FAQs for BMT CTN PROTOCOL 2001 DRAFT v1.0

A Multi-Center, Phase 2 Gene Transfer Study Inducing Fetal Hemoglobin in Sickle Cell Disease (SCD)

1. **What is gene therapy?**
   Treatment for an inherited genetic disease by making changes to a person's genetic material with the intent to alter the gene's expression. The goal is to make permanent changes for a lifetime of benefit. New genetic material is introduced to replace or alter the expression of the disease gene. The procedure is similar to a bone marrow transplant, in that the patient's malfunctioning blood stem cells are reduced or eliminated using chemotherapy, but it is different because instead of using a different person's (donor) cells for the transplant, the patient's own blood cells are given back after the new genetic material has been introduced into those cells. The method that is used to introduce the gene into the patient's own blood cells is to engineer and use a modified version of a virus (called a 'vector') that efficiently inserts the "correcting" genetic material into the cells. The vector is a specialized biological medicine that has been formulated for use in human beings.

2. **What is the advantage of targeting BCL11A vs using a more standard gene therapy approach of adding back a non-sickling globin gene?**
   We recently discovered a gene that is very important in the control of fetal hemoglobin expression and is key in the universally observed switch from fetal hemoglobin (Hb F) to adult hemglobin (Hb A, normal hemoglobin) or, in the case of individuals with SCD, sickle (Hb S) hemoglobin production in the first year of life. Importantly, the Hb F of SCD patients does not sickle and actually inhibits the sickling of Hb S. Consequently, patients with SCD generally do not have disease manifestations while their Hb F levels are high. The goal of this gene therapy is to maintain high levels of Hb F production. In a pilot study, decreasing the expression of this gene in sickle cell patients increased the amount of fetal hemoglobin while simultaneously reducing the amount of sickle hemoglobin in their blood, thereby reducing the severity of the disease. The gene we are targeting for change in this study that controls the level of fetal hemoglobin is called BCL11A (B cell lymphoma/leukemia 11A).

   The protein BCL11A a zinc-finger protein encoded by a gene on chromosome 2p15 is an important developmental regulator of both the initial "hemoglobin switch" and of the persistent silencing of γ-globin (fetal globin) expression. By serving as a repressor of the γ-globin gene, BCL11A is an important developmental regulator of the hemoglobin switch. This protocol involves introduction of a small interfering RNA call an shRNA embedded in a microRNA (called a shmiR) targeting BCL11A expression in erythroid cells derived from hematopoietic stem cells for the purpose of *concurrently increasing* γ-globin and *reducing* mutant β^S globin expression by knockdown of BCL11A. RNA interference (RNAi) is a post-transcriptional silencing mechanism in which a small double-stranded RNA molecule (in this case, a short hairpin, or "shRNA") acts to suppress expression of a target gene (in this case, the *BCL11A* gene) with sequence homology. Our approach (the BCL11A knockdown) takes advantage of the physiological regulation machinery that controls globin expression by reversing a portion of the hemoglobin "switch" that occurs in hematopoietic development. Our approach has the advantage of increasing fetal hemoglobin which has *potent anti-sickling effects* and concurrently and coordinately *reducing the red cell content of β^s sickling globin*. In contrast, the gene addition strategies (e.g., the LentiGlobin BB305) being pursued for treatment of β-hemoglobinopathies require expression

of the transgene to compete with the endogenous mutant β-globin production without the explicit target of reducing the concentration of β^s sickling globin. At a similar rate of successful gene transfer, we hypothesize that the BCL11A knockdown approach has the potential for greater clinical impact by simultaneously harnessing the anti-sickling effects of HbF and silencing the mutated β-globin.

Thus, the aim of our study is to use a lentiviral vector shRNA approach to decrease expression of the BCL11A gene. Since BCL11A is a repressor of gamma globin expression, knocking down the expression of BCL11A will de-repress gamma globin expression and thus allow increased production of Hb F. Increasing Hb F is a promising strategy for significantly alleviating the clinical manifestations of SCD.

