

Chapter 12

Novel Treatment Options

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Opportunities and Challenges

Physicians have made remarkable progress in hematopoietic cell transplantation over the last decade, and the care of FA patients in need of transplantation has improved dramatically. A minority of patients, however, cannot be transplanted because of their medical conditions or do poorly during or after transplant. Therefore, we aim to investigate all possibilities that can translate into better care for FA patients.

The prime candidates in this realm are gene therapy, stem cell therapy, and stem cell gene therapy.

Gene Therapy

Gene therapy vectors

Delivering a gene into a cell is not a trivial matter. Investigators in the past have realized this and have used viruses as vectors for this purpose. Viruses have developed their own means of delivering their genes into cells, and researchers have “borrowed” these properties to insert genes of interest into cellular genomes.

The retroviral vector is the traditional vector, although improved lentiviral vectors, with an added advantage of being able to transduce non-dividing cells, have been used recently. Small DNA viruses, called pyroviruses, represented by adenovirus and adeno-associated virus, represent another group of viruses that have been investigated in great detail in preclinical gene therapy testing.

Their main advantage is that they do not integrate into the genomes of cells. Their main disadvantage is that they very easily elicit an immune response in the recipient.¹

In addition to the viral strategies, non-viral means of gene delivery have been under investigation as well—most prominently “jumping genes,” termed transposons (for example, *Sleeping Beauty* transposon).²

The genetic manipulation of the cell can occur either outside the patient, called *ex vivo*, or the vector can be injected directly into the patient, termed *in vivo*.

Mechanisms of gene therapy

There are two main mechanisms whereby gene therapy can occur. The first is gene replacement when a gene of interest is inserted at a more or less random spot in the genome of the recipient. This results in predictable complications: e.g., dysregulation of the delivered gene in that new genomic site as well as disruption of the genes that are located in the region of the insertion.³ The second mechanism of gene therapy is gene correction, which is based upon a capacity of genomes to repair themselves using a process called homologous recombination. As a result of such homologous recombination, the faulty gene is corrected at its original locus. Its regulation remains intact, and no other genomic region is affected by the gene therapy process.⁴

Side effects of gene therapy

The most important side effect of gene therapy is insertional mutagenesis,^{3,5-9} a side effect that is unavoidable if a gene replacement approach is used. It is possible, however, to target the gene insertion into a so-called “safe haven” region of the genome, where fewer or no genes of importance are located. The important

consideration in assessing insertional mutagenesis is that the genotoxicity related to it may vary from patient to patient. The latency of the side effect can be quite long, as we have learned from the first several gene therapy trials. The additional side effects relate to the immune reaction to the virus and to inappropriate expression—either related to the site in the genome or the differentiation status of the cell where the gene is expressed.¹ In theory, gene transfer can also result in germ line transmission, but this has not been documented to date.

Stem Cell Therapy

Stem cell therapy vectors

The traditional stem cell therapy vector is a bone marrow cell, which has been experimentally and clinically proven in many thousands of successful bone marrow transplants over the last 50 years.¹⁰ Hematopoietic stem cell transplant remains the prototype of cellular therapy and a testament to the consistently remarkable fact that a stem cell can be transferred from one organism to another (from a donor to a recipient) and that it can reconstitute the full functional lymphohematopoietic system from relatively few cells.

Embryonic stem cells represent an opportunity for understanding more deeply how stem cells work but, due to biological (e.g., generation of teratomata in the recipient) and legal constraints, they do not constitute a useful strategy at present.¹¹

Stromal stem cells (e.g., mesenchymal stromal cells) are non-hematopoietic cells of the bone marrow and other organs in the body.^{12,13} They are presumed to be located in the wall of the vessels and to exert multiple critical functions, including support of the hematopoietic stem

cell in the bone marrow, and immunomodulation, which has been harnessed clinically in the therapy of graft-versus-host disease.^{14,15}

Mechanisms of stem cell therapy

Stem cell replacement is the mechanism of traditional hematopoietic stem cell transplantation in which the entire lymphohematopoietic system of the recipient is replaced with that of the host.

The second mechanism is immunomodulation. An example is the use of mesenchymal stem cells to support engraftment or to treat steroid-resistant graft-versus-host disease.¹⁶

An additional mechanism is likely to be the role of stem cells, especially mesenchymal stem cells, in tissue repair and healing after injury.¹⁷ This occurs in the setting of bone marrow transplant, due to tissue damage from chemotherapy, and immune reactions such as graft-versus-host disease. Mesenchymal stem cells are known to home to the site of injury. The injury is amplified in the setting of transplantation in Fanconi anemia patients because of their DNA repair defect and, therefore, represents an especially appealing modality applicable to the Fanconi anemia patients in need of transplant.

Side effects of stem cell therapy

The possible side effect of stem cell therapy can be tumorigenesis (i.e., generation of benign and malignant tumors). Most cancers originate from so-called cancer stem cells, which are in many processes and metabolic pathways indistinguishable from a normally functioning stem cell. Therefore, some donor stem cells will likely lead to malignancies in the recipients. We have seen this in multiple examples of donor-derived leukemias

in hematopoietic cell transplantation recipients.¹⁸ We and others have observed this in animal models when mesenchymal stem cells were transplanted from one organism to another and gave rise to cancers.¹⁹ Additional side effects are related to the specific functions of these cells. For example, immunomodulation generated by mesenchymal stem cells will lead to immunosuppression of the recipient, which in turn can translate into a reactivation of latent infections, especially DNA viral infections, or can favor tumor growth and result in a permissive state for leukemia.²⁰

Stem Cell Gene Therapy

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.” The intention is to correct the gene in the stem cells of the recipient *ex vivo* and then return them to the patient.

