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ORAL ABSTRACTS

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

Evaluation of GBT021601 As a Therapeutic Agent to Restore Bone Marrow Health and Effective Erythropoiesis in a Sickle Mouse Model

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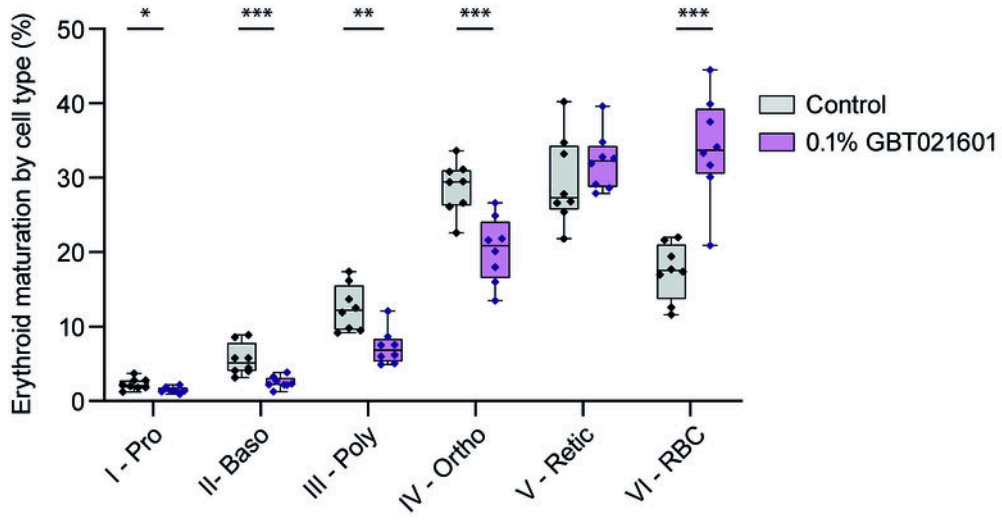
Introduction: Hypoxia-mediated damage resulting from vaso-occlusion in sickle cell disease (SCD) leads to abnormal angiogenesis, such as occurs with moyamoya disease and proliferative retinopathy. Abnormal angiogenesis in mouse models of SCD has been observed to impact the bone marrow niche, resulting in a structurally abnormal vascular network. A dysfunctional bone marrow environment results in ineffective erythropoiesis and can damage hematopoietic stem cells (HSCs). Healthy HSCs are essential to the maintenance of hemoglobin (Hb) levels regardless of the rapid turnover of red cells and because of their potential as material for gene therapy. Recently, red cell transfusions were shown to reverse pathogenic angiogenesis in a sickle cell mouse model. However, chronic red cell transfusions are difficult to sustain in individuals with SCD due to complications including iron overload, risk of alloimmunization, and the time and cost involved. GBT021601 is a potent sickle Hb polymerization inhibitor that improves the rheology of red blood cells (RBCs) and has been shown to increase total Hb by as much as 3 g/dL. Here, we explore the effectiveness of GBT021601 for restoring bone marrow health and decreasing proangiogenic factors while maintaining the stemness of marrow cells to preserve erythrocyte maturation. Understanding the impact of GBT021601 on aberrant angiogenesis and the function of HSCs will indicate if it has a role in improving the bone marrow niche and HSC fitness in an era of expanded cell-based therapies for SCD.
Methods: Nine-week-old, humanized Townes HbSS SCD mice were split into 3 cohorts (n=8 per group: 4 male, 4 female) and for 12 weeks were fed either standard chow (control) or chow containing 0.1% or 0.2% of GBT021601. After 12 weeks, the mice were phlebotomized and euthanized. Spleens were collected and weighed. Whole bone marrow was collected via centrifugation from both tibias and one femur; the other femur was saved for subsequent 3D confocal microscopy. Bone marrow was analyzed using flow cytometry to measure erythroid cell maturation (Annexin V, TER119, and CD44) and HSC markers (Lin, Sca-1, c-kit). Immunoblot analysis was performed on peripheral plasma to measure hypoxia-induced signaling and markers of angiogenesis (VCAM-1, VEGF-A, ANG-1, and -2).
Results: Hb increased by 3 g/dL on average with administration of GBT021601, and spleen weights were significantly reduced (P=0.0023 and P=0.0015 for control vs 0.1% and 0.2%, respectively). Flow cytometry of the extracted bone marrow revealed a significant increase in mature RBCs and a decrease in erythroid progenitors at all stages (P<0.001 for RBCs and basophils; P=0.04, P=0.002, and P=0.001 for pro-, poly-, and ortho-chromatic erythroblasts, respectively) (Figure 1). HSCs, defined as Lin-Sca-1 $^{+}$c-kit $^{+}$, were not significantly different between groups (control vs 0.1%, control vs 0.2%, and for 0.1% vs 0.2%). Immunoblot analysis revealed a reduction in VCAM-1 and ANG-1 markers with administration of GBT021601 relative to control. However, no difference between ANG-2 and VEGF-A markers was observed between treated and control mice (Figure 2).
Conclusions: Treatment with GBT021601 was effective at reducing extramedullary hematopoiesis as determined by the reduction in spleen size compared with control mice. VCAM-1 and ANG-1 are increased in HbSS compared with HbAA mice; treatment with GBT021601 reduced factors that contribute to pathogenic angiogenesis. Treatment with GBT021601 did not change ANG-2 and VEGF-A expression; notably, VEGF-A levels are similar between HbSS and HbAA mice. Our findings support GBT021601 as a sustainable treatment to reduce ineffective erythropoiesis and abnormal angiogenesis-which can otherwise damage the bone marrow niche-thereby suggesting a potential benefit for individuals with SCD participating in future cell-based therapies with curative intent and improved outcomes. More work is planned to examine the ability of

