White Paper

Optimizing CD34⁺ Cell Dose and Purity in Ex Vivo Cell and Gene Therapy

Strategies to Improve Manufacturing Robustness and GMP Supply

Elisa Tomellini, PhD, Senior Director, R&D Laboratory, ExCellThera Inc.

Introduction

Hematopoietic stem cells (HSCs) are rare self-renewing, multipotent cells essential for lifelong blood cell production. Their unique ability to durably sustain hematopoiesis underpins their critical role in HSC transplantation and in an expanding range of cell and gene therapy (CGT) applications[1].

Manufacturing HSC-based CGT products is a complex and resource-intensive process requiring tight control of multiple Critical Quality Attributes (CQAs) to ensure product quality, safety and consistency (**Table 1**). Failure to meet release specifications, particularly for CD34⁺ cell dose or purity, may result in lot rejection. Such failures can delay treatment in patients with severe or life-threatening conditions, increase manufacturing costs, disrupt supply chains, and raise regulatory concerns regarding process consistency and control.

This white paper examines how starting material characteristics, cell manipulation steps, and formulation processes influence CD34+ cell dose and purity. It also describes how incorporation of the small molecule UM171 during *ex vivo* culture can improve expansion, preserve stem cell properties and enhance manufacturing robustness.

Factors influencing CD34⁺ cell dose and purity

The therapeutic effectiveness of HSC-based CGT products is tied to delivering an adequate dose of functional CD34⁺ cells. In transplantation settings, higher CD34⁺ doses are associated with faster engraftment, reduced graft failure, and improved hematopoietic recovery.[2]

Gene therapy efficacy is governed by both the functional impact of the genetic modification and the dose-dependent engraftment and persistence of modified CD34⁺ cells. Purity is equally important. Reduced CD34⁺ cell purity limits the number of stem and progenitor cells available for durable hematopoietic reconstitution. The presence of non-target CD34⁻ cells introduces variability in product composition and may increase regulatory scrutiny related to safety and consistency.

CQA	Description	Example of general test parameters in HSC-based CGT
Identity	Confirmation that the intended therapeutic cell type is present	Presence of CD34⁺ cells
Purity	Adequate proportion of target cells relative to non-target cells	Percentage of CD34⁺ cells
Quantity	Availability of required clinical dose at formulation	Total viable CD34⁺ cell number; dose per kg of patient weight
Potency	Functional activity of the cells	Viability, colony forming unit (CFU) assay, on-target editing frequency
Safety	Absence of contaminations or unintended alterations	Sterility, mycoplasma, endotoxin, adventitious virus testing

Table 1. Generalized CQAs for HSC-derived CGT products.

From cell collection to final formulation, each manufacturing step can affect CD34⁺ cell dose, purity and functional integrity. CD34⁺ cell attrition and phenotypic drift may occur cumulatively across the workflow rather than at a single isolated step (**Figure 1**). Although specific processes across CGT platforms, the principal drivers of variability can be broadly categorized and generalized (**Table 2**). Failure to adequately control these variables increases the risk of not meeting release specifications for identity, purity, potency, or dose at final product release.

