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Gene Therapy for Fanconi Anemia: One Step Closer to the Clinic

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Game-changing trends in biomedical science usually start as daring, visionary ideas that struggle through the confusion of trials and experiments, experience a crisis at the collision of an expectant public and disbelieving colleagues, and—if successful—finally emerge as widely accepted new standards (Evans, 2011). The gene therapy field has followed this sequence and, in the teens of the twenty-first century, seems to be emerging as a realistic new therapy for genetic disorders of hematopoiesis, such as congenital immunodeficiencies and bone marrow failure syndromes (Naldini, 2011; Sheridan, 2011).

Fanconi anemia (FA) is a prototypical inherited bone marrow failure disorder characterized by aplastic anemia and a dramatically increased risk of hematological and solid malignancies. FA is due to defects in one of at least 15 genes that encode the proteins involved in the cellular response to DNA damage and the maintenance of genomic integrity. More than half of the reported cases of FA are due to *FANCA* gene mutations. FANCA protein is a member of a "functional core complex" (formed with seven other proteins) that is essential in the DNA damage response. A lack of core complex results in a phenotype characterized by bone marrow failure, myelodysplasia, leukemia, and other cancers (D'Andrea, 2010).

With support from the FA Research Fund and Fanconi Hope Charitable Trust, two dozen scientists and clinicians convened in Barcelona on November 22, 2011 for the second meeting of the FA Gene Therapy International Work Group. This International Work Group, chaired by Jakub Tolar (University of Minnesota, Minneapolis, MN), has been established with the goal of coordinating the best available knowledge in gene therapy with the best format of clinical trial for FA. The first meeting, a year ago in London, brought together researchers from fields that rarely interact.

The objective of that meeting was to establish an open platform whereby teams initiating gene therapy trials in FA, institutions that already have strong track records in gene therapy, and groups that are developing novel strategies in genome modification could be brought together. We found common ground in our efforts to accelerate the transition of gene therapy research into clinical trials for patients with FA. The initial FA gene therapy platform was outlined as follows: *FANCA* gene delivered by third-generation lentiviral vector pseudotyped with vesicular stomatitis virus (VSV-G); short transduction without prolonged prestimulation with growth factors; and exclusion of individuals who have a human leukocyte antigen-matched sibling donor, an abnormal karyotype, or a serious infection (Tolar *et al.*, 2011). The particular focus of this year's event was to synthesize the data to inform the impending clinical trials and make the future iterations of FA gene therapy trials possible.

Hematopoietic stem cell gene therapy has the potential to transform conventional therapy for FA, which for decades has involved transfusion support, anabolic steroids, and hematopoietic cell transplantation (HCT). Androgens can

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stabilize the numbers of red blood cells, white blood cells, and platelets in some individuals with FA. Only HCT, however, has been able to cure the hematologic complications of FA. The life-saving impact of HCT has been enormous, especially when we remind ourselves that 50 years ago nearly all leukemia and bone marrow failure conditions were lethal.

HCT—itself one of the most successful stories in medicine—has been optimized for people with FA (e.g., by use of T cell-depleted bone marrow grafts and fludarabine-based conditioning) with an increase in overall survival from 20% in the 1990s to approximately 90% today in patients with immunologically well-matched donors. Thus, HCT is a life-saving measure and the standard of care for bone marrow failure, myelodysplasia, and leukemia in FA therapy today (Macmillan and Wagner, 2010). Inevitably, with the success of the therapy in hematopoietic, life-threatening complications, the focus shifts to minimizing the side effects of HCT. These can result either from the physical injury by chemoradiotherapy (e.g., pulmonary toxicity, renal toxicity, or systemic endothelial injury, termed *veno-occlusive disease*) or immune injury (e.g., from graft-versus-host disease, whereby allogeneic immune cells from the donor graft attack the tissues of the recipient). Furthermore, long-term side effects such as secondary cancers and endocrinopathies can impact the quality of life after HCT.

Gene therapy can, in principle, circumvent many of these unwanted effects while preserving the benefits of HCT because the transplanted cells come from the patient (autologous) and because either no or low-dose conditioning is needed. Indeed, the potential of gene therapy in this disease is confirmed by experiments of nature that involve the acquisition of "corrective" somatic mutations in single hematopoietic stem cells. These mutations are capable of curing the bone marrow defect while serving as a source of stem cells that can successfully repopulate the bone marrow of a Fanconi recipient. Additional evidence exists. Specifically, in the last decade more than 50 individuals with congenital immunodeficiencies were treated with an infusion of autologous cells that had been gene-corrected with retroviral vectors. The majority of these individuals derived clinically meaningful benefits from the therapy, a response that was a solid improvement over the typical results achievable with HCT in these diseases. However, a few patients experienced unwanted side effects. Leukemia—driven by the integration of the therapeutic vector in the vicinity of an oncogene, followed by the expansion of the resulting cellular clone—developed in 5 of the 20 children in the severe combined immunodeficiency (SCID) trial. Although one child died of this complication, the others were rescued by conventional therapies. Importantly, the overall survival and quality of life for individuals receiving this therapy have been superior compared with those who received other therapies, such as HCT (Fischer *et al.*, 2010).