3. What preliminary data support the use of BCL11A as a target for this trial?

Preliminary data for this study are derived from animal studies (Guda et al; 2015; Brendel et al., 2016; Brendel et al., 2020) and human studies completed during a pilot and feasibility (phase 1) study. Human data have been reported in abstract form (Esrick et al. Late Breaking Abstract, ASH, 2019) and are contained in a manuscript currently under review (Esrick et al., manuscript in revision, NEJM, 2020). To summarize, these data in humans (N=5, with follow-up of 16 months (range 9-26 months) show significant induction of fetal hemoglobin (21.6-44% at latest follow-up), a near pancellular distribution of F cells (65-81%) and high Hb F content/ F cell (9.4-18.6 pg/cell). There was significant amelioration of anemia (Hb 10-11 gm/dl, unsupported) and no veno-occlusive events (VOEs) since treatment. There were no serious adverse events related to the gene therapy product.

4. Why is VOE reduction a primary endpoint?

As noted above, increasing Hb F is clearly beneficial in SCD. Hydroxyurea (HU) is successful in increasing Hb F and F cells; the average Hb F on HU is 18-24%. Higher Hb F is associated with lower mortality, less pain, and lower rates of other complications. Data from our pilot study provide evidence that a one-time gene therapy infusion with this product can produce uniformly high and sustained Hb F levels in both adults and children.

Clinically severe disease is defined as at least 4 VOEs within the preceding 24 months and fulfilling this criterion is a requirement for the study. Our definition of the primary endpoint is a complete reduction in the number of VOEs from the time of stabilization of Hb F after infusion of gene modified cells in the 18-month period from 6 - 24 months post infusion, compared to the 24-month period prior to consent. We think this is a clinically meaningful impact on the disease.

Our primary endpoint, developed with direct input from the FDA at our Type C meeting, is supported by the findings of the bluebird bio HGB-206 study (group C) reported at the 2020 European Hematology Association meeting. In that study, 13/14 patients (92.8%, with 95% CI of [66.3%, 99.8%]) had no VOEs post-infusion of gene modified cells. These preliminary observations reflect a high rate of complete reduction of severe VOE; however, a slightly lower rate would still be considered clinically important and meaningful. In addition, we considered recent outcome data for allogeneic bone marrow transplant **(***Eapen et al. Lancet Haematol 2019***)**. Among 910 sickle cell patients who received allogeneic bone marrow transplants between 2008 and 2017, event-free survival (EFS) (defined as survival without graft failure) was 85% in patients < 13 years old and 75.6% in patients 13-49 years old. Analyzed further by donor type, EFS was 90.7% in matched sibling donor (MSD) transplant recipients and 65.3% in non-

MSD transplants. Based on these data, for our trial we have chosen an alternative hypothesis of 80.5% and a null hypothesis of 50% of patients with 100% reduction in VOEs.

5. **How does Hb F mitigate the sickle cell phenotype?**
   Hb F mitigates the phenotype of sickle cell disease by inhibiting deoxygenated sickle hemoglobin (Hb S) polymerization. Inhibition of polymerization of Hb S molecules occurs because a glutamine residue at position γ87 acts to prevent lateral contact in the double strand of the sickle fiber.

6. **What level of Hb F is needed to mitigate the sickle cell disease phenotype?**
   While this number is not known with certainty, it is felt that a total Hb F concentration of at least ~25% is required for the number of protected cells to approach 70% to mitigate SCD severity. However, the whole blood HbF level is less predictive for phenotype correction than the distribution of fetal hemoglobin in large numbers of red cells (so-called "F-cells") and the concentration of fetal hemoglobin in these F cells (F/F cell), which are being measured as biological endpoints in study.

7. **Why were the biological endpoints of %F, F/F cell and the number of F cells chosen?**
   Hb F is unevenly distributed among red blood cells and some cells may not have sufficient concentration to inhibit Hb S polymerization. Therefore, the number of cells with detectable Hb F (F-cell) and the proportion of those F-cells that have sufficient Hb F is the most critical predictor to prevent polymerization of Hb S.

8. **Why is plerixafor used for mobilization instead of or in addition to G-CSF and how are hematopoietic stem cells collected in this protocol?**
   High CD34+ cell numbers are important to ensure a stable graft as supported by data analysis from several ongoing gene therapy trials. Previous gene therapy studies have used bone marrow harvests as a source of stem cells. However, in SCD this has been problematic with inadequate numbers of cells harvested, requiring up to four different harvests, each 8-10 weeks apart and each requiring general anesthesia. Thus, many gene therapy trials now use mobilized peripheral blood to generate adequate number of stem cells for gene transfer.