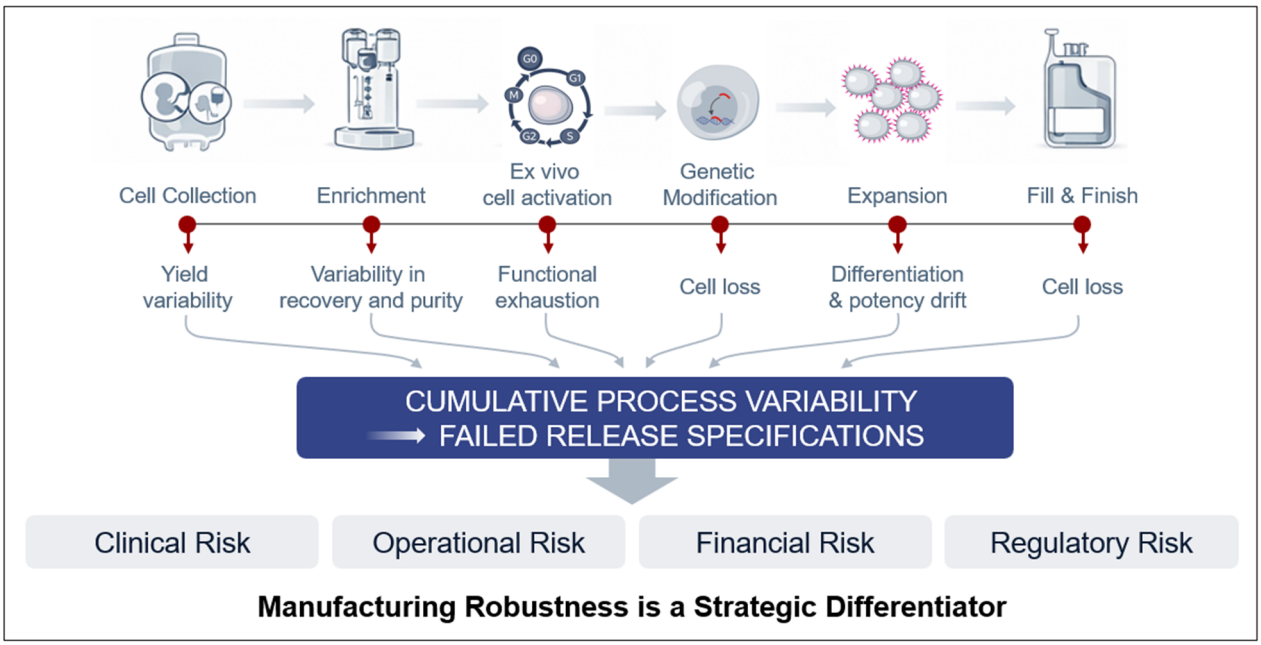

Figure 1. Simplified manufacturing workflow. CD34⁺ cell loss, reduction in CD34 purity, potency or stem cell activity, can take place at different steps during CGT products manufacturing.

Manufacturing Step	Impacting Variable	Reasons and Consequences
CD34⁺ cell collection	Low CD34⁺ cell count	Yield varies by source and donor factors. Peripheral blood depends on mobilization efficiency and prior treatments. Bone marrow yield is influenced by harvest technique and donor characteristics. Cord blood frequently fails to meet minimum CD34⁺ dose requirements[4]. Higher patient body weight increases required cell dose.
Isolation and enrichment	Low recovery and purity	Magnetic bead-based isolation efficiency and starting material quality (e.g., RBCs, platelets) directly affects CD34⁺ purity and recovery.
***Ex vivo* activating culture**	Reduced HSC function	Pre-editing activation (48-72h) enhances homology-directed repair efficiency by promoting cell cycling. However, even short culture periods can induce differentiation and reduce long-term HSC functionality[5,6].
Genetic manipulation	Cell loss	Gene modification methods, including viral transduction and electroporation, can reduce CD34⁺ yield, viability, and functional capacity.
Expansion/ resting phase	Reduced purity and potency	Culture media composition influences CD34⁺ cell expansion and maintenance. Suboptimal conditions can lead to poor growth, differentiation and reduced viability[5,6].
Formulation and cryopreservation	Cell loss	Processing, freezing, and thawing steps reduce viable CD34⁺ cell numbers, requiring higher starting input to meet dose specifications.

Table 2. Manufacturing steps most prone to affect CD34⁺ cell dose and purity in CGT products.

The impact of ex *vivo* culture

Ex vivo culture is an unavoidable component of most CGT workflows. It is required for genetic modification and for quality control assessments of identity, functionality, and genomic integrity. However, cell culture can have a detrimental impact on stem cell quality, by rapidly inducing differentiation and reducing stem cell activity. Even limited exposure to *ex vivo* conditions has been shown to diminish long-term repopulating potential[5]. Culture-induced attrition of primitive HSCs directly contributes to variability in $CD34^+$ dose, purity, and potency at final release.

Strategies that enhance $CD34^+$ expansion while preserving stemness are therefore critical to improving manufacturing reliability.

UM171 as a strategy to improve $CD34^+$ yield and stem cell preservation

UM171 is a pyrimido-indole small molecule enhancing *ex vivo* expansion of $CD34^+$ cells while preserving their stemness[3].

