Scientific Abstract

bluebird bio, Inc., a privately owned biotech company located in Cambridge, MA, is developing an ex-vivo gene therapy product, LentiGlobin BB305 Drug Product, consisting of autologous CD34+ hematopoietic stem cells transduced with lentiviral vector LentiGlobin BB305 encoding the human $\beta^{A\text{-}T87Q}$-globin gene and suspended in cryopreservative solution, for the treatment of β-thalassemia, a rare, debilitating and life threatening, genetic disease caused by the absent or insufficient production of the β chain of hemoglobin.

β-thalassemia results from the impaired production of β-globin (β^A) and leads to a relative excess of α-globin. Unable to form hemoglobin tetramers, the excess α-globin chains become insoluble and form a precipitate that damages the developing red blood cell precursors (erythroblasts). The clinical implications of the α-globin/ β-globin imbalance are twofold: 1) patients lack sufficient red blood cells (RBC) and hemoglobin to effectively transport oxygen throughout the body; and 2) abnormal red blood cells suffer an elevated rate of hemolysis that can lead to morbidities through chronic damage to the vasculature and iron overload.

The clinical course of β-thalassemia correlates with the degree of globin chain imbalance. In the most severe cases, termed β-thalassemia major, patients require ≥ 8 red blood cell transfusions per year (Rachmilewitz & Giardina, 2011). In terms of genotype, most β^0/ β^0, β^+/β^+ and half of the β^E/ β^0 thalassemic patients suffer from β-thalassemia major. While β^0 alleles produce no β-globin and β^+ very little, the β^E mutation leads to 50% to 60% reduction in expression (Traeger *et al*., 1980; Wasi *et al*., 1982). Clinically, β-thalassemia major patients experience profound hypochromic, microcytic anemia, and produce as little as 1 to 7 g/dL of total hemoglobin (Olivieri, 1999; Rachmilewitz & Giardina, 2011).

Where adequate treatment is available, β-thalassemia major patients receive chronic blood transfusion regimens aimed at maintaining steady state hemoglobin levels of about 9-10 g/dL. This regimen consists of infusions with units of packed red cells every three to five weeks, and is highly effective at preventing the hallmark symptoms of childhood β-thalassemia major. However, chronic transfusions introduce a large iron overload (Cao *et al*., 1996), and over time lead to mortality through iron associated heart and liver toxicity (Vichinsky *et al*., 2005). In order to prevent iron overload associated risks, patients must adhere to iron chelation regimens administered orally (Hoffbrand *et al*., 2003; Cappellini *et al*., 2006) or subcutaneously (Olivieri & Brittenham, 1997). However, poor compliance remains a key challenge, and even with current therapies overall survival until the age of 30 is only 55% (Modell *et al*., 2000; Delea *et al*., 2007).

The only cure for β-thalassemia major is allogeneic hematopoietic stem cell transplant (HSCT). Because of the significant risk of transplant related mortality, graft versus host disease (GVHD) and graft rejection with allogeneic HSCT, transplants are offered primarily to subjects with available HLA-matched sibling donors (< 25% of cases). For these patients, the most recently large scale allogeneic HSCT study for 179 subjects (Sabloff *et al*., 2011) reported the following

five year probabilities of overall and disease free survival rates: 91% and 88%, respectively, for patients with Pesaro risk class II and 64% and 62%, respectively, for Pesaro risk class III. Approximately 9.5% of subjects suffered graft failure, and 38% and 13% experienced acute GVHD and chronic GVHD, respectively. The experience with unrelated donor umbilical cord blood (UCB) HSCT has been less promising, with reported overall and disease free survival rates of 77% and 65%, respectively (Rachmilewitz & Giardina, 2011).

In order to provide an effective and safer alternative to allogeneic HSCT, bluebird bio is proposing a clinical protocol to evaluate the use of LentiGlobin BB305 Drug Product in subjects with β-thalassemia major, i.e. patients who are transfusion dependent, defined as requiring greater than 100 ml/kg/year of packed red blood cells or 8 or more transfusions per year. The LentiGlobin BB305 lentiviral vector encodes a single codon variant of the β-globin gene, $\beta^{A\text{-}T87Q}$, which conserves the protein's function while allowing for quantification relative to other globin species. Expression of the $\beta^{A\text{-}T87Q}$-globin gene is driven by the erythroid lineage specific globin Locus Control Region (LCR) to correct the β-globin/ α-globin imbalance in erythrocytes. Treatment with LentiGlobin BB305 Drug Product uses autologous hematopoietic stem cells (with the added sequence of the human $\beta^{A\text{-}T87Q\text{-}}$globin gene), thereby significantly reducing/eliminating the risk of GVHD and graft rejection. The added sequence should provide expression of a functional $\beta^{A\text{-}T87Q}$-globin protein correcting the anemia.

bluebird bio has reported preliminary clinical safety and efficacy data from three subjects treated with autologous CD34+ stem cells transduced with LentiGlobin HPV569 lentiviral vector in an ongoing Phase 1/2 trial in France (LG001). The first treated subject (1002) failed to engraft and received rescue cells. The second treated subject (Subject 1003) became transfusion independent 12 months following treatment with LentiGlobin HPV569 Drug Product (Cavazzana-Calvo *et al*., 2010) with sustained expression of $\beta^{A\text{-}T87Q}$-globin protein for close to four years now. The last subject in this trial was treated in November 2011. For this subject, neutrophil engraftment occurred on Day 20 post-transplant, and delayed platelet engraftment on Day 129 post-transplant. There have been no gene therapy related adverse events attributed to the genetically modified cells (i.e. no GVHD-like adverse events), and no leukemia or lymphoma related to insertional mutagenesis.

Based on these preliminary clinical results, bluebird bio intends to initiate a Phase 1/2 clinical study (HGB-204) in the US in 2012 for the treatment of β-thalassemia major with LentiGlobin BB305 Drug Product.