Gene therapy for congenital marrow failure syndromes – no longer grasping at straws?

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The clinical potential that stems from the discovery of DNA's double helix in 1953, and the subsequent genomic knowledge about health and disease, is now beginning to be realized as targeted corrections of genetic lesions are being translated into therapies. Hematopoietic disorders, arising from an accessible tissue that is amenable to *ex vivo* manipulation, provide a framework for the development of gene therapy cures. With a particular focus on the hemoglobinopathies, hematologists have been at the forefront of these efforts. In this issue of *Haematologica*, Liu *et al.* report on the application of a non-traditional CRISPR/Cas9 delivery method to establish a faithful model of Diamond-Blackfan anemia (DBA) in primary human hematopoietic stem and progenitor cells (HSPC) that can be rescued by lentiviral gene replacement.[1]

Newborn screening for hemoglobinopathies has been universally performed in the United States for several decades, and recent clinical trials using lentiviral delivery of a non-sickle hemoglobin gene[2] or an inhibitor of hemoglobin switching[3] are now showing promising results. Indeed, the need is great for individuals who suffer from sickle cell anemia or β-thalassemia major. And the global market is large.

A much, much smaller market with a comparable need is found in the congenital bone marrow failure syndromes, which are also leukemia and cancer predisposition syndromes. This expanding list of monogenic disorders includes: Fanconi anemia, DBA, Shwachman-Diamond syndrome, dyskeratosis congenita, severe congenital neutropenia, congenital amegakaryocytic thrombocytopenia, GATA2 deficiency, and SAMD9/9L syndromes. This list continues to expand. Inherited conditions affecting hematopoiesis and resulting in myeloid neoplasms are now recognized in the adult population with germline pathogenic variants found in *ANKRD26*, *RUNX1*, *CEBPA*, and *DDX41*. More comprehensive neonatal screening for blood and non-blood disorders looms. The question will then be how to prevent disease manifestation or progression, and this will require faithful disease modeling in relevant primary human cells. Hematologists will again lead this charge. But how?

One intriguing hematologic disorder with suboptimal models is DBA. DBA is a rare inherited bone marrow failure syndrome that presents in infancy with pallor due to a profound hypoplastic macrocytic anemia. The mainstays of therapy are chronic red blood cell transfusions and judicious use of corticosteroids, while the only cure is allogeneic bone marrow transplantation.[4] Chronic steroid therapy is effective in about one-third of patients, but it can confer long-term morbidity, affecting immune function, the adrenal axis, glucose utilization, and fat deposition. Chronic steroid use can lead to gastric ulcers, cataracts, osteopenia, delayed growth, and neuropsychologic impairment.[5] One goal has been to find another effective drug with fewer side effects. Increased erythroid output has been demonstrated in preclinical models of DBA following treatment with leucine,[6] trifluoperazine,[7] and sotatercept,[8] and efforts to translate these therapies to the clinic are ongoing, currently with mixed results at best.[9] These drug-discovery efforts too require an accurate experimental model.

The discovery of mutations in the ribosomal protein gene *RPS19* in DBA[10] confirmed its genetic basis, raising the possibility of definitive gene therapy-based cures. However, at least 20 genes have been identified to cause DBA, almost all of which encode ribosomal structural proteins.[11] Others are related to ribosomal function or are specifically impacted by reduced ribosome numbers (e.g., *HEATR3* or *GATA1*).[12,13] That the disease can be due to a number of genes makes gene therapy somewhat cumbersome and complicates the development of a unified gene therapy cure using traditional approaches.[11] However, approximately 25% of patients have *RPS19* mutations, leading to efforts by the Karlsson group and others to develop an *RPS19*-directed gene replacement strategy to treat this largest subset of DBA patients.

Two major challenges must be addressed in the preclinical development of novel gene therapy approaches: 1) a

faithful *ex vivo* model for the disease must be established to enable evaluation of therapeutic efficacy; and 2) a safe, efficient means for the delivery of the genetic payload must be developed to achieve therapeutic benefit while minimizing short- and long-term toxicities. Early retroviral gene therapy trials were marred by the development of insertional mutagenesis, leading to acute lymphoblastic leukemia in some children who received gene therapy for severe combined immunodeficiency[14] or Wiskott-Aldrich syndrome.[15] Viral vectors were improved, relying on safer lentiviral backbones and alternative promoters, and the risk for leukemic transformation appears to be lessened, although clonal expansion remains a theoretical, if not an actual, risk. The accompanying paper from the Karlsson group extends their prior work[16] developing a lentiviral gene replacement strategy to ameliorate the erythroid maturation defect that is the hallmark of DBA (Figure 1).

Using their previously validated EF1α-driven *RPS19* lentivirus as a gene replacement tool, Liu *et al.* set out to design a more faithful model of *RPS19* haploinsufficiency that would allow for direct evaluation of this and future DBA-directed therapies. Taking advantage of the efficiency of CRISPR/Cas9 editing of *RPS19*, the authors knocked in a GFP reporter to the *RPS19* locus, enabling tracking of *RPS19* disrupted clones by the presence of the GFP signal. They found significant cellular toxicity related to ribonucleoprotein delivery of CRISPR/Cas9 components, unlike what was recently described in the *RPS19* CRISPR model by Bhoopalan *et al.*,[17] perhaps owing to differences in electroporation conditions. Nonetheless, to avoid some of this toxicity, the authors optimized mRNA delivery of CRISPR components by nanostraws and demonstrated the efficacy of this approach in primary human cells for the first time. These proof-of-principle nanostraw CRISPR delivery approaches may one day extend beyond the hematopoietic system to allow for efficient disease modeling in other difficult-to-transfect tissue types, although the requirement for specialized equipment for nanostraw production and use may limit their widespread applicability.

