Blood Advances Talks audio review for *Blood Advances*

Title: Investigational curative gene therapy approaches to sickle cell disease

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Contributions:

DAW drafted the paper

EE provided editorial review

COI:

DAW received research funding from bluebird bio for research in hemoglobinopathies in the past. BCH licensed certain IP relevant to hemoglobinopathies to bluebird bio. The current license includes the potential for future royalty/milestone income. Bluebird has indicated they will not pursue this as a clinical program and BCH is negotiating return of IP. Received payment in past through BCH institutional licensing agreement. Bluebird bio provided GMP vector for SCD clinical trial. EE participated in consulting for bluebird bio.

Running title: Gene editing for beta-hemoglobin diseases

Abstract (250)

Sickle cell disease (SCD) is an inherited blood condition resulting from abnormal hemoglobin production. It is one of the most common genetic diseases in the world. The clinical manifestations are variable and range from recurrent acute and debilitating painful crises to life-threatening pulmonary, cardiovascular, renal and neurologic complications. The only curative treatment of SCD at this time is bone marrow transplantation (also called hematopoietic stem cell transplantation, or HSCT) using healthy blood stem cells from an unaffected brother or sister, or from an unrelated donor if one can be identified who is a match in tissue typing. Unfortunately, only a minority of sickle cell patients has such a donor available. The use of autologous hematopoietic stem cells and alternative types of genetic modifications is currently under study in clinical research trials for this disease. The approaches include the use of viral vectors to express globin genes that are modified to prevent sickle hemoglobin polymerization or to express interfering RNAs to “flip the switch” in adult red cells from adult β-sickle hemoglobin to fetal hemoglobin using a physiologic switch, and several gene editing approaches with the goal of inducing fetal hemoglobin or correcting/modifying the actual sickle mutation. In this audio review, we will discuss these different approaches and review the current progress of curative therapy for sickle cell disease using gene therapy. (221)

Text (1000-1500)

The only curative treatment of sickle cell disease SCD at this time is bone marrow transplantation (also called hematopoietic stem cell transplantation, or HSCT) using healthy blood stem cells from an unaffected brother or sister, or from an unrelated donor if one can be identified a highly HLA-matched donor.[1] Unfortunately, only a minority of sickle cell patients has such a donor available. Even when an appropriate donor is found, the transplant can be unsuccessful because the patient's immune system rejects the donor bone marrow (called graft rejection). Other times the transplant causes toxicity because patients develop graft versus host disease (GVHD), a condition in which some of the immune blood cells from the donor attack the body of the recipient patient and cause severe damage. Treatment for GVHD includes long-term use of powerful immunosuppressive drugs which can increase the risk of serious, even fatal, infections. Both rejection and GVHD are more common when the donor is not a matched sibling but can occur any time a transplant is done using cells from another person. These complications also seem to occur more frequently in patients with sickle cell disease.

An alternative promising approach for the treatment of genetic diseases is called gene therapy.[2] Gene therapy is a relatively new field of medicine that alters genetic material (mostly DNA) within the patient to treat his or her own disease. In gene therapy, we introduce new genetic material or alter the genome in order to fix or replace the patient's disease gene or otherwise alter the disease phenotype, with the goal of curing the disease. 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 has the advantage of not requiring a lengthy donor search, eliminating any risk of GVHD, reducing the risk of graft rejection, and using a single drug for the conditioning portion of the transplant procedure.

