White Paper

UM171: Improving Hematopoietic Stem Cells for Enhanced Cell and Gene Therapies

Shanti Rojas-Sutterlin, PhD. Director, Stem Cell Laboratory, ExCellThera.
Guy Sauvageau, M.D., Ph.D., FRCP(C). Chief Scientist, ExCellThera; Principal Investigator, University of Montreal.

Introduction

Expanding hematopoietic stem cells (HSCs) *ex vivo* is crucial for advancing stem cell engineering and boosting the number of therapeutic CD34+ cells for effective transplantation, enhancing treatment efficacy and patient's outcome. However, creating the ideal culture environment remains a major challenge. The key hurdle is preserving HSC properties (i.e., self-renewal and multipotency) essential for their ability to replicate and differentiate into various blood cell types. This delicate balance is critical for maximizing research potential leading to therapeutic efficacy.

Small molecules are used to create more favorable conditions for HSCs in culture, potentially improving their yield and functionality. Although many small molecules are under investigation, only a few have made their way to clinical trials[1]. UM171 stands out as a breakthrough solution to mitigate *ex vivo* HSC exhaustion. Leveraged by ExCellThera in the production of *dorocubicel* (UM171-enhanced cell therapy to treat hematological malignancies), UM171 is transforming the treatment landscape for blood cancer, driving superior patient outcomes[2,3], and offering unprecedented potential in stem cell therapy.

Why does *ex vivo* culture cause HSC exhaustion?

It is well known that culture of HSCs leads to a decline in their stem cell properties and repopulation ability, as evidenced in pre-clinical mouse models[4]. Indeed, unwanted process-induced changes in stem cells occur as early as the first 24-72 hours of cell culture[5], highlighting the importance of using optimal culture conditions from the beginning. Key factors contributing to HSC exhaustion in culture are discussed hereafter.

Culture-Induced Stress

To support HSC survival and proliferation, cultures are typically supplemented with cytokines like TPO, SCF, and FLt3L. While essential for maintaining and expanding HSCs, cytokines can also induce excessive proliferation, altering HSC metabolism, reducing oxidative phosphorylation, and increasing reactive oxygen species (ROS) (reviewed in Nakamura-Ishizu *et al.*[6]). Over time, this stress can cause premature differentiation and consequently a decrease in the frequency of CD34+ cells. Johnson *et al.* have shown that this culture-induced HSC attrition occurs within 24 hours[5]. This is particularly important in the context of gene editing, where a short term *ex vivo* activation phase is performed to increase homology-directed repair (HDR) efficiency[7].

Genetic, Epigenetic and DNA Damage

Ex vivo culture leads to epigenetic modifications[8] and altered gene expression profiles[5] in HSCs. More precisely, RCOR1 and CoREST complexes are rapidly upregulated in human HSCs when introduced in culture, which significantly reduces key histone marks[8], contributing to impaired stem cell function. Modification of the chromatin structure could contribute to the rapid firing of the differentiation program that is taking place in cultured HSCs[5]. Additionally, the stress of *ex vivo* culture can induce DNA damage leading to apoptosis and/or senescence (loss of cell's power of division and growth) in HSCs.

How to improves HSC culture conditions

Improving HSC culture conditions is crucial to maintaining stem cell functionality, identity, and expanding their numbers for therapeutic applications. Several strategies are used to enhance HSC culture conditions.

Enhancing HSC Functionality with UM171

Why use UM171 in stem cell expansion?

- ✓ **Enhances** CD34+ cell expansion
- ✓ **Preserves** cell functions and lymphocytosis
- ✓ **Maintains** stem cell epigenetic marks
- ✓ **Prevents** excessive ROS accumulation
- ✓ **Reduces** cell death and DNA damage

UM171 improves HSC culture conditions by counteracting epigenetic changes **resulting in long-term HSC expansion (Figure 1), maintenance of metabolism fitness and prevention of differentiation.**

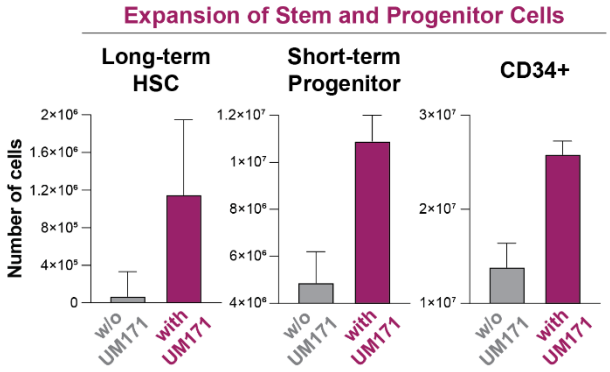

Figure 1. 1x10⁶ blood-derived CD34+ cells were expanded for 7 days with UM171 (35nM) or without (w/o). Shown are median with 95% CI. LT-HSC: CD34⁺CD45RA^{lo}CD90⁺CD201⁺; ST-progenitor: CD34⁺CD45RA^{lo}.

