagent. The target and non-target fractions were separated using centrifugation. CD3⁺ cells were also studied for expansion using CD3/CD28 and IL-2 activation.

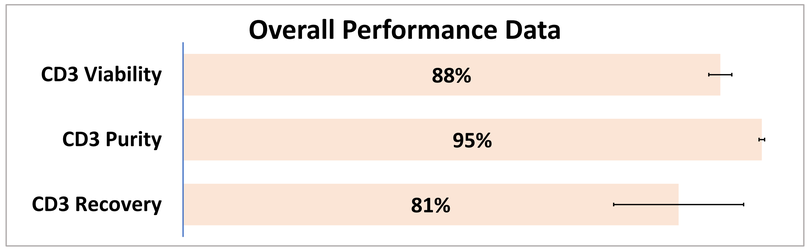
Results

The DMSO removal from cryopreserved samples and CD3⁺ cell isolation was accomplished in two phases. Each phase was studied for recovery efficiency and cell viability.

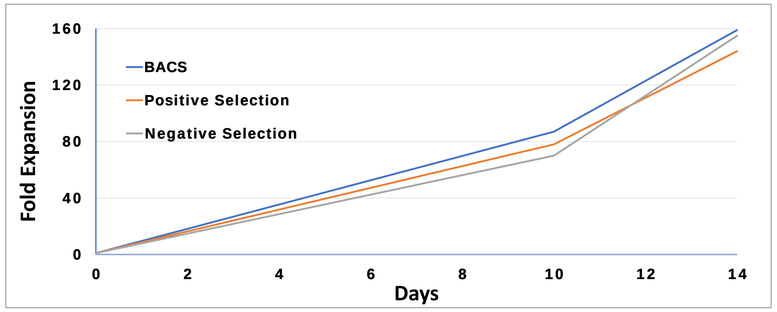




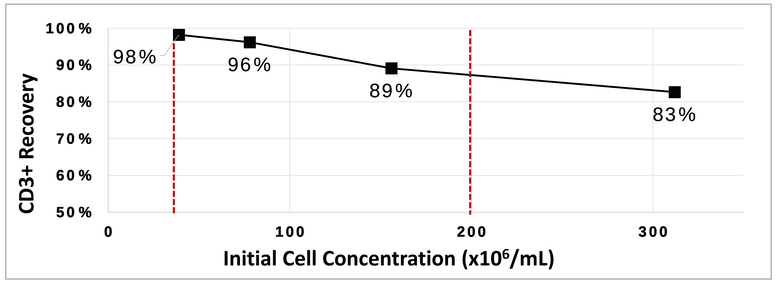
Overall recovery was calculated by multiplying values obtained in X-WASH and X-BACS steps. A mean CD3⁺ cell recovery of 81% was observed (n =3).



In a separate experiment, the expansion of CD3⁺ cells isolated using X-BACS was equivalent to CD3⁺ cells isolated using technologies from other manufacturers. Viable CD3⁺ cells were quantified over 14 days using flow cytometry.



To assess the consistency of CD3⁺ cell recoveries using the X-BACS process, the antibody and microbubble concentrations were kept constant while cell concentrations were increased. The results indicate only a marginal drop in CD3⁺ cell recovery within the normally expected dose range (30 – 200 x10⁶/mL).



Conclusions

DMSO removal using the X-WASH System resulted in cell recoveries of greater than 90% with no significant loss of viability. A mean CD3⁺ cell recovery of 84% was accomplished using the X-BACS process with high purity (>94%). In brief, we have developed an efficient method for removing DMSO and isolating CD3⁺ T-cells from cryopreserved apheresis samples that can be used for CAR-T cell manufacturing.

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