Novel Small Molecules

For the Ex Vivo Expansion of Human Hematopoietic Stem Cells

Summary

A major advance towards the goal of ex vivo expansion of human hematopoietic stem cells (HSCs) has been made with the recent discovery of the novel pyrimidoindole small molecules UM171 (Figure 1A) and UM729 (Figure 1B).[1,2] UM729 was originally discovered in a screen of compounds capable of promoting human CD34$^+$ cell expansion, and later underwent structure-activity relationship (SAR) optimization to develop UM171.[1,2] Culturing human CD34$^+$ cells isolated from cord blood (CB) or mobilized peripheral blood (MPB) with either of these small molecules, in addition to cytokines, has been shown to promote a concentration-dependant increase in the number of phenotypically primitive (CD34$^+$CD45RA$^-$) hematopoietic cells in culture.[1] When ex vivo expanded CD34$^+$ cells were transplanted into immunodeficient mice, a corresponding ~10-fold increase in the frequency of HSCs was observed, as identified by their ability to reconstitute the hematopoietic system of these recipients (Figure 2).[1]

UM171 and UM729 act differently than other small molecule stimulators of hematopoiesis, such as the aryl hydrocarbon receptor (AhR) antagonist StemRegenin 1 (SR1).[1-3] Indeed, the addition of UM171 or UM729 to cultures containing SR1 and cytokines further enhances the ex vivo expansion of normal HSCs, including CD34$^+$ cells (Figures 2 - 4).[1] Furthermore, the combination of UM729 and SR1 in similar conditions was found to support the ex vivo culture of CD34$^+$CD15$^-$ acute myeloid leukemia (AML) progenitor cells.[2] These small molecules are important new tools for investigators studying the biology of both normal human HSCs and leukemic progenitor cells.

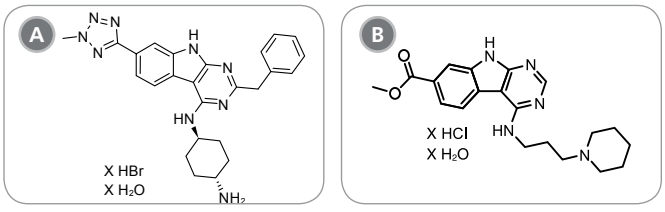

Figure 1. Chemical Structures of UM171 and UM729

Chemical structure of (A) UM171, (1r,4r)-N1-(2-benzyl-7-(2-methyl-2H-tetrazol-5-yl)-9H-pyrimido[4,5-b]indol-4-yl)cyclohexane-1,4-diamine and (B) UM729, Methyl 4-((3-(piperidin-1-yl)propyl)amino)-9H-pyrimido[4,5-b] indole-7-carboxylate.

APPLICATIONS:

- Enhancement of human HSC self-renewal in culture[1]
- Maintenance of human leukemia stem cell (LSC) activity in culture[2]

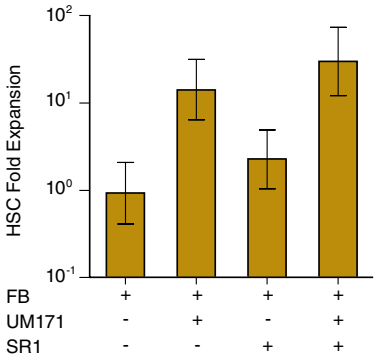

Figure 2. Effect of UM171 and SR1 on the Ex Vivo Expansion of HSCs Isolated From CB

CD34$^+$ cells isolated from CB were cultured in a fed-batch (FB) system for 12 days in the presence of cytokines and UM171 [35 nM], SR1 [750 nM] or the combination of both UM171 [35 nM] and SR1 [500 nM]. Cells were then transplanted into immunodeficient mice and human cell engraftment was measured at 20 weeks. Data represent mean ± 95% CI, (n = 5 independent experiments). (Modified from Fares et al., 2014.)