Mechanistically, UM171 targets RCOR1 for degradation, counteracting epigenetic changes and controlling MYC activation[9]. This balance prevents excessive ROS accumulation and maintains a favorable environment for HSC self-renewal[8,9]. Moreover, UM171's ability to reduce cell death (**Figure 2A**) and DNA damage (**Figure 2B**) while preventing clonal dominance makes it particularly valuable for *ex vivo* gene correction, especially in pathological stem cells such as those from sickle cell disease (SCD)[10].

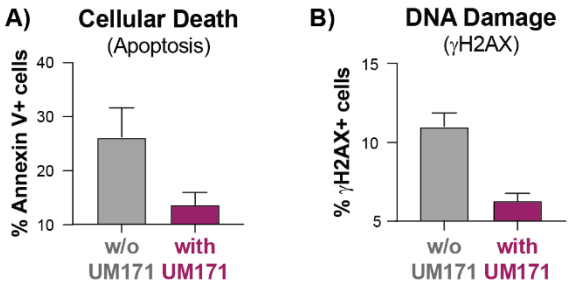

Figure 2. CD34+ cells from SCD patients transduced with a lentivirus. Analysis performed at day 7. Adapted from Liu et al.[10].

More precisely, in the presence of UM171, RCOR1 levels are maintained at physiological levels throughout the expansion process (tested for as long as 7 days), whereas levels drastically increase within 48 hours in absence of UM171 (**Figure 3A**). This is in line with epigenetic alterations occurring early upon culture initiation, even if frequencies of CD34+ cells appear unchanged at point (**Figure 3B**, Day 2). Nonetheless, the full benefits of UM171 can also be appreciated on longer *ex vivo* culture, where high proportions of primitive CD34+ cells are maintained (**Figure 3B,** Day 7).

Monitoring Relevant Cells

Ex vivo culture induces changes in cell surface markers, making traditional markers like CD38 less reliable for tracking HSC differentiation[4,11,12]. Instead, monitoring CD34 and CD90 can better identify HSCs[12,13]. For example, EPCR/CD201, combined with CD34, CD45RA^{lo} and CD71^{lo}, defines a rare, functionally active HSC population[13].

Incorporating UM171 in the culture has a positive impact on the expansion of primitive stem cell sub-populations, including CD34+CD90+ (**Figure 3C**) and EPCR/CD201+ HSCs[14]. Interestingly, the positive impact on this primitive population observed after 2 days and exacerbated with time (**Figure 3C**).

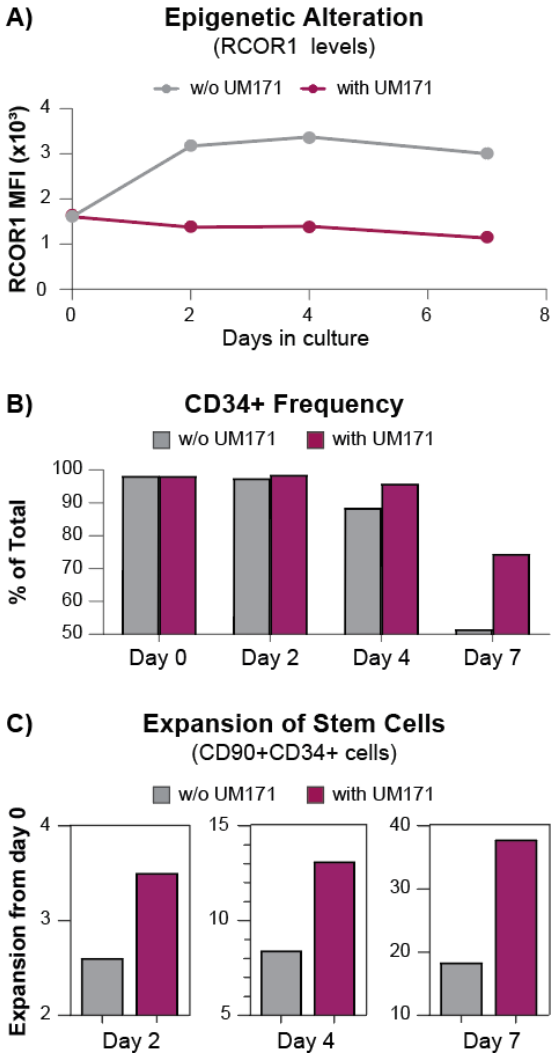

Figure 3. Blood-derived CD34+ cells were expanded with UM171 (35 nM) or without (w/o). A) Mean Fluorescence Intensity (MFI) in CD90+CD34+ cells. B) Percentage of CD34+ on total cells, C) Expansion of CD90+CD34+ cells from Day 0.