Nanostraw-enabled generation of *RPS19* haploinsufficient erythroid progenitors allowed the authors to profile transcriptional changes associated with RPS19 loss and sub-

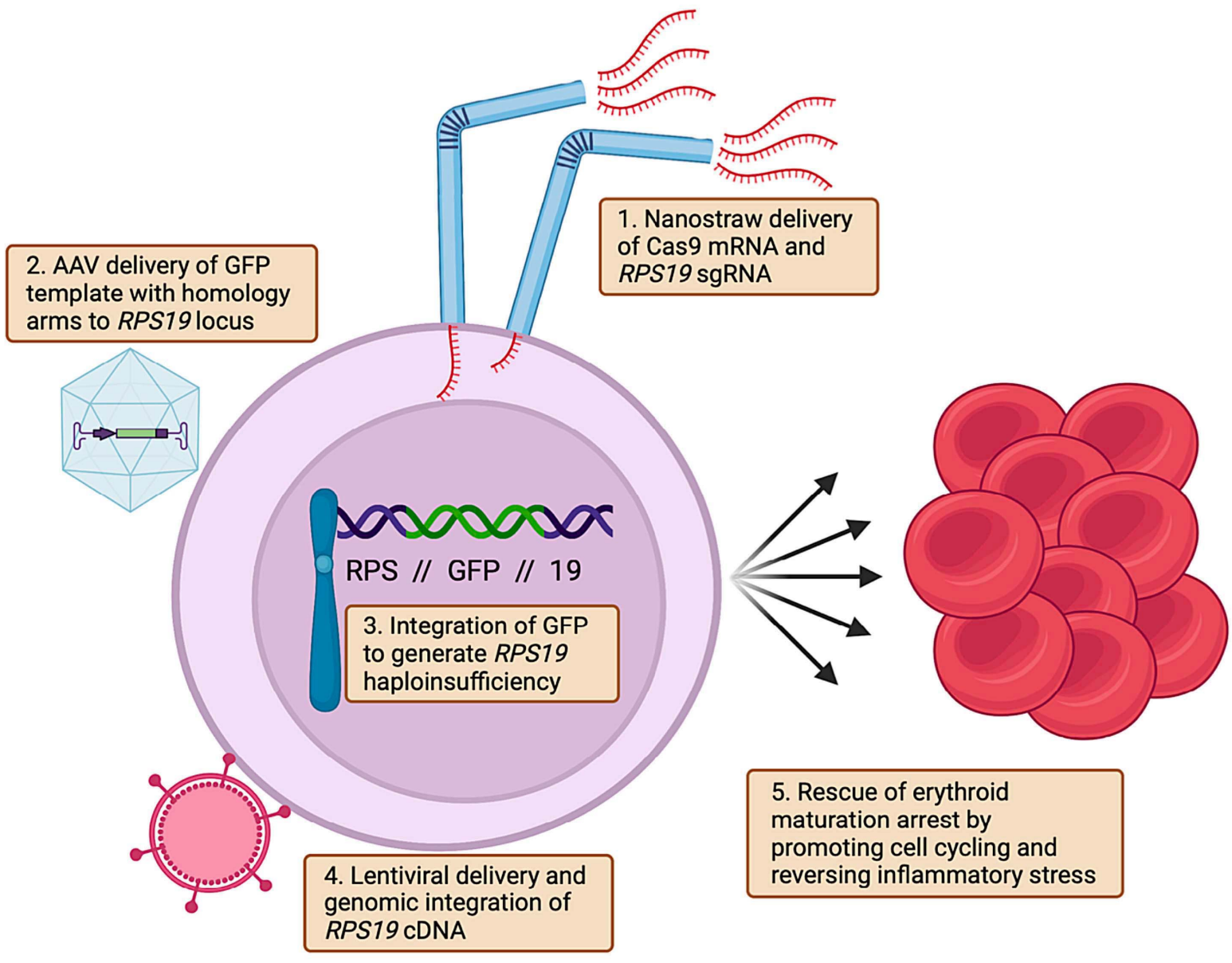

Figure 1. Generation and rescue of primary human hematopoietic stem and progenitor cell model of Diamond-Blackfan anemia. Steps of model generation and gene therapy treatment. 1. Nanostraw delivery of Cas9 mRNA and *RPS19* sgRNA ameliorates toxicity associated with other delivery methods. 2. Delivery of a homology-directed repair template with a gene fluorescent protein (GFP) cassette flanked by arms of homology to the *RPS19* locus. 3. Integration of a GFP cassette at *RPS19* generates trackable clones with *RPS19* haploinsufficiency. 4. Delivery of EF1α-RPS19 by lentivirus. 5. *RPS19* gene replacement improves erythroid differentiation and reverses many of the transcriptional consequences of RPS19 haploinsufficiency. AAV: adeno-associated virus.

sequent treatment with their gene therapy vector in an otherwise isogenic background. As other DBA-directed treatments (both gene therapy and small molecules) emerge, it is essential to have a primary human cell system that will allow for sensitive profiling of direct cellular effects of treatment as the authors show here. Liu *et al.* establish such a model, which may mean that hematologists who are pursuing gene therapy cures for inherited bone marrow failure syndromes are no longer grasping at straws.

Disclosures

No conflicts of interest to disclose.

Contributions

RAV and SJC wrote and edited the manuscript.

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