THINK FORWARD TO HSPCs IN THE CLINIC

With cGMP-Manufactured StemSpan™-AOF

Hematopoietic stem and progenitor cells (HSPCs) are widely used in cell and gene therapy applications. When culturing HSPCs for cell therapy research, it is important to minimize risk and variability in your cell culture medium to ensure consistent, reproducible performance and safety. Whether you are performing fundamental research or ready to transition to the clinic, StemSpan™-AOF medium helps minimize the risk of viral contamination in your cell therapy research. Choosing animal origin-free (AOF) cell culture conditions can facilitate a smoother pathway to the clinic by helping you avoid regulatory roadblocks. StemSpan™-AOF is also manufactured under relevant cGMPs, ensuring the highest quality and consistency for reproducible results.

StemSpan™-AOF for Gene Editing Applications

The ability to genetically manipulate HSPCs has significantly advanced our understanding of the mechanisms that regulate hematopoiesis and is contributing to the development of novel cellular therapies. Using a medium that supports genome editing of hematopoietic cells can help take your cell therapy research to the next level. See how StemSpan™-AOF supports optimal culture conditions for HSPC maintenance and expansion in CRISPR-Cas9 gene editing applications:

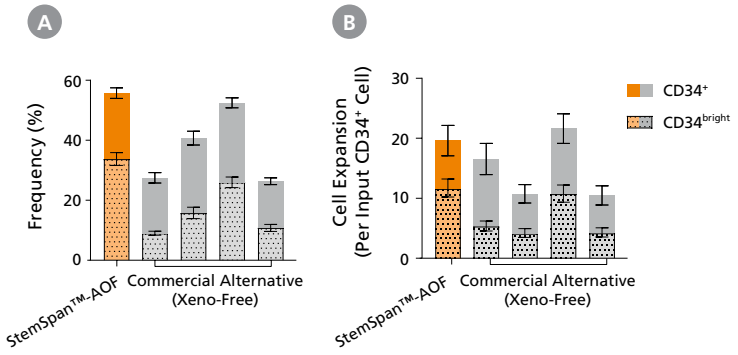

Figure 1. StemSpan™-AOF Supports Equivalent or Greater Expansion of Human CD34$^+$ and CD34^{bright} Cells Compared to Other Commercial Media

Purified cord blood-derived CD34$^+$ cells were cultured for 7 days in StemSpan™-AOF (orange bar) and in four alternative commercial media (gray bars). Each medium was supplemented with StemSpan™ CD34$^+$ Expansion Supplement and 175 nM UM171*. The (A) frequency and (B) cell expansion of viable CD34$^+$ and CD34^{bright} cells in culture were measured based on viable cell counts and flow cytometry results. StemSpan™-AOF, the only animal origin-free formulation, showed equivalent or greater performance to all xeno-free alternative media tested.

Why Use StemSpan™-AOF for Cell Therapy Research?

SAFE. Minimize the risk of viral contamination by using a medium that does not contain any primary or secondary raw materials derived from animals.

ROBUST. Ensure consistency in your experiments by using serum-free and animal origin-free culture conditions.

FLEXIBLE. Customize your cell culture conditions by adding StemSpan™ Expansion Supplements, individual cytokines, or additives to suit your specific cell therapy research needs.

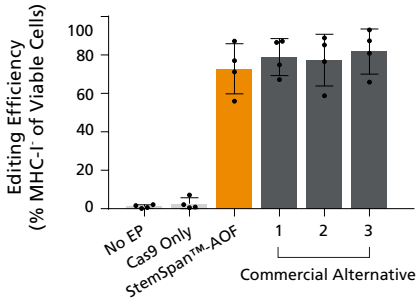

Figure 2. Human CD34$^+$ Cells Cultured in StemSpan™-AOF Show Equivalent Gene Editing Efficiency Compared with Alternative Media

Cells cultured for 2 days in either StemSpan™-AOF (orange bar) or xeno-free alternative commercial media (gray bars), each supplemented with StemSpan™ CD34$^+$ Expansion Supplement and 175 nM UM171*, were electroporated with CRISPR-Cas9 RNP complexes containing crRNA:tracrRNA targeting β2 Microglobulin (B2M). Non-electroporated (No EP) cells and cells electroporated with Cas9 without gRNA (Cas9 Only) were cultured in StemSpan™ SFEM II supplemented with StemSpan™ CD34$^+$ Expansion Supplement plus 175 nM UM171*. B2M knockout efficiency (% MHC-I$^-$ viable cells) was monitored by flow cytometry using a fluorophore-conjugated anti-MHC-I antibody.

*Similar results are expected when using UM729 (Catalog #72332) prepared to a final concentration of 1 µM. For more information, including data comparing UM171 and UM729, see Fares et al. Science 345, 1509-1512, 2014.

In Vivo Engraftment of HSPCs

One of the best assays to determine the quality of a hematopoietic cell therapy product is evaluation of its engraftment and multilineage differentiation potential after intravenous injection into immunodeficient mice (e.g. NOD scid gamma (NSG) mice). The "stemness" of HSPCs can be affected by many parameters, such as cell processing methods and culture conditions used for expansion and gene editing, which may impact the ability of the cells to successfully engraft. StemSpan™-AOF, the only animal origin-free cGMP medium on the market, supports multilineage engraftment of CD34⁺ cells at equivalent or higher levels when compared to uncultured cells.

