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ORAL ABSTRACTS

113.SICKLE CELL DISEASE, SICKLE CELL TRAIT AND OTHER HEMOGLOBINOPATHIES, EXCLUDING THALASSEMIAS: BASIC AND TRANSLATIONAL

UM171 Enhances Fitness and Engraftment of Gene Modified Hematopoietic Stem Cells from Sickle Cells Disease Patients

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Sickle cell disease (SCD) is one of the most common genetic blood diseases. Autologous hematopoietic stem cell gene therapy using lentiviral vectors is an effective therapeutic strategy for SCD. However, the occurrence of post gene therapy myelodysplastic syndrome and acute myeloid leukemia not related to vector insertion has suggested an underlying genetic risk or acquisition of damage during *ex vivo* culture or from proliferative stress associated with reconstitution. Here, we aimed to improve the transduction efficiency, stem cell fitness and reconstitution of SCD CD34+ cells transduced with a lentiviral vector (LV) containing BCL11A shmiR currently in clinical trials (Esrick et al. NEJM, 2021, NCT03282656), to minimize culture-induced stress and reduce genomic damage during *ex vivo* culture. UM171, an HSC self-renewal pyrimidoindole derivative agonist, has been shown to expand HSCs and enhance multilineage blood cell reconstitution in mice. We examined the effect of addition of UM171 during *ex vivo* transduction on HSCs from SCD patients. Culture of SCD CD34+ HSC in HSC expansion conditions with UM171 increased the proportion of CD34+CD133-CD45RA-CD90+ long term HSCs (LT-HSCs), from 1.1% to 4.1% ($N = 3$, $P < 0.001$), and the absolute number of LT-HSCs increased 4-fold ($N = 3$, $P < 0.001$). Treatment with UM171 also significantly enhanced the transduction efficiency of BCL11A shmiR containing lentiviral vector (LV-BCL11A) in SCD CD34+ cells from 0.7 vector copies/diploid genome (VCN) to 1.0 VCN ($N = 5$, $P < 0.001$), and significantly decreased apoptosis of stem cells from 22.5% to 14.3% ($N = 3$, $P < 0.001$), and DNA damage as measured by γ-H2AX from 10.6% to 6.6% ($N = 3$, $P < 0.001$). Given these results, we assessed the engraftment capability and clonality of SCD CD34+ transduced in the presence or absence of UM171 in NBSGW mice. UM171 increased the human CD45 engraftment of mice measured in BM at 16 weeks post-transplantation from 32.5% to 69.4% in hCD45+ BM cells ($N = 6$, $P < 0.01$) and enhanced the engraftment of transduced in SCD CD34+-derived CD45+ cells from 11.2% to 18.2% in gene-marked BM cells ($N = 6$, $P < 0.01$), while maintaining multilineage differentiation in this model. Barcode analysis revealed the engraftment of 500-1800 unique barcodes with a high degree of clonal diversity and a trend towards increased polyclonal engrafting populations in transplanted mice. To assess the effect of UM171 more rigorously on functionally defined HSCs, we performed a competitive transplantation assay using CD34+ cells transduced in the presence vs absence of UM171. Analysis of BM obtained from the mice at 16 weeks post-transplantation showed that cells transduced in the presence of UM171 consistently outcompeted those transduced under control conditions, 62.6% in gene-marked erythroid cells of UM171 treated cells compared with 37.4% of control cells ($N = 9$, $P < 0.001$), and UM171 maintains LV-BCL11A induced γ-globin expression in erythroid cells. In summary, a short-term exposure of SCD CD34+ PB cells to UM171 enhances stem cell fitness. Implementing these findings in clinical gene therapy protocols may improve the efficacy and sustainability of gene therapy and generate new opportunities in the field of gene editing.

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