SUPERIOR EXPANSION OF LONG-TERM HEMATOPOIETIC STEM CELLS USING StemPro™ HSC MEDIUM KIT

Navjot Kaur, Thermo Fisher Scientific, Frederick, USA
navjot.kaur@thermofisher.com
Janet J. Sei, Thermo Fisher Scientific, Frederick, USA
Abigail H. Becker, Thermo Fisher Scientific, Frederick, USA
David Kuninger, Thermo Fisher Scientific, Frederick, USA
Mohan Vemuri, Thermo Fisher Scientific, Frederick, USA

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The use of $CD34^+$ hematopoietic stem cells (HSC) for transplantation has been limited due to the low $CD34^+$ cell numbers in tissue sources such as peripheral blood and cord blood. Two strategies have been employed to increase the $CD34^+$ cell dosage. These include mobilization of HSC into peripheral blood via injection of G-CSF, and *ex vivo* expansion of $CD34^+$ cells. A major limitation of current systems used for the expansion of HSC is that *ex vivo* culture leads to expansion and differentiation of cells, at the expense of the most primitive pluripotent long-term HSC. This has limited the clinical application of *ex vivo* expanded HSC, since short-term progenitor cells only provide transient protection, ultimately reducing the positive health outcomes, increasing the duration of hospitalizations, and health care costs per patient. Development of a culture system that expands, both short term progenitor cells and long-term HSC would enable immune protection during the early phase of recovery, and provide a suitable solution for transfusion-independent hematopoiesis. Therefore, we have developed an HSC culture medium that enables the expansion of both long-term HSC and short-term progenitor cells, while maintaining their functional properties. We conducted several iterative rounds of Design of Experiments (DOE) involving multifactorial analysis, and mathematical modeling methods. The DOEs allowed us to identify optimal combinations and concentrations of essential media components, small molecules, and growth factors. The performance of candidate HSC expansion media were evaluated after 7 days of culture, upon which the $CD34^+$ cells and $CD34^+CD90^+CD45RA^-$ cells (long-term HSC) were quantified. We were successful in developing a media system- StemPro™ HSC Medium Kit-which is xeno-free, serum-free medium that expands both long term $CD34^+CD90^+CD45RA^-$ HSC and short term $CD34^+$. The expression of aldehyde dehydrogenase was conducted to identify primitive stem cells, and colony-forming unit assays were performed to assess the *in vitro* differentiation capacity of expanded cells. We plan to determine whether the expanded cells are engraftable by transplanting the cells into immuno-deficient mice. Taken together, we seek to highlight our design philosophy in HSC culture media development, and we believe our efforts are critical for the successful utilization of hematopoietic stem cell transplants in translational cell therapies.