Chapter 13: Novel Stem Cell Treatment Options

Introduction

Physicians have made remarkable progress in hematopoietic stem cell transplantation over the last decade, and patients with Fanconi anemia (FA) who need transplantation now have access to dramatically improved care. Some patients with FA, however, have a difficult time with transplantation, fare poorly during or after the procedure, do not have a medical reason for transplantation, or do not wish to pursue this treatment option. This chapter explores emerging therapies that can translate into better care for those patients.

We will describe three of the most promising therapies in this realm: ***gene therapy, stem cell therapy***, and a combination thereof known as ***stem cell gene therapy*** [1]. With the objective of moving stem cell gene therapy into clinical trials for individuals with FA, and with support from the Fanconi Anemia Research Fund and Fanconi Hope Charitable Trust, the International FA Gene Therapy Working Group was established in 2010 [2,3]. Through the power of global collaboration, this group encourages the world's top experts in FA to design gene therapy trials that follow a consistent protocol so that the findings can be easily accessed, shared, compared, and expanded on by FA researchers around the globe.

Good to Know

Hematopoietic stem cells are rare blood cells found in the bone marrow and umbilical cord. These cells are unique because they have the potential to develop into any of the various types of blood cells found in the body.

Doctors can harvest and store a patient's **hematopoietic stem cells** before radiation or chemotherapy, or these cells can be obtained from a human donor. A medical procedure called **hematopoietic stem cell transplantation** transfers stored or donated cells to a patient's body.

Gene Therapy

Gene therapy vectors

Delivering a gene into a cell is not a simple matter. There are many barriers to successful gene transfer: moving the genetic material into the cell, evading the cell's defenses, moving the genetic material through the shell of the nucleus, and finally prodding it to integrate into the cell's own genetic code, or genome. To overcome these challenges, researchers have used viruses as so-called "vectors" to deliver genetic material into cells. Viruses naturally have their own means of delivering genes into cells—after all, this is how viruses cause illnesses such as the common cold. Researchers have simply borrowed these properties to insert genes of interest into the patient's cellular genome.

Researchers have traditionally used the gamma retroviral vector in gene therapy studies, although new and improved lentiviral vectors boast the advantage of being able to transduce non-dividing cells. Small DNA viruses known as pyroviruses—the adenovirus and adeno-associated virus, for example—have also been carefully studied in preclinical gene therapy testing. Among these pyroviruses, adenoviruses are considered advantageous because they deliver the gene into the cell without causing the virus to integrate into the cellular genome. The disadvantage of adenoviruses, however, is that they are more likely than other viruses to elicit an immune response in the recipient [(4)].

When some of the patient's cells are removed from the body so that this genetic manipulation can take place in a laboratory, the procedure is known as ex vivo (Latin for "outside the living") gene therapy. Conversely, when a viral vector containing the healthy gene is injected directly into the patient, the procedure is known as in vivo (Latin for "within the living") gene therapy.

Good to Know

Gene therapy allows physicians to "correct" a patient's genetic information, or DNA, by replacing a disease-associated gene with a healthy version of the gene.

Since the 1970s, researchers have searched for safe and effective ways to correct disease-related genes in human cells. Researchers are currently testing gene therapies for FA in clinical trials, and hope to bring these therapies to market in the years to come.

Methods of gene therapy

There are two main methods of gene therapy: **gene replacement** and **gene editing**. In gene replacement, a gene of interest is inserted at an almost random location in the patient's genome. This method predictably causes non-physiological regulation of the delivered gene in its new location, or the inadvertent functional disruption of other genes near the insertion site [5-7]. Gene editing, on the other hand, takes advantage of the genome's natural ability to repair itself through a process called homologous recombination, in which the faulty gene is corrected at its original locus without the insertion of new material. Gene editing does not typically result in gene dysregulation, and no other region of the genome is likely to be affected [8,9].

Side effects of gene therapy

The most noteworthy side effect of gene therapy is insertional mutagenesis—an accidental mutation caused by inserting new DNA. This is an unavoidable side effect of gene replacement. Since 2000, more than 70 people—mostly with fatal genetic disorders—have undergone autologous transplantation, in which the patients' own cells were removed and treated with viral vectors carrying a therapeutic gene, then transferred back to their bodies. This gene correction strategy relied on the ability to deliver a functional gene along with other related elements needed to promote sustained, high-level gene expression. The drawbacks of this approach included loss of physiological regulation of the treated gene, and disruption and possible dysregulation of other genes. In one clinical trial, the therapeutic gene was inserted near, and inadvertently activated, a gene that causes cancer, resulting in leukemia in 5 of 20 individuals with severe combined immune deficiency (SCID). Four of the 5 children with leukemia were successfully treated; 1 died. Even with this unfortunate event, the overall outcome of the trial provided evidence that gene therapy is equivalent or superior to the previous standard of care (hematopoietic cell transplantation), providing superior immune function, improved disease-free survival, and a better quality of life [5,6,10,11].