Conclusion

Optimizing HSC culture remains a critical challenge in cell therapy manufacturing. *Ex vivo* cell culture, editing and/or manipulation leads to HSC exhaustion, but UM171 emerges as a powerful solution, preserving stem cell functionality and enhancing yield by mitigating stress and epigenetic damage. While some researchers advocate for shorter culture periods to maintain stem cell properties, UM171's ability to sustain HSC self-renewal suggests a viable path forward. By integrating advanced small molecules and improved monitoring techniques, both the efficacy and reliability of stem cell therapies can be enhanced, paving the way for breakthroughs in regenerative medicine.

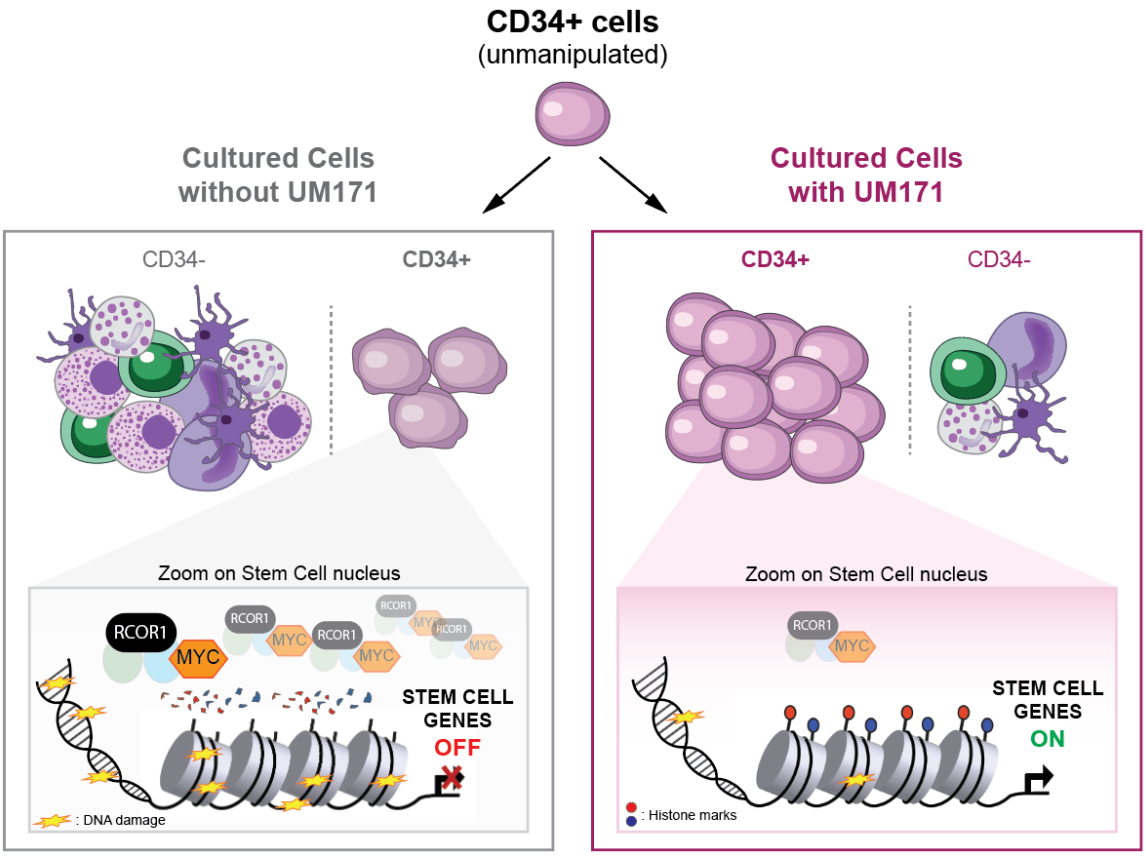

Figure 4. Schematic representation of how UM171 enhances cultured CD34+ stem cell functionality and enhances expansion by mitigating stress and preserving stem cell epigenetic profile.

Where to Source UM171

UM171 is a proprietary patented small molecule for *ex vivo* use. ExCellThera holds the exclusive worldwide rights to UM171 patents and therefore **ExCellThera is the only lawful source of UM171**. If you wish to source and use UM171, please contact info@excellthera.com.