GBT021601 to normalize the bone marrow environment and reduce the risk of deleterious effects on allogenic or autologous edited HSCs.

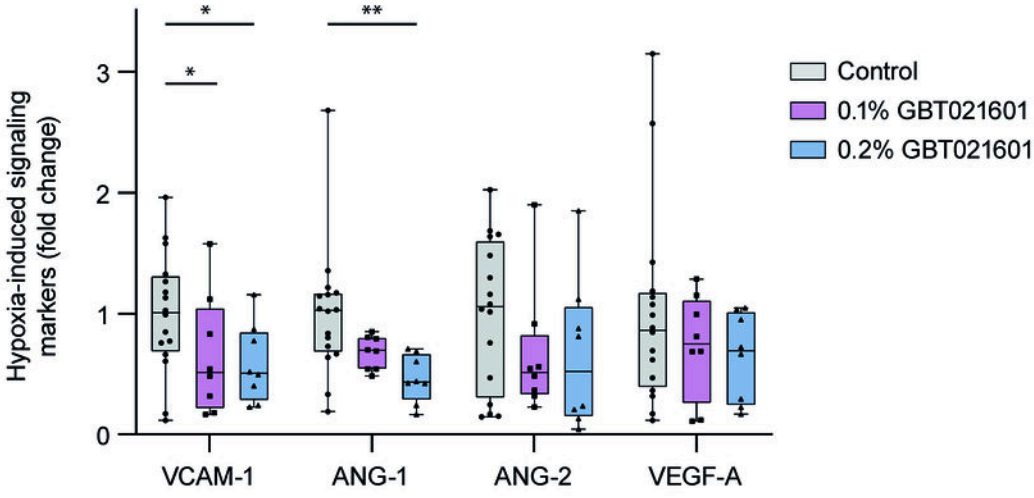
Disclosures Jupelli: *Pfizer:* Current Employment. **Huang:** *Pfizer:* Current Employment. **Nguyen Dang:** *Pfizer:* Current Employment. **Pochron:** *Pfizer:* Current Employment, Current holder of *stock options* in a privately-held company; *Global Blood Therapeutics:* Ended employment in the past 24 months. **Dufu:** *Pfizer:* Current Employment. **Sheehan:** *Novartis:* Research Funding; *Refoxy Pharmaceuticals:* Research Funding; *Pfizer:* Research Funding; *NHLBI TOPMed program:* Research Funding; *Beam Therapeutics:* Research Funding.

Figure 1. Treatment with GBT021601 results in a significant reduction in ineffective erythropoiesis and erythroid maturation arrest in a sickle mouse model[a,b]



[a] A gating strategy using Annexin V-TER119+CD44+ was applied to characterize erythroid maturation.
[b] Graph represents proerythroblasts, basophilic erythroblasts, polychromatic erythroblasts, orthochromatic erythroblasts, reticulocytes, and red blood cells in ascending order. Wilcoxon rank-sum was used for statistical analysis (n=8/group, 4 males and 4 females).
*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, nonsignificant P values not shown.

Figure 2. Hypoxia-induced signaling markers were improved in sickle mice fed GBT021601 as determined from peripheral plasma using immunoblot analysis[a,b]



[a] Vascular cell adhesion molecule-1 (VCAM-1), angiopoietin-1 (ANG-1), angiopoietin-2 (ANG-2), and vascular endothelial growth factor-A (VEGF-A) antibodies shown as protein-fold change relative to control and normalized to IgG heavy chain (loading control).
[b] Dunn's test was used for statistical analysis (n=8/group, 4 males and 4 females for 0.1% and 0.2%; n=16/group for respective controls combined).
*$P \leq 0.05$, **$P \leq 0.01$, nonsignificant P values not shown.

Figure 1

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