It is important to note that the effects of insertional mutagenesis may vary from patient to patient. It can take a long time for side effects to occur, as demonstrated by the gene therapy trials performed to date. Furthermore, in patients with FA, the bone marrow cells used for gene correction are few in number, extraordinarily intolerant of ex vivo manipulations, and are already at risk of accumulating pre-leukemic mutations, the impact of which can be increased by gene correction. FA cells are constantly in danger of becoming

genetically unstable and triggering the development of leukemia and other cancers. Correcting the gene in a FA cell that has already accumulated some of the mutations needed for cancer progression could have adverse effects by keeping alive cells that might otherwise have been eliminated from the body. This can, in principle, evolve into full-blown leukemia [37].

Stem Cell Therapy

Stem cell therapy vectors

Traditionally, stem cell therapy has entailed the use of **bone marrow cells**; this method has been experimentally and clinically proven in many thousands of successful bone marrow transplants over the last 50 years. Hematopoietic stem cell transplant remains the prototype of cellular therapy and a testament to the consistently remarkable fact that stem cells can be transferred from a donor to a recipient, and that they can reconstitute a fully functional lymphohematopoietic system—a system that produces the body's white blood cells, red blood cells and platelets—from relatively few starting cells [12-19].

While **embryonic stem cells** provide an opportunity to understand more deeply how stem cells work, their use remains controversial and various biological and legal constraints prevent their therapeutic use.

More relevant to clinical care are **induced pluripotent stem cells,** which are embryonic stem cell-like cells from the skin or blood of adults that have been engineered with the potential to develop into any other type of cell in the body. Induced pluripotent stem cells have become a popular tool for the investigation of tissue formation in health and disease, early stages of cell development, and drug intervention strategies, all of which are relevant to the biology and treatment of FA [20-24].

Good to Know

Pluripotent stem cells are cells capable of developing into almost any type of cell in the body. Stem cells can be found in embryos, in umbilical cord blood, and in the blood and bone marrow of adults.

Through a procedure called **stem cell therapy,** physicians introduce new, healthy stem cells into a patient's body to help replace, repair, or regenerate diseased tissues.

Hematopoietic stem cell transplantation usually uses stem cells from the bone marrow or umbilical cord blood of a matched donor.

Stromal stem cells, also known as mesenchymal stromal cells, are non-hematopoietic (non-blood-producing) cells of the bone marrow and other organs in the body. These cells are thought to be located in the walls of the blood vessels and to perform key functions, such as supporting hematopoietic stem cells in the bone marrow and modulating the immune response. These useful properties of stromal stem cells have been harnessed clinically in the therapy of graft-versus-host disease (GvHD) [25].

Methods of stem cell therapy

There are at least two methods of cell therapy: **traditional hematopoietic stem cell transplantation** and **immunomodulation**. Traditional hematopoietic stem cell transplantation involves replacing the entire blood-producing system of the recipient patient with that of a healthy donor. Immunomodulation, on the other hand, involves modifying the patient's immune response. An example of immunomodulation would be the use of mesenchymal stromal cells to support bone marrow engraftment or to treat steroid-resistant GvHD disease.

Good to Know

Graft-versus-host disease occurs when immune cells in the transplanted tissue attack the patient's own cells. This disease is often treated with steroids to suppress the immune response.

Stem cells, for example mesenchymal stromal cells, can also play a role in tissue repair and healing after injury. This is especially relevant in the setting of bone marrow transplant, particularly in healing tissue damage from chemotherapy and treating immune reactions such as GvHD. Patients with FA have defects in the body's DNA repair system, which cause their injuries to be amplified after transplantation. Mesenchymal stromal cells are known to home to the site of injury and therefore, in principle, may provide an especially appealing modality for FA patients who receive transplants [25].