See how StemSpan™-AOF supports the engraftment and expansion of cord blood-derived CD34⁺ cells in NSG mouse recipients:

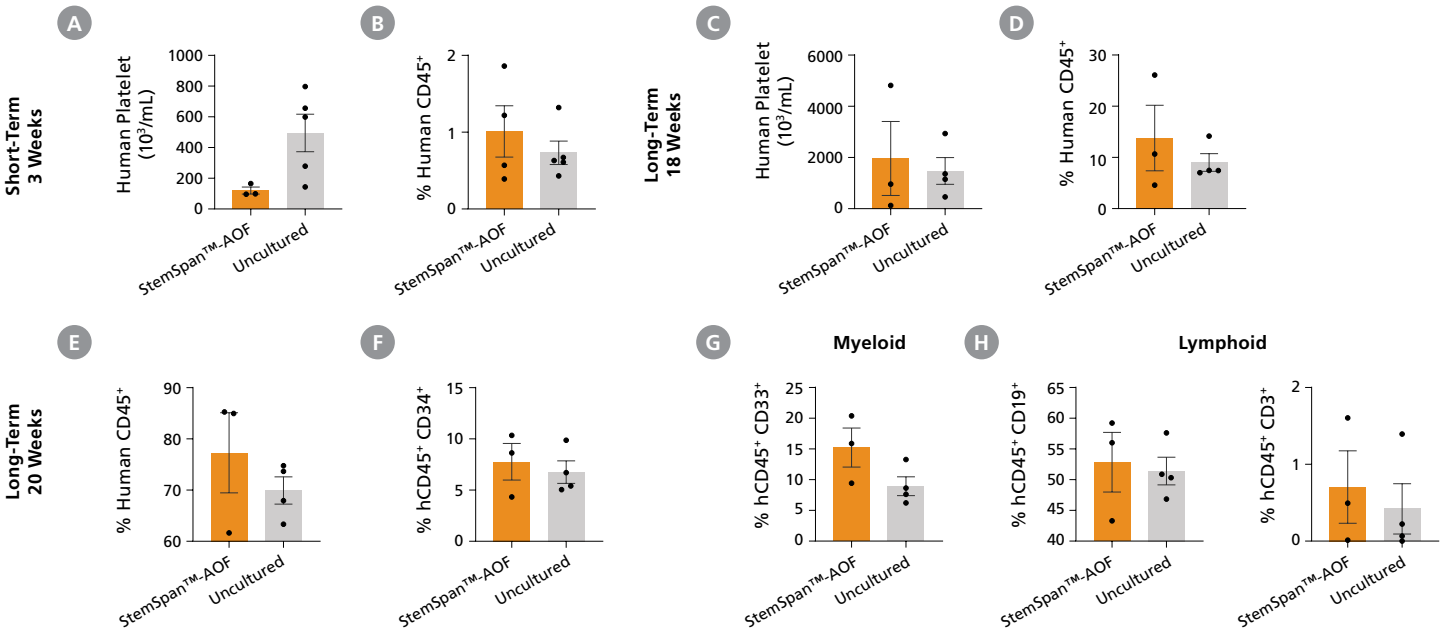

Figure 3. StemSpan™-AOF-Expanded Cord Blood CD34⁺ Cells Engraft in NSG Mouse Recipients

Purified cord blood-derived CD34⁺ cells were cultured for 7 days in StemSpan™-AOF supplemented with StemSpan™ CD34⁺ Expansion Supplement and UM729 (1 µM). After 7 days of expansion, progeny of 10,000 fresh or uncultured CD34⁺ cells were transplanted in sub-lethally irradiated NSG mice. (A-D) The number of human platelets and the frequency of human cells expressing the pan-leukocyte marker CD45 were measured in peripheral blood at 3 and 18 weeks post-transplantation. Data shown are mean ± SEM (n = 3 - 5 mice). (A) At 3 weeks, engraftment of human platelets was lower in recipients of cells cultured in StemSpan™-AOF than in recipients of uncultured cells. (C) At week 18, there were no significant differences in platelet engraftment between the expanded and uncultured cells. (B,D) Human CD45⁺ cell frequencies in recipients of cells expanded in StemSpan™-AOF were similar to those in recipients of uncultured cells. (E-H) At week 20, long-term multilineage engraftment was measured in bone marrow of transplanted NSG mice. Data shown are mean ± SEM (n = 3 - 4 mice). (E,F) Recipients of StemSpan™-AOF expanded cells showed similar frequencies of human CD45⁺ and CD34⁺ cells in the mouse bone marrow compared to recipients of uncultured cells. (G,H) Cells expanded with StemSpan™-AOF showed similar levels of myeloid (CD45⁺CD33⁺) and lymphoid (CD45⁺19⁺ B cells and CD45⁺CD3⁺ T cells) engraftment relative to uncultured cells.

Taking Your Research to the Clinic?

STEMCELL's Services for Cell Therapy program has a team of experts who can help support your regulatory filing by providing custom solutions such as quality documentation, additional product testing, and customized product manufacturing. To learn more about how we can support your preclinical and clinical research needs, visit us at **www.stemcell.com/cell-therapy-services**

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