Thus, the understanding already obtained from the gene therapy trials of different hematopoietic genetic diseases (Sheridan, 2011), the clinical observation in mosaic FA patients that self-corrected FA HSCs can expand and restore the hematopoietic compartment (Lo Ten Foe *et al.*, 1997; Waisfisz *et al.*, 1999; Gregory *et al.*, 2001; Gross *et al.*, 2002; Mankad *et al.*, 2006), and the preclinical studies of FA gene therapy with lentiviral vectors (Galimi *et al.*, 2002; Jacome *et al.*, 2009; Muller *et al.*, 2008; Becker *et al.*, 2010; Gonzalez-Murillo *et al.*, 2010), have collectively established a platform for a state-of-the-art clinical trial of FA gene therapy.

Adrian Thrasher (University College London, London, UK) described current activities using self-inactivating gammaretroviral vectors for X-linked SCID and the use of lentiviral vectors incorporating myeloid-specific promoters for the treatment of patients with chronic granulomatous disease. He also discussed the use of low-intensity conditioning regimens that may be applicable to FA, and the development of antibody-mediated conditioning (e.g., targeting CD45 or c-Kit), which is likely to be minimally genotoxic. Raffaele Renella (Children's Hospital Boston, Boston, MA) described the current gene therapy trials open in Boston for X-linked SCID, Wiskott-Aldrich syndrome, and X-linked adrenoleukodystrophy.

A significant problem and obstacle for successful gene therapy in FA is the lack of sufficient numbers of hematopoietic stem cells. Hans-Peter Kiem (University of Washington/Fred Hutchinson Cancer Research Center, Seattle, WA) addressed the issue, and presented data from mouse and nonhuman primate studies exploring novel strategies to expand hematopoietic stem cells (HSCs). Kiem's group presented some encouraging data for HSC expansion using a combination of HOXB4 and DELTA-1 (Watts *et al.*, 2010), reviewed some of the other strategies currently explored, and outlined studies such as the use of aryl hydrocarbon receptor antagonist SR1 alone or in combination with HOXB4 or DELTA-1.

As FA HSCs are fragile, their targeted *in vivo* transduction would represent a major advance. Els Verhoeyen (Human Virology Department, INSERM U758, Ecole Normale Supérieure de Lyon, Lyon, France) reported on an "early-acting, cytokine-displaying" lentiviral vector that targets HSCs in unmanipulated FA bone marrow and mediates in vivo HSC gene transfer (Frecha *et al.*, 2011). In addition, Verhoeyen discussed new lentivector pseudotypes outperforming classical lentiviral vectors pseudotyped with VSV-G for transduction of HSCs.

To improve the efficacy of FA gene correction (Jacome *et al.*, 2009), the members of the Spanish home team (Paula Rio, Susana Navarro, and Juan Bueren [Centro de Investigaciones Energéticas, Medioambientales, y Tecnológicas, Madrid, Spain], Julián Sevilla [Universitario Niño Jesús, Madrid, Spain], and Cristina Díaz de Heredia [Hospital Val d'Hebrón, Barcelona, Spain]) have investigated enrichment of hematopoietic progenitors present in human FA bone marrow either by lineage negative selection (Lin^-), or by $CD34^+$ positive selection. Both procedures were highly efficient and potentially complementary. Lin^- selection allows collection of both hematopoietic stem/progenitor cells and nonhematopoietic mesenchymal cells, which could aid in hematopoietic engraftment (van der Loo *et al.*, 1998; in 't Anker *et al.*, 2003; Lee *et al.*, 2008). $CD34^+$ cell selection results in a higher purity of hematopoietic stem/progenitor cells, which in turn facilitates transductions at higher multiplicities of infection (MOIs).

As $CD34^+$ cell selection is currently validated for clinical purposes, purified FA $CD34^+$ cells were transduced with VSV-G-packaged lentiviral vector carrying *FANCA* regulated by the human phosphoglycerate kinase promoter in combination with an optimized woodchuck hepatitis virus

posttranscriptional regulatory element (Orphan Drug Registry, EU/3/10/822, 2010) produced by Généthon. This protocol and vector will now be taken forward for a clinical trial to be conducted in Europe.

