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POSTER ABSTRACTS

501.HEMATOPOIETIC STEM AND PROGENITOR CELLS AND HEMATOPOIESIS: BASIC AND TRANSLATIONAL

Deciphering the Boundaries of KBTBD4-CoREST1 Axis Modulation to Maximally Expand Human HSCs

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Background:
The UM171 technology has led to major progress in the expansion of cord blood units (CBUs) with low cellularity (Fares et al., Science 2014). This breakthrough has notably increased availability of better HLA-matched CBUs and improvements in clinical outcome (Cohen et al., Lancet Hematol 2020 and Blood Advances 2023, PMID: 37467030; see also Cohen et al. and Milano et al., this meeting). Enhanced HLA matching is a direct consequence of the selection of CBUs which are otherwise too small without expansion. Indeed, with this technology, approximately 50% of units in CB banks are eligible for clinical use, versus 5% without expansion.
As recently reported, UM171 promotes the activation of the novel CUL3-KBTBD4 E3 ligase complex leading to the ubiquitylation and degradation of its natural substrate, CoREST1, a repressor complex that includes RCOR1, LSD1, HDAC1/2. This complex is upregulated in human HSC upon their ex vivo culture where it compromises their potential in the absence of UM171 (Chagraoui et al., Cell Stem Cell, 2021).
Aims and Methods:
The goal of this research is to understand the boundaries of KBTBD4-CoREST1 axis modulation that determine the optimal expansion of human HSCs in vitro and in vivo. More precisely, we monitored the relationship between CoREST1 levels in primary CD34+CD45RA-CD90+ CB cells and determined the impact of maximal CoREST1 degradation on the yield of expanded HSC using a series of functional in vitro and in vivo approaches along with transcriptomic data.
Results:
Using flow cytometry, we noticed a gradual degradation of RCOR1 with increasing concentrations of UM171 with a plateau at 125nM suggesting that maximal RCOR1 degradation was reached at this dose which represented a 4-fold increase above the concentration of UM171 used in current clinical manufacturing. Of interest, at high concentration of UM171 (i.e.,125nM), total nucleated cell expansion was reduced by approximately 30% while that of primitive CD34+CD45RA-CD90+EPCR+ HSC was maximal and significantly above that measured at 35nM suggesting a preferential impact of CoREST1 degradation on the more primitive cell compartment along with improvement in graft quality.
CITE-Seq experiments confirmed the preferential expansion of primitive HSPC including HSCs and LMPPs with a reduction of more mature cellular subsets when cells were exposed to 125nM of UM171 in comparison to 35nM.
The in vivo functionality of the 7-day expanded grafts (35nM vs 125nM UM171) were compared by transplanting the outcome of 100 Day 0 CD34+ CB cells in NSG-SGM3 (NOD-scid IL2Rgnull-3/GM/SF) mice, a cell dose representing less than 1 competitive repopulation unit (CRU) of unexpanded cells in this model. Using this low cell dose (limit dilution), we observed that the 125nM expanded graft provided 10-times greater engraftment than observed in mice transplanted with cells exposed to 35nM UM171 with values reaching a median of 5±5% *vs.* 0.4±0.13%(n=10 mice per condition, p=0.01; Mann Whitney test) of human engraftment at 12 weeks post-transplantation, respectively.
Conclusion:
Maximal stimulation of CUL3-KBTBD4 by higher concentration of UM171 leads to much better expansion of functional human HSC and opens new avenues for optimal expansion of very small cord blood units and possibly adult bone marrow-derived HSCs which require more than minimal manipulation (e.g., gene therapy). We believe this new data represents a novel milestone in HSC expansion with obvious subsequent clinical benefits.

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