ExCellThera Inc.
2950 Chemin de Polytechnique
Montreal, QC, Canada H3T1J4

References

1. Sakurai, M., Ishitsuka, K., Becker, H.J., and Yamazaki, S. (2024). Ex vivo expansion of human hematopoietic stem cells and clinical applications. Cancer Sci *115*, 698-705. 10.1111/cas.16066.
2. Cohen, S., Bambace, N.M., Ahmad, I., Burns, L.J., Roy, J., Lachance, S., Bernard, L., Delisle, J.S., Roy, D.C., Kiss, T.L., et al. (2022). Improved Outcomes of UM171-Expanded Cord Blood Transplantation Compared with Other Graft Sources: Real World Evidence. Blood *140*, 1610-1612. 10.1182/blood-2022-160417.
3. Cohen, S., Roy, J., Lachance, S., Delisle, J.S., Marinier, A., Busque, L., Roy, D.C., Barabe, F., Ahmad, I., Bambace, N., et al. (2020). Hematopoietic stem cell transplantation using single UM171-expanded cord blood: a single-arm, phase 1-2 safety and feasibility study. Lancet Haematol *7*, e134-e145. 10.1016/S2352-3026(19)30202-9.
4. Chen, Y., Fang, S., Ding, Q., Jiang, R., He, J., Wang, Q., Jin, Y., Huang, X., Liu, S., Capitano, M.L., et al. (2021). ADGRG1 enriches for functional human hematopoietic stem cells following ex vivo expansion-induced mitochondrial oxidative stress. J Clin Invest *131*. 10.1172/JCI148329.
5. Johnson, C.S., Williams, M., Sham, K., Belluschi, S., Ma, W., Wang, X., Lau, W.W.Y., Kaufmann, K.B., Krivdova, G., Calderbank, E.F., et al. (2024). Adaptation to ex vivo culture reduces human hematopoietic stem cell activity independently of the cell cycle. Blood *144*, 729-741. 10.1182/blood.2023021426.
6. Nakamura-Ishizu, A., Ito, K., and Suda, T. (2020). Hematopoietic Stem Cell Metabolism during Development and Aging. Dev Cell *54*, 239-255. 10.1016/j.devcel.2020.06.029.
7. Demirci, S., Khan, M.B.N., Hinojosa, G., Le, A., Leonard, A., Essawi, K., Gudmundsdottir, B., Liu, X., Zeng, J., Inam, Z., et al. (2024). Ex vivo culture resting time impacts transplantation outcomes of genome-edited human hematopoietic stem and progenitor cells in xenograft mouse models. Cytotherapy *26*, 641-648. 10.1016/j.jcyt.2024.02.011.
8. Chagraoui, J., Girard, S., Spinella, J.F., Simon, L., Bonneil, E., Mayotte, N., MacRae, T., Coulombe-Huntington, J., Bertomeu, T., Moison, C., et al. (2021). UM171 Preserves Epigenetic Marks that Are Reduced in Ex Vivo Culture of Human HSCs via Potentiation of the CLR3-KBTBD4 Complex. Cell Stem Cell *28*, 48-62 e46. 10.1016/j.stem.2020.12.002.
9. Chagraoui, J., Girard, S., Mallinger, L., Mayotte, N., Tellechea, M.F., and Sauvageau, G. (2024). KBTBD4-mediated reduction of MYC is critical for hematopoietic stem cell expansion upon UM171 treatment. Blood *143*, 882-894. 10.1182/blood.2023021342.
10. B. Liu, D.K., Y. Zhou, J. P. Manis, G. Sauvageau, D. Pellin, C. Brendel, D.A. Williams (2024). UM171 enhances fitness and engraftment of gene modified hematopoietic stem cells from sickle cells disease patients. Blood Adv. (Under press)
11. Zhang, C.C., and Lodish, H.F. (2005). Murine hematopoietic stem cells change their surface phenotype during ex vivo expansion. Blood *105*, 4314-4320. 10.1182/blood-2004-11-4418.
12. Dorrell, C., Gan, O.I., Pereira, D.S., Hawley, R.G., and Dick, J.E. (2000). Expansion of human cord blood CD34(+)CD38(-) cells in ex vivo culture during retroviral transduction without a corresponding increase in SCID repopulating cell (SRC) frequency: dissociation of SRC phenotype and function. Blood *95*, 102-110.
13. Fares, I., Chagraoui, J., Lehnertz, B., Macrae, T., Mayotte, N., Tomellini, E., Aubert, L., Roux, P.P., and Sauvageau, G. (2017). EPCR expression marks UM171-expanded CD34+ cord blood stem cells. Blood *129*, 3344-3351. 10.1182/blood-2016-11-750729.
14. Fares, I., Chagraoui, J., Gareau, Y., Gingras, S., Ruel, R., Mayotte, N., Csaszar, E., Knapp, D.J.H.F., Miller, P., Ngom, M., et al. (2014). Pyrimidoindole derivatives are agonists of human hematopoietic stem cell self-renewal. Science *345*, 1509-1512. 10.1126/science.1256337.