Because of the difficulties in collecting enough FA $CD34^+$ cells for gene therapy purposes (Croop *et al.*, 2001; Kelly *et al.*, 2007), potent mobilization regimens using chemokine receptor (CXCR4)-blocker plerixafor and granulocyte-colony stimulating factor have been considered for harvesting FA $CD34^+$ cells. This combined mobilization regimen was highly efficient in FA mouse models (Pulliam *et al.*, 2008), successfully used without significant adverse events for the collection of $CD34^+$ cells from non-FA adult (Dipersio *et al.*, 2009) and pediatric patients (Sevilla, 2012; Shenoy, 2011), and is under evaluation in patients with FA (NCT00479115; http://clinicaltrials.gov/).

It is unclear whether myeloablation/immunosuppression is needed for stable engraftment of genetically corrected FA HSCs. Taking into account the known toxicity of pretransplantation conditioning, it was proposed to infuse gene-corrected cells into the first FANCA patients without preparative chemo/radiotherapy. If no engraftment is observed, the next group of patients may receive conditioning before cell infusion. The options include fludarabine in combination with cyclophosphamide or ionizing radiation, or perhaps even antibody-based regimens (Macmillan and Wagner, 2010; Svahn and Dufour, 2011; Thakar *et al.*, 2011).

For our goal to be achieved without waste of time and potential, the support of society at large is of paramount importance. Pankaj Qasba (Division of Blood Diseases and Resources, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD) spoke about the NHLBI Gene Therapy Resource Program (GTRP) that facilitates translation of gene therapy research into clinical interventions by providing resources for gene therapy research, primarily in heart, lung, and blood diseases. The resources are available for (1) preclinical vector production, (2) pharmacology/toxicology testing, and (3) clinical-grade vector production for adeno-associated viral and lentiviral vectors. The program also provides clinical trial funding assistance and regulatory support at no cost to the investigator. Wade Clapp (Indiana University, Indianapolis, IN) discussed ideas for bridging funding gaps and addressing regulatory issues to help move preclinical studies to phase I clinical trials.

This meeting illustrated the enormous potential of clinical trial coordination focused on scientific merit and real-time data exchange, and reinforced the responsibility we owe to individuals with FA. The promise of gene therapy has raised hopes in patients with FA and their families, who then must learn that, as of yet, nothing genuinely useful to the patient with FA has been created. Obviously the people who are daily affected by FA view progress in gene therapy differently than do the scientists. For them, the hope in gene therapy has become disappointment. As an adult patient with FA expressed it: "I am taking the same drugs as my sister with FA did 15 years ago. For me, the FA gene therapy failed."

In response to this obvious and uncomfortable fact, a lentiviral gene therapy trial has now been reviewed and approved by the U.S. Food and Drug Administration (FDA) to start enrolling patients (NCT01331018; http://clinicaltrials.gov/), as described and discussed by Pamela S. Becker (University of Washington, Seattle, WA) and Hans-Peter Kiem (University of Washington/Fred Hutchinson Cancer Research Center, Seattle, WA). This trial will incorporate the new stem cell mobilization procedure described above with a combination of plerixafor and granulocyte colony-stimulating factor and updated transduction procedures, including a relatively brief overnight incubation of cells in low oxygen in the presence of a reducing agent. Two other trials are also planned in 2012 and were discussed by Helmut Hanenberg and Wade Clapp (both from Indiana University), and the group of Juan Bueren (Madrid, Spain) at this meeting. We see each institution as a unit of innovation that benefits from this integration of information gained by other units through their specific way of overcoming the challenges of gene therapy for FA. Simultaneously, in the current funding environment that requires short-term justification for any project, we realize that no one person or institution can do this alone.

Without combining forces, there will be flashes of discovery but not the much-needed rapid translation to world-scale, collaborative, clinical gene therapy trials for FA. In this second meeting, the group moved this process forward, each group adding intellectual mass to accelerate the common goal of changing medical practice for people with FA. Clearly, the complexity of FA promises exciting and challenging times ahead. With new stem cell gene therapy tools, such as site-specific nucleases, foamy viruses, cell reprogramming, inducible apoptosis, and expansion of hematopoietic stem cells, we hope the upcoming gene therapy of FA with lentiviral vectors "is perhaps, the end of the beginning" (Churchill and Langworth, 2008) for the treatment of FA.

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Author Disclosure Statement

No competing financial interests exist.

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