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ORAL ABSTRACTS

114.SICKLE CELL DISEASE, SICKLE CELL TRAIT AND OTHER HEMOGLOBINOPATHIES, EXCLUDING THALASSEMIAS: CLINICAL AND EPIDEMIOLOGICAL

Preclinical and Clinical Use of AB1, a DNMT1 Protein Depleter, to Upregulate Fetal Hemoglobin in Townes Sickle Cell Disease (SCD) Mice and Patients with SCD

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Background: Sickle Cell Disease (SCD) is an inherited hematologic disorder characterized by lifelong hemolysis, vaso-occlusion and severe complications including pain, acute chest syndrome and stroke among others. Its complex pathophysiology is driven by polymerization of mutated sickle hemoglobin (HbS) in red blood cells. Fetal hemoglobin (HbF) has been well documented to interfere with HbS polymerization, however, γ-globin gene transcription is silenced after birth through the action of a repressor complex including DNA methyltransferase 1 (DNMT1) during hemoglobin switching. We previously demonstrated the ability of AB1, a potent non-cytotoxic agent which depletes DNMT1 protein, to induce HbF in sickle erythroid progenitors. To expand these findings, we aimed to 1) perform preclinical studies in the Townes SCD mouse model to determine the ability of AB1 to induce HbF and deplete DNMT1 protein; and 2) evaluate the safety of oral AB1 in patients with SCD through an open-label, dose escalating phase 1/2 study (NCT05261711).
Methods: *Preclinical.* Townes SCD mice, 4 months old (6 mice per group) were treated by intraperitoneal injections of escalating AB1 doses of 0.5, 1.5 and 3.0 mg/dose, hydroxyurea (HU; 100 mg/kg/dose) alone or HU combined with AB1 (0.5mg/dose) and water control, 5 days/week for 6 weeks. Every 2 weeks, complete blood counts with differentials were performed along with flow cytometry for HbF positive cells (F-cells) and reticulocytes; RT-qPCR for γ- and β-globin mRNA levels was completed and the spleen was harvested for weights and DNMT1 protein levels by Western blot.
Clinical Trial. After informed consent, patients with confirmed HbSS or HbSb ^{0} thalassemia, on stable HU doses (if on treatment), and no recent/chronic transfusions or pain crisis were approached for the study. Patients were enrolled at Duke and Augusta University, starting on 2 mg of AB1 with 4 additional cohorts (4mg, 8mg, 16mg, and 32mg) to be recruited. Doses were taken daily by mouth for 8 weeks. Safety and tolerability (primary outcomes), as well as pharmacokinetics, pharmacodynamics and HbF response (secondary outcomes) were assessed. The F-cells and mean fluorescence intensity were measured by flow cytometry and HbF% by HPLC.
Results: *Preclinical.* In general, SCD mice treated with AB1 showed good weight gain without toxicity. Three deaths occurred in the HU and combination treatment groups along with a significant decrease in platelets, granulocytes, and monocytes; by contrast, one death occurred in the 3mg/kg AB1 group, but the blood or reticulocyte counts were not affected. By week 6, HU increased F-cells by 2.1-fold (p=0.02) compared to a 2.2-fold (p=0.006) and 1.5-fold (p=0.03) increase by AB1 1.5mg/kg and 3.0 mg/kg respectively. Likewise, γ-globin mRNA increased, and β-globin mRNA decreased at the highest dose. Spleen analysis showed decreased weights and an 80% decrease in reactive oxygen species by AB1. Finally, DNMT1 protein levels were depleted by AB1 in spleen tissue.
Clinical Trial. The clinical trial opened for enrollment in the winter of 2022. Two patients completed cohort 1 and two patients have enrolled and started cohort 2. Both cohort 1 patients had HbSS with a median age of 29.5 years (range 28-31). No change

in total hemoglobin or reticulocyte counts was observed in either subject treated with 2mg AB1 daily. During the treatment period two adverse events occurred (rash-drug related and elevated liver enzymes-unrelated), however there were no serious adverse events. AB1 increased F-cell levels in subject 1 by 5.7-fold (2.9% to 16.4%). Expanded analysis of subject 2 showed an increase in F-cells 3-fold (4.8% to 14.4%) and 3-fold increase in F-reticulocytes. At the lowest 2mg dose, by HPLC analysis both patients had an increase in HbF% from a mean of 6.3% to 7% supporting protein induction by AB1.
Discussion: The goal of this project is to develop an oral-active HbF inducer for the treatment of individuals with SCD. Data generated in the SCD mouse showed a significant increase in F-cells and γ-globin gene transcription, along with DNMT1 protein depletion. Subsequent oral AB1 treatment of patients with HbSS confirmed increased F-cells and HbF protein levels without clinical toxicities. These findings support continuing the ongoing phase 1/2 study, which will provide further insights into the ability of AB1 to increase HbF without hematologic side effects in patients with SCD.

Disclosures Shah: *Agios Pharmaceuticals:* Consultancy; *Alexion Pharmaceuticals:* Speakers Bureau; *Global Blood Therapeutics/Pfizer:* Consultancy, Research Funding, Speakers Bureau; *Bluebird bio:* Consultancy; *Vertex:* Consultancy; *Forma:* Consultancy. **Kutlar:** *Akira Bio:* Membership on an entity's Board of Directors or advisory committees, Research Funding; *Forma/Novo-Nordisk:* Research Funding; *GBT/Pfizer:* Honoraria, Membership on an entity's Board of Directors or advisory committees, Research Funding; *Novartis:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees, Research Funding. **Blackburn:** *Akirabio:* Current Employment. **Vadivelu:** *Akirabio:* Current Employment. **Schaub:** *Akirabio:* Current Employment. **Santos:** *Akirabio:* Consultancy.

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