Side effects of stem cell therapy

The most notable side effect of stem cell therapy is tumorigenesis, or the uncontrolled growth of stem cells, which can give rise to benign or malignant tumors. Most cancers are thought to originate from so-called "cancer stem cells," which are in many ways similar to normally functioning stem cells in their cellular processes and metabolic pathways. Because of this, some donor stem cells potentially can cause malignancies in the patient; indeed, donor-

derived leukemias have been reported in some recipients of hematopoietic cell transplantation. Multiple researchers have observed this phenomenon in animal models when mesenchymal stromal cells were transplanted from one organism to another and gave rise to cancers [26].

In theory, additional side effects are possible because of the specific functions of stem cells. For example, immunomodulation by mesenchymal stromal cells can suppress the immune system of the transplant recipient, which can reactivate latent infections—especially DNA viral infections—or favor tumor growth and create an environment conducive to leukemia.

Stem Cell Gene Therapy

An effective gene therapy strategy must target the cell type relevant to the specific disease. In most instances, the effects of gene correction are enhanced by the corrected cells' ability to reproduce and repopulate the body in meaningful numbers. For this reason, many gene therapies have attempted to deliver genes to stem cells. It seems only logical that the parallel tracks of gene therapy and stem cell therapy should be joined in one concerted effort termed "**stem cell gene therapy**." This effort aims to correct the gene in the stem cells of the recipient ex vivo and then return the corrected cells to the patient.

Challenges specific to FA can be viewed as opportunities in the context of stem cell gene therapy. For example, deficits in DNA repair make FA stem cells more sensitive than their healthy, or wild-type, counterparts. This sensitivity can be manipulated to the advantage of the patient by using low-dose chemotherapy to selectively eliminate uncorrected cells in vivo in a patient with FA who has received a mixture of cells that are either corrected or not corrected.

For reasons mentioned above, the leading strategy for gene therapy represents a shift away from **gene addition**, in which an entirely new gene is pasted into the genome with the help of viruses or transposons, and a move toward **genome editing**, whereby the pathogenic mutation is corrected in its natural gene location with the aid of newly engineered molecules called zinc-finger nucleases, transcription activator-like effector nucleases, or homing endonucleases. These hybrid molecules are engineered to target a specific location in the genome, where they introduce a break in the strand of DNA near the targeted mutation. The break in the DNA is then resolved by homologous recombination between the endogenous genes and an exogenously introduced

fragment from the donor containing the healthy genetic sequence. In this fashion, the pathogenic mutation is permanently changed to the normal sequence. This process also preserves the architecture of the genome and maintains control of the gene by the cell's normal regulatory elements.

One of the advantages of gene editing is its spectacular flexibility and range of use; it can be used for targeted delivery, tissue-specific regulatory sequences, or transduction of cell types committed to tissue-specific differentiation programs. Gene therapy can even be designed to treat diseases that are limited to specific sites in the body, such as for the prevention of head and neck cancers in patients with FA.

The great early promise of stem cell gene therapy comes—as with many advances in medicine—with some risk. Although this risk might be deemed unacceptable to a healthy person, individuals and families who are already living with the perils of a disorder like FA may be willing to accept the risk of emerging therapies when balanced with their potential benefits.

Stem cell gene therapy trials in FA

The first clinical trials of stem cell gene therapy for FA used retroviruses to deliver the *FANCA* or *FANCC* genes. Viral transduction, however, resulted in transient or no correction of hematopoietic cells, an observation consistent with only short-term functional gene complementation [27-30].

Since 2010, the International FA Gene Therapy Working Group has focused on developing a translational platform that can deliver clinically meaningful benefits to individuals with FA. Members of the Working Group share the common goal of accelerating the transition of gene therapy research into clinical trials that follow a consistent protocol so that the findings can be shared among FA researchers around the globe. The current FA stem cell gene therapy platform entails the following: the *FANCA* gene is delivered by a third-generation lentiviral vector pseudotyped with vesicular stomatitis virus, and short viral transduction is achieved without prolonged prestimulation with growth factors. Individuals with a human leukocyte antigen-matched sibling donor, an abnormal karyotype, or a serious infection are not eligible for the trial [3,31,32].

The first FA lentiviral gene therapy trial, led by Dr. Pamela S. Becker (University of Washington, Seattle) and Dr. Hans-Peter Kiem (University of Washington/Fred Hutchinson Cancer Research Center, Seattle), has been

approved by the U.S. Food and Drug Administration (FDA) and was open for enrollment at the time of publication (NCT01331018; *available at:* http://clinicaltrials.gov). This trial incorporates the updated transduction procedures and a relatively brief overnight incubation of cells in low oxygen in the presence of a reducing agent. Dr. Juan Bueren (CIEMAT, Madrid, Spain) and his team have opened a hematopoietic stem cell mobilization trial and plan to have the *FANCA* gene therapy trial opened for accrual in 2014. Preparations are under way to open a second U.S.-based trial in Indianapolis (Dr. Helmut Hanenberg and colleagues).

