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POSTER ABSTRACTS

509.BONE MARROW FAILURE AND CANCER PREDISPOSITION SYNDROMES: CONGENITAL

Small Molecule Inhibitor Targeting Nemo-like Kinase Improves Erythropoiesis in Human and Mouse Models of Diamond Blackfan Anemia

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Diamond Blackfan Anemia (DBA) is a rare inherited bone marrow failure syndrome characterized by anemia, congenital anomalies, and an increased risk of developing cancer. Approximately 80% of patients with DBA have a mutation in one of 20 different genes that encode ribosomal proteins, resulting in abnormal protein translation. The current standard of care for patients with DBA includes steroids, chronic red cell transfusions, or stem cell transplantation, but these are all associated with significant side effects including infections, iron overload, and the risk of graft versus host disease. Thus, the development of more effective and less-toxic therapies is needed to treat the anemia seen in patients with DBA. Our previous work demonstrated that Nemo-Like Kinase (NLK) is overly active in red cell precursors in models of DBA as well as in patient samples (Wilkes, et al., 2020). We hypothesize that NLK plays an important role in the pathogenesis of DBA, and is a potential target for DBA therapy.

Human cord blood CD34+ hematopoietic stem and progenitor cells (HSPCs) were transduced with lentivirus co-expressing GFP and shRNA against RPS19/RPL11 or luciferase (control), and were differentiated in erythroid media in the presence of small molecule NLK inhibitors. After screening small molecule compounds that inhibit NLK as an off-target from previously approved or clinically advanced drugs, we found that a known MELK (maternal embryonic leucine zipper kinase) inhibitor, OTS167, improved erythropoiesis (CD71+ cell expansion) by 31% and 27% at a concentration of 50nM in RPS19- and RPL11-knockdown HSPCs, respectively. We also investigated the effect of OTS167 on the erythropoiesis in *Rpl11*-haploinsufficient mouse model of DBA (*Rpl11* $^{wt/Loxp}$*Cre-ERT2* $^{+}$, Morgado-Palacin, et al., 2015 and *Rpl11* $^{wt/Loxp}$*Mx-Cre* $^{+}$ Liu,et al.,2023). Lineage-negative (Lin-) hematopoietic progenitors isolated from the mouse bone marrow were cultured in erythroid differentiation medium with OTS167. Our data showed that the erythroid progenitors (CD71+ cells) expanded from the cultured Lin- cells were increased by 20% (n=2, p=0.065) at an OTS167 concentration of 30nM. We confirmed that NLK activity was increased in erythroid progenitors from the *Rpl11*-haploinsufficient mice for *in vitro* phosphorylation of the substrate compared with tamoxifen-treated wild type mice. Moreover, we performed colony formation assay with OTS167 in the mouse bone marrow cells. OTS167 increased BFU-E colony numbers in a dose-dependent manner with the highest effect at a concentration of 50nM (n=3, p=0.003). OTS167 did not increased CFU-E colony number, suggesting that OTS167 affected the BFU-E stage and/or earlier stages of erythroid development. Also, OTS167 did not affected CFU-GM colony formation as myeloid lineage. The goal in treating DBA patients with NLK inhibitors is to sufficiently raise the hemoglobin to minimize the need for chronic red cell transfusions or treatment with steroids. Our *in vitro* data suggest that pharmacologic inhibition of NLK with OTS167 may achieve this goal. We have initiated studies to test the efficacy of OTS167 *in vivo* using *Rpl11*-haploinsufficient mouse models.

Given that DBA is associated with a number of mutations in the ribosome, using one drug that targets NLK as a common therapeutic target could be a more practical treatment option for patients in contrast to gene therapy or gene editing followed by autologous stem cell rescue. Even if the drug targeting NLK does not completely rescue the anemia in ribosome-insufficient cell models of DBA, the improvement could be enough to help patients especially in combination with other drugs that are currently being used or studied .

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