   Granulocyte colony-stimulating factor (G-CSF**),** which is generally used to mobilize hematopoietic stem cells into the peripheral blood, is contraindicated in SCD because G-CSF has been reported to cause severe adverse effects in these patients. Even with doses smaller than the dose utilized for standard mobilization of hematopoietic stem cell (HSCs), G-CSF has been shown to result in vaso-occlusive pain crises, severe acute chest syndrome, and in one report, massive splenomegaly and death.

   Plerixafor is an agent that leads to HSC mobilization by reversibly inhibiting the binding of the chemokine stromal-derived factor 1 (SDF-1/CXCL12) to its receptor CXCR4, which is expressed on the surface of HSCs. Disruption of the SDF-1/CXCL4 interaction results in the rapid release of HSCs from the bone marrow. At Boston Children's Hospital a pilot trial was completed to assess safety as well as feasibility of procuring an adequate CD34+ cell dose in subjects with SCD. Six patients completed treatment with plerixafor, 3 at a lower dose of 180 µg/kg, and 3 at the FDA-approved dose of 240 µg/kg. All of the subjects tolerated the plerixafor and apheresis without any sickle cell-related complications of any grade, and there have been no adverse events of any kind ≥ grade 3. After optimization of the apheresis procedure to allow for sickle cell related cell processing modifications, a

successful apheresis collection of > 2 x10^6 CD34+ cells/kg of CD34+ cells was achieved in all subjects mobilized at the full plerixafor dose. Several other small studies have also demonstrated the safety and efficacy of plerixafor in SCD patients. Finally, the Pilot Study on which this phase 2 study is based also utilized plerixafor safely and successfully.

9. What are risks of using lentivirus vector for gene transfer and what engineering measures were used to mitigate this risk for this trial?

Lentivirus vectors are used so that the genetic alterations are inserted into the chromosome where they become a permanent part of the genome. Past gene therapy trials have utilized different versions of virus vectors and the insertion of these led to inappropriate activation of neighboring genes, called insertional mutagenesis. In some cases, this led to leukemia due to activation of oncogenes. The vector utilized in this trial has markedly reduced this risk by: i) removing the strong enhancer elements that can lead to inappropriate activation of nearby genes; ii) incorporating a design that allows expression only in erythroid cells, avoiding expression in stem cells and lymphocytes that retain significant proliferative capacities; iii) incorporating very potent transgene function, thereby allowing phenotype correction with low vector insertions/cell (called 'vector copy number", VCN). These changes were shown to work in robust preclinical testing that determined a very low propensity of this vector to cause abnormal proliferation of hematopoietic cells.

10. What are the risks of busulfan conditioning for autologous stem cell transplantation?

The purpose of conditioning (myeloablative busulfan dose) prior to infusion of genetically modified HSCs is to favor the engraftment of genetically treated hematopoietic stem cells as much as possible. In SCD, engraftment of transduced hematopoietic stem cells from which gene modified erythroid cells will be derived at high level is desirable. Early complications (in particular veno-occlusive disease) associated with busulfan are mitigated by using pharmacokinetic monitoring to target a total exposure (AUC) of ~20,740 - 23,180 micromole-min/L, or 85-95 mg*h/L, given over 4 days. To our knowledge, no VOD has been reported using busulfan as a single agent in gene therapy trials. Some patients may require blood product support consisting of red cell and platelet transfusions during this time. If no recovery is observed after six weeks, then a previously-collected unmanipulated back-up source of autologous CD34+ HSC will be administered to restore hematopoiesis. At the discretion of the treating physician, and based upon the clinical status of the patient, infusion of the back-up marrow may be delayed by up to one week (7 weeks after gene transfer).

11. How was the size of the trial determined?

The sample size of 25 evaluable patients provides at least 90% power for a one-stage rule, with a one-sided type 1 error of 0.022, to evaluate the primary endpoint.

References

1. Eapen M, Brazauskas R, Walters MC, Bernaudin F, Bo-Subait K, Fitzhugh CD, Hankins JS, Kanter J, Meerpohl JJ, Bolaños-Meade J, Panepinto JA, Rondelli D, Shenoy S, Williamson J, Woolford TL, Gluckman E, Wagner JE, Tisdale JF. Effect of donor type and conditioning regimen intensity on allogeneic transplantation outcomes in patients with sickle cell disease: a retrospective multicentre, cohort study. Lancet Haematol. 2019 Nov;6(11):e585-e596.