Challenges Ahead

The ultimate goal of our effort in stem cell gene therapy for FA is to cure bone marrow failure and leukemia safely by preventing unintended effects on surrounding genes and by fine-tuning the expression of FA genes. In addition to the procedures mentioned above, the vector design and treatment of the cells may greatly reduce the risk to the patient. This will likely require the use of genetic components such as weak promoters, strong insulators, and strong polyadenylation sequences to isolate the functions of the inserted genes from the genome and that of the genome from the inserted genes.

A new gene therapy tool involves the use of microRNAs (miRNAs), short segments of ribonucleic acid that bind to and turn off specific products of the genetic code (i.e., transcribed genes, known as RNA transcripts). MicroRNAs are extremely important because they allow researchers to target gene expression accurately in the desired cell population and to avoid cells that should not be targeted by the gene therapy vector, such as antigen-presenting cells that might trigger an undesirable immune response to the vector [33].

As mentioned above, most future efforts will likely focus on combined modalities and attempt to minimize oxidative stress in these cells. For example, a combination of stem cell expansion, correction of hematopoietic stem cells and mesenchymal stromal cells from the same patient, and co-infusion of these cells may provide an ideal environment for engraftment of the gene-corrected hematopoietic stem cells [34-36].

Summary

Gene therapy, stem cell therapy, and stem cell gene therapy are powerful tools that will improve care for FA patients. Several steps must be taken to achieve this goal. The first step involves the coordination of clinical trials so that individual research centers can pool their collective knowledge and statistical power. The second step involves focusing on a common goal, such as the development of treatments that can be rapidly translated to clinics around the world. The third step involves implementing real-time data exchanges and allowing for the evaluation of these data on the basis of scientific merit. Through these actions, FA researchers can expedite the clinical impacts of basic and clinical gene therapy research.

The field of gene therapy started with a visionary and a daring idea, but suffered from a dearth of preclinical data. The first clinical trials were permitted only because of the high risks of living with such challenging genetic diseases and the risks and incomplete efficacy of alternative therapies such as hematopoietic cell therapy. Through the years, the field of gene therapy has overcome several crises at the collision of public expectations and unintended side effects, and has emerged as an acceptable therapy in the treatment of several genetic disorders.

There is abundant room for optimism that the same outcome will be possible for the treatment of FA. Without doubt, the collective knowledge and unique enthusiasm of FA researchers and clinicians will provide a winning combination of ideas and well-designed experiments that will translate into improved care for people with FA.

Chapter Committee

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References

1. Tolar J (2013) Translating genome engineering to better clinical outcomes. *Transl Res* 161(4):199-204.

2. Tolar J, *et al.* (2012) Gene therapy for Fanconi anemia: one step closer to the clinic. *Hum Gen Ther* 23(2):141-144.

3. Tolar J, *et al.* (2011) Stem cell gene therapy for Fanconi anemia: report from the 1st international Fanconi anemia gene therapy working group meeting. *Mol Ther* 19(7):1193-1198.

4. Kay MA (2011) State-of-the-art gene-based therapies: the road ahead. *Nat Rev Genet* 12(5):316-328.

5. Hacein-Bey-Abina S, *et al.* (2008) Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. *J Clin Inv* 118(9):3132-3142.

6. Cavazza A, Moiani A, Mavilio F (2013) Mechanisms of retroviral integration and mutagenesis. *Hum Gen Ther* 24(2):119-131

7. Moiani A, *et al.* (2012) Lentiviral vector integration in the human genome induces alternative splicing and generates aberrant transcripts. *J Clin Inv* 122(5):1653-1666.

8. Handel EM, Cathomen T (2011) Zinc-finger nuclease based genome surgery: it's all about specificity. *Curr Gen Ther* 11(1):28-37.

9. Bogdanove AJ, Voytas DF (2011) TAL effectors: customizable proteins for DNA targeting. *Science* 333(6051):1843-1846.

10. Cavazzana-Calvo M, Lagresle C, Hacein-Bey-Abina S, Fischer A (2005) Gene therapy for severe combined immunodeficiency. *Annu Rev Med* 56:585-602.

11. Rivat C, Santilli G, Gaspar HB, Thrasher AJ (2012) Gene therapy for primary immunodeficiencies. *Hum Gen Ther* 23(7):668-675.