There are different methods used to attempt correction of the sickle cell disease phenotype using autologous cells. In one approach, a modified version of a virus (called a 'vector') is used to efficiently insert the "correcting" genetic material into the cells. The vector is a specialized biological medicine that has been formulated for use in human beings. In several current trials the genetic material added is a modified, sickling-resistant, version of the β-globin gene or the γ-globin gene.[3-5] Expression of the modified gene leads to reduction in sickling within cells that would normally express only the mutant sickle hemoglobin. In another approach, called post-transcriptional gene silencing, the viral vector carries genetic instructions to reverse the globin expression from adult β-globin (in this case the β-sickle globin) to γ-globin and thereby simultaneously reducing sickle hemoglobin while expressing high levels of fetal hemoglobin.[6] This approach takes advantage of the potent anti-sickling characteristics of fetal hemoglobin. The therapeutic target of this approach is a major repressor of γ-globin expression in adult red cells, called BCL11A, and the vector carries RNA interference sequences in the form of a small hairpin (sh) RNA embedded in a microRNA (termed a shmiR).

An alternative to the use of viral vectors to insert new genes or new genetic material into hematopoietic stem cells is to edit the DNA sequences in the genome. There are currently at least four editing approaches. In one, a small intronic enhancer that regulates the red cell expression of BCL11A is excised (ie 'edited out') using either CRISPR or zinc-finger nucleases.[7-9] This leads to induction of fetal hemoglobin and reduction of sickle hemoglobin. A second approach edits the promotor of γ-globin to create a mutation identical to one found in a genetic condition called "hereditary persistence of fetal hemoglobin or HPFH", which leads to high levels of fetal hemoglobin expression into adulthood.[10-12] In the third approach, the sickle mutation itself is edited

to a normal sequence using homology driven repair and a normal gene template thus eliminating- in the correctly edited cells- the sickle globin expression and replacing this with normal β-globin expression.[13-14] In these three methods, nucleases are directed to the sequence of interest by homologous sequences and cut the DNA leading to repair to the intended new sequence. In the fourth, called base editing, the homologous sequences direct an enzyme to chemically change the mutated sickle mutation to a base pair that does not sickle called Hb G-Makassar, a naturally occurring variant hemoglobin, which appears to function perfectly well for oxygen delivery.[15]

Several of these methods are currently in clinical trials and all appear to be successful in reversing some of the clinical characteristics of sickle cell disease. However, these efforts are still very early in development with short follow-up times and relatively few patients in the trials. For all the above methods, success and broad adoption of the technology ultimately depends on several factors. First is the efficiency of the genetic modification of long-lived and multi-lineage reconstituting hematopoietic stem cells which relates both to the number of corrected red blood cells derived from these stem cells and the duration of correction. The efficiency of viral vectors for transferring genetic material into human hematopoietic stem cells has steadily improved over the past 30 years, and now the fraction of engrafting hematopoietic stem cells that can routinely be modified in clinical scale should be sufficient to alter the disease phenotype in sickle cell disease. For gene editing approaches, efficiencies are still improving but early results from clinical trials suggest the some of the methods currently in testing, particularly those that use CRISPR, can be successful in large scale in human hematopoietic stem cells. The second critical factor for success is safety. For viral vectors, this primarily depends on eliminating insertional mutagenesis leading to abnormal expression of genes associated with the development of myelodysplastic syndrome. While current vectors, based primarily on human immunodeficiency, or lentiviruses, appear safe, relatively low numbers of patients have been followed long-term. For gene and base editing, an unknown risk is unintended cutting of DNA due to so-called “off target” pairing of the nuclease with gene sequences other than the intended target. Early studies appear to show this is rare but does occur, but current methodologies to monitor for these off-target effects are more complicated and likely less robust than monitoring for insertional mutagenesis. For any autologous genetic therapy, myeloablative alkylator-based conditioning is associated with a small risk of secondary myeloid malignancy. Based on the occurrence of myeloid leukemias on one particular gene therapy trial,[16] research is also ongoing to elucidate whether patients with sickle cell patients have a higher baseline risk of malignancy. Given the large numbers of cells being infused in patients, even rare events could theoretically lead to adverse events. Finally, should any of these approaches be determined by careful clinical trials to be efficacious and safe, in the long run the costs of these therapies will be large and access to a large number of individuals will be an issue that will require consideration. (1125)

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