In mobilized peripheral blood cultures, UM171 has demonstrated greater than 30-fold expansion of $CD34^+$ cells over a 7-day culture period relative to input cell numbers, with improved maintenance of $CD34^+$ purity compared to culture without UM171 (**Figure 2**). Similar expansion has been observed in cord blood- and bone marrow-derived $CD34^+$ cells[7].

UM171 has been incorporated into various CGT workflows, including Cas9-AAV6-based gene editing[8] and lentiviral transduction[9]. Clinical studies involving over 100 patients have demonstrated safety and feasibility of UM171-expanded grafts in hematologic malignancies[10-12].

At the cellular level, UM171 acts as a molecular glue that stabilizes the CRL3-KBTBD4 complex, preserving epigenetic programs associated with HSC self-renewal and limiting culture-induced transcriptional drift [13,14]. By mitigating stem cell attrition during *ex vivo* manipulation, UM171 supports expansion of primitive $CD34^+$ cells and improves the likelihood of achieving target release specifications.

Manufacturing, clinical and economic implications

Enhanced $CD34^+$ expansion with UM171 offers several practical advantages:

- Increased probability of meeting dose and purity specifications
- Reduced dependence on large starting material volumes
- Lower consumption of costly gene-editing reagents by starting with fewer cells
- Improved manufacturing consistency across variable donor sources
- Alleviate the burden of extensive cell harvesting procedure

Importantly, variability in stem cell mobilization remains a key limitation in donor-derived workflows. A subset of donors—commonly referred to as poor mobilizers—fail to achieve adequate $CD34^+$ yields. Mobilization failure occurs in 5–30% of donors and patients[15]. Improved expansion with small molecules such as UM171 reduces pressure on collection yields and may mitigate clinical risk associated with poor mobilization.

UM171 is used as an ancillary reagent during manufacturing and can be washed out to trace levels following expansion. GMP-grade material is available and supported by a Drug Master File (DMF), facilitating regulatory integration into CGT workflows.

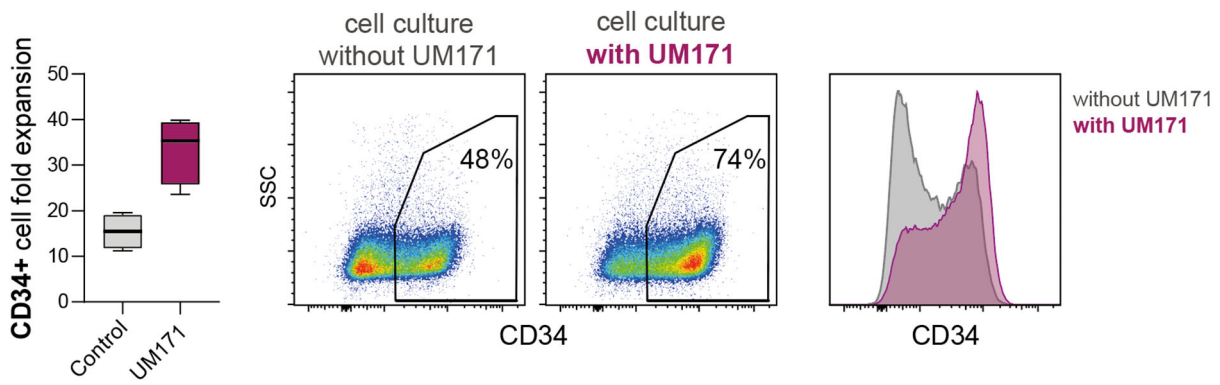

Figure 2. Mobilized peripheral blood-derived CD34⁺ cells expanded for 7-days with or without UM171. Culture with UM171 increases total CD34⁺ cell numbers while preserving CD34 expression, as assessed by ISHAGE gating strategy[16].

Conclusion

Manufacturing HSC-based CGT products requires strict control of CD34⁺ cell dose, purity, and functional integrity. Variability in starting material and sensitivity to *ex vivo* manipulation create significant risk of lot failure and regulatory concern.