12. Lorenz E, Uphoff D, Reid TR, Shelton E (1951) Modification of irradiation injury in mice and guinea pigs by bone marrow injections. *J Natl Cancer Inst* 12(1):197-201.

13. Billingham RE, Brent L, Medawar PB (1953) Actively acquired tolerance of foreign cells. *Nature* 172(4379):603-606.

14. Thomas ED, Lochte HL, Jr., Lu WC, Ferrebee JW (1957) Intravenous infusion of bone marrow in patients receiving radiation and chemotherapy. *N Engl J Med* 257(11):491-496.

15. Medawar PB (1986) *Memoir of a thinking radish: an autobiography.* Oxford; New York: Oxford University Press.

16. Appelbaum FR (2007) Hematopoietic-cell transplantation at 50. *N Engl J Med* 357(15):1472-1475.

17. Gatti RA, Meuwissen HJ, Allen HD, Hong R, Good RA (1968) Immunological reconstitution of sex-linked lymphopenic immunological deficiency. *Lancet* 2(7583):1366-1369.

18. MacMillan ML, *et al.* (2000) Haematopoietic cell transplantation in patients with Fanconi anaemia using alternate donors: results of a total body irradiation dose escalation trial. *Br J Haematol* 109(1):121-129.

19. Tolar J, Mehta PA, Walters MC (2012) Hematopoietic cell transplantation for nonmalignant disorders. *Biol Blood Marr Transpl* 18(1 Suppl):S166-171.

20. Zhu H, Lensch MW, Cahan P, Daley GQ (2011) Investigating monogenic and complex diseases with pluripotent stem cells. *Nat Rev Genet* 12(4):266-275.

21. Muller LU, *et al.* (2012) Overcoming reprogramming resistance of Fanconi anemia cells. *Blood.* 119(23):5449-5457.

22. Raya A, *et al.* (2009) Disease-corrected haematopoietic progenitors from Fanconi anaemia induced pluripotent stem cells. *Nature.* 460(7251):53-59.

23. Thomson JA, *et al.* (1998) Embryonic stem cell lines derived from human blastocysts. *Science.* 282(5391):1145-1147.

24. Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126(4):663-676.

25. Prockop DJ (2009) Repair of tissues by adult stem/progenitor cells (MSCs): controversies, myths, and changing paradigms. *Mol Ther* 17(6):939-946.

26. Tolar J, *et al.* (2007) Sarcoma derived from cultured mesenchymal stem cells. *Stem Cell* 25(2):371-379.

27. Williams DA, Croop J, Kelly P (2005) Gene therapy in the treatment of Fanconi anemia, a progressive bone marrow failure syndrome. *Curr Opin Mol Ther* 7(5):461-466.

28. Liu JM, *et al.* (1999) Engraftment of hematopoietic progenitor cells transduced with the Fanconi anemia group C gene *(FANCC).* *Hum Gen Ther* 10(14):2337-2346.

29. Walsh CE, *et al.* (1994) A functionally active retrovirus vector for gene therapy in Fanconi anemia group C. *Blood* 84(2):453-459.

30. Muller LU, Williams DA (2009) Finding the needle in the hay stack: hematopoietic stem cells in Fanconi anemia. *Mut Res* 668(1-2):141-149.

31. Yamada K, *et al.* (2003) Phenotype correction of Fanconi anemia group A hematopoietic stem cells using lentiviral vector. *Mol Ther* 8(4):600-610.

32. Becker PS, *et al.* (2010) Preclinical correction of human Fanconi anemia complementation group A bone marrow cells using a safety-modified lentiviral vector. *Gene Ther* 17(10):1244-1252.

33. Naldini L (2011) Ex vivo gene transfer and correction for cell-based therapies. *Nat Rev Genet* M12(5):301-315.

34. Boitano AE, *et al.* (2010) Aryl hydrocarbon receptor antagonists promote the expansion of human hematopoietic stem cells. *Science* 329(5997):1345-1348.

35. Digiusto DL, Kiem HP (2012) Current translational and clinical practices in hematopoietic cell and gene therapy. *Cytotherapy* 14(7):775-790.

36. Bernstein ID, Delaney C (2012) Engineering stem cell expansion. *Cell Stem Cell* 10(2):113-114.

37. Rochowski A, Olson SB, Alonzo TA, Gerbing RB, Lange BJ, and Alter BP (2012) Patients with Fanconi anemia and AML have different cytogenetic clones than de novo cases of AML. *Pediatr Blood Cancer.* 59:922-924.