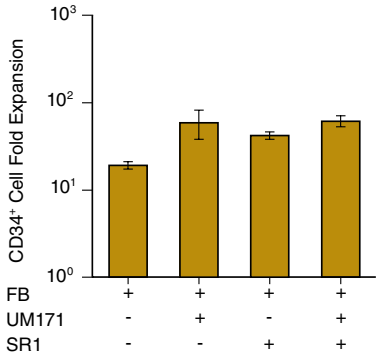

Figure 3. Effect of UM171 and SR1 on the Ex Vivo Expansion of CB-Derived CD34$^+$ Cells

CD34$^+$ cells isolated from CB were cultured in a fed-batch (FB) system for 12 days in the presence of cytokines and UM171 [35 nM], SR1 [750 nM] or the combination of both UM171 [35 nM] and SR1 [500 nM]. Data represent mean ± SEM, (n = 3 independent experiments). (Modified from Fares et al., 2014.)

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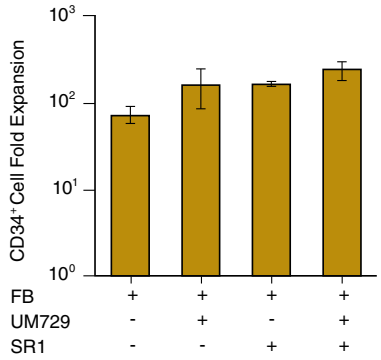

Figure 4. Effect of UM729 and SR1 on the Ex Vivo Expansion of CB-Derived CD34$^+$ Cells

Lineage-depleted CB cells were cultured in a fed-batch (FB) system for 12 days in the presence of cytokines and UM729 [500 nM], SR1 [750 nM] or the combination of both UM729 [500 nM] and SR1 [750 nM]. Data represent mean ± SEM, (n = 2 independent experiments). (Data provided by the University of Montreal.)

Advantages of UM171 & UM729:

- Promote expansion of phenotypically primitive (CD34$^+$ and CD34$^+$CD45RA$^-$) human hematopoietic cells in culture
- Stimulate approximately 10-fold ex vivo expansion of long-term in vivo repopulating HSCs
- Additive effects on the expansion of normal human HSCs when used in combination with an AhR antagonist (e.g. SR1)

Products*

PRODUCT	CATALOG #	SIZE	APPLICATION
UM729	72332 72334	250 µg 1 mg	Maintenance and expansion of human HSPCs in culture[3,4] Maintenance of LSC activity in culture when combined with SR1[2]
SR1	72342 72344	1 mg 5 mg	Maintenance and expansion of human HSPCs in culture[3,4] Differentiation of human CD34$^+$ HPCs into functional dendritic cells[5] Maintenance of LSC activity in culture when combined with UM729[2]
SR1 (Hydrochloride)	72352 72354	1 mg 5 mg	
EasySep™ Human Cord Blood CD34 Positive Selection Kit	18096	For labeling up to 2 x 10^9 cells	Immunomagnetic positive selection of human CD34$^+$ cells from CB
StemSpan™ SFEM	09600 09650	100 mL 500 mL	Culture and expansion of human HSPCs
MyeloCult™ H5100	05100 05150	100 mL 500 mL	Myeloid long-term cultures of human HPCs in the presence of stromal feeder cells
MethoCult™ Classic	04434 04444	100 mL 24 x 3 mL	Colony-forming unit (CFU) assays with CB, BM, PB and MPB cells

*Products listed above are used in Fares et al. (2014).

For related products for HSPC research, including specialized culture and storage media, supplements, antibodies, cytokines, and small molecules, visit **www.stemcell.com/HSPCworkflow** or contact us at **techsupport@stemcell.com**. For available fresh and cryopreserved peripheral blood, cord blood and bone marrow products in your region, visit **www.stemcell.com/primarycells**.

References

1. Fares I et al. (2014) Pyrimidoindole derivatives are agonists of human hematopoietic stem cell self-renewal. Science 345, 1509-1512.
2. Pabst C et al. (2014) Identification of small molecules that support human leukemia stem cell activity ex vivo. Nat Methods 11, 436-442.
3. Boitano AE et al. (2010) Aryl hydrocarbon receptor antagonists promote the expansion of human hematopoietic stem cells. Science 329, 1345-1348.
4. Csaszar E et al. (2012) Rapid expansion of human hematopoietic stem cells by automated control of inhibitory feedback signaling. Cell Stem Cell 10, 218-229.
5. Thordardottir S et al. (2014) The aryl hydrocarbon receptor antagonist StemRegenin 1 promotes human plasmacytoid and myeloid dendritic cell development from CD34$^+$ hematopoietic progenitor cells. Stem Cells 23, 955-967.