Incorporating UM171 during *ex vivo* culture represents a clinically validated strategy to enhance CD34⁺ expansion while preserving stem cell properties. By improving yield, maintaining purity, and reducing reliance on high starting cell numbers, UM171 supports more robust, scalable, and economically sustainable CGT manufacturing.

As demand for HSC-based therapies continues to grow, improving manufacturing consistency will be essential to ensuring reliable patient access and long-term clinical and commercial success.

Where to source UM171

UM171 is a patented small molecule for *ex vivo* use. ExCellThera holds the exclusive worldwide rights to UM171 patents and is the only authorized source of UM171. If you wish to source and use UM171, please see **www.UM71.com**.

ExCellThera Inc.
2950 Chemin de Polytechnique, Montreal, QC, Canada H3T 1J4

References

1 Naldini, L. Genetic engineering of hematopoiesis: current stage of clinical translation and future perspectives. EMBO Mol Med 11, doi:10.15252/emmm.201809958 (2019).
2 Remberger, M. et al. The CD34(+) Cell Dose Matters in Hematopoietic Stem Cell Transplantation with Peripheral Blood Stem Cells from Sibling Donors. Clin Hematol Int 2, 74-81, doi:10.2991/chi.d.200221.001 (2020).
3 Fares, I. et al. Cord blood expansion. Pyrimidoindole derivatives are agonists of human hematopoietic stem cell self-renewal. Science 345, 1509-1512, doi:10.1126/science.1256337 (2014).
4 Barker, J. N. et al. CD34(+) cell content of 126 341 cord blood units in the US inventory: implications for transplantation and banking. Blood Adv 3, 1267-1271, doi:10.1182/bloodadvances.2018029157 (2019).
5 Johnson, C. S. et al. Adaptation to ex vivo culture reduces human hematopoietic stem cell activity independently of the cell cycle. Blood 144, 729-741, doi:10.1182/blood.2023021426 (2024).

6 Rojas-Sutterlin, S. et al. UM171: Improving Hematopoietic Stem Cells for Enhanced Cell and Gene Therapies. White Paper. https://excellthera.com/wp-content/uploads/2024/10/WP_HSC-Culture.pdf

7 Demirci, S. et al. 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, doi:10.1016/j.jcyt.2024.02.011 (2024).

8 Charlesworth, C. T. et al. Priming Human Repopulating Hematopoietic Stem and Progenitor Cells for Cas9/sgRNA Gene Targeting. Mol Ther Nucleic Acids 12, 89-104, doi:10.1016/j.omtn.2018.04.017 (2018).

9 Capo, V. et al. Expanded circulating hematopoietic stem/progenitor cells as novel cell source for the treatment of TCIRG1 osteopetrosis. Haematologica 106, 74-86, doi:10.3324/haematol.2019.238261 (2021).

10 Cohen, S. et al. 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, doi:10.1016/s2352-3026(19)30202-9 (2020).

11 Cohen, S. et al. Improved outcomes of UM171-expanded cord blood transplantation compared with other graft sources: real-world evidence. Blood Adv 7, 5717-5726, doi:10.1182/bloodadvances.2023010599 (2023).

12 Claveau, J. S. et al. Single UM171-expanded cord blood transplant can cure severe idiopathic aplastic anemia in absence of suitable donors. Eur J Haematol 105, 808-811, doi:10.1111/ejh.13504 (2020).

13 Chagraoui, J. et al. 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, doi:10.1016/j.stem.2020.12.002 (2021).

14 Chagraoui, J. et al. KBTBD4-mediated reduction of MYC is critical for hematopoietic stem cell expansion upon UM171 treatment. Blood 143, 882-894, doi:10.1182/blood.2023021342 (2024).

15 Bik To, L. et al. How I treat patients who mobilize hematopoietic stem cells poorly. Blood. 2011 Oct 27;118(17):4530-40. doi: 10.1182/blood-2011-06-318220

16 Sutherland, D. R. et al. The ISHAGE guidelines for CD34+ cell determination by flow cytometry. International Society of Hematotherapy and Graft Engineering. J Hematother 5, 213-226, doi:10.1089/scd.1.1996.5.213 (1996).