The specific challenges of Fanconi anemia can be seen as specific opportunities for stem cell gene therapy. For example, as a consequence of DNA repair disease, Fanconi anemia stem cells are more sensitive than their wild-type counterparts.²¹⁻²³ The phenotype of Fanconi anemia cells is that of clastogen sensitivity, which can be manipulated to the advantage of the patient by using low-dose chemotherapy as an *in vivo* selection in the patient who has received a mixture of cells that are corrected and cells that are not corrected.

An additional phenotypic feature is the paucity of the stem cells, especially hematopoietic stem cells in the bone marrow of Fanconi anemia patients. There are approximately six-fold fewer CD34⁺ cells in Fanconi anemia patients when compared with non-Fanconi anemia patients, even before cytopenia occurs. In other

words, there are about ten-fold fewer colony-forming cells generated from the bone marrow source of the FA patient, pointing to the decreased repopulating capacity of these cells.²⁴⁻²⁶

Last, the data from mosaicism observed in about 25% of Fanconi anemia patients point to the possibility of mimicking this naturally occurring “gene therapy” in a clinical setting.²⁷⁻³²

Stem cell gene therapy trials in Fanconi anemia

The three Fanconi anemia clinical trials conducted to date have used retroviral means of delivery of Fanconi anemia A or Fanconi anemia C genes. Viral transduction, however, resulted in no or only transient correction of hematopoietic cells, an observation consistent with only short-term functional complementation.³³⁻⁴⁰

A lesson learned from these experiments was that the cells collected from the Fanconi anemia patients are extremely sensitive and extremely few. This led the investigators in a Spanish clinical trial, which is in preparation, to argue that no pre-stimulation of hematopoietic cells with growth factors is needed or wanted in this process, and that only a short exposure to a retrovirus is warranted.^{41,42}

Stem cell gene therapy trials for other diseases

The gene therapy trials performed to correct other genetic diseases have resulted in correction of the disease phenotype in many, but also have produced significant side effects.¹ First, the adenoviral-based trials for ornithine transcarbamylase deficiency and Factor XI deficiency have been unsuccessful in correcting the phenotype of the patients, primarily as a consequence of host immune response to the virus, which in one case resulted in the death of the patient.

Second, the retroviral-based gene therapy trials for immunodeficiencies, such as severe combined immune deficiency, adenine deaminase deficiency, and chronic granulomatous disease, have resulted in the correction of the immune deficiency in all but one patient. Unfortunately, four leukemias occurred in the severe combined immunodeficiency trial (with one death), and two clonal myeloproliferations occurred in the chronic granulomatous disease trial (with one death). It is worth noting that the clonal disorders have occurred in the defective lineage in these patients; that is, in T cells in the severe combined immunodeficiency patients and in myeloid cells in the patients with chronic granulomatous disease. To date, there are no side effects observed in the two patients who were treated for adenine deaminase deficiency.

Third, the latent period after which these side effects occur is much longer than expected. It follows from this that the cancer risk assessment testing systems at the moment are inadequate to assess the changes that can occur years after the gene treatments. In turn, animal tests, typically in the murine models, have to be modified so that the short life span of mice when related to humans is offset by sensitizing the mice to development of tumors in a much shorter period of time and then testing these putative gene therapy agents in these cancer-prone animals. Alternatively, the cellular testing can be performed by sensitizing the cell cultures to unearth the hidden potential of the gene therapy agents for transformation.¹

The Evolution of Thinking about Fanconi Anemia Stem Cell Gene Therapy

Available gene therapy and stem cell therapy data suggest that there are several specifics that can make stem cell gene therapy in Fanconi anemia more successful.

First, we need to focus on the stem cell exhaustion and stem cell stress which are inherent features of the Fanconi anemia phenotype. In Fanconi anemia, the poor mobilization of the hematopoietic stem cell, impaired clonogenicity, and repopulation are features that cannot be removed from the stem cell gene therapy manipulations. Fanconi anemia genes themselves are anti-apoptotic, so it comes as no surprise that these cells deficient in DNA repair have increased cell cycling and are easily exhausted in numbers and function. It is important to note that this is not an engraftment defect, but a replicative deficiency. In turn, this offers hope for increasing the homing potential of these stem cell grafts in order to achieve more complete and longer-lasting phenotypic correction.⁴³

Second, the gene therapy viral infection is associated with cellular proliferation in transduced cells, which only adds to the potential of the few rapidly cycling Fanconi anemia cells to transform into malignancy or, alternatively, by default into apoptosis and graft failure.

Third, cell expansion prior to transduction (which is usually a part of standard gene therapy transfer) is likely a counter-productive measure in Fanconi anemia gene therapy trials. Fanconi anemia cells have been known to be sensitive to reactive oxidative species and pro-inflammatory cytokines, so it seems logical to limit apoptosis by limiting their pre-stimulation by choice of the growth factors and by short time of their *ex vivo* manipulation. On the other hand, we might be able to rely on a competitive advantage of the corrected Fanconi anemia cells, such as has been seen in the immune deficiency gene therapy trials and, perhaps, even enhance this selection by administration of alkylator agents to the patients at a low dose after infusion of gene-corrected autologous stem cells.

Last, the pre-stem cell infusion cytoreduction that has been a component of the only successful and side-effect-free gene trial (for adenine deaminase deficiency) may need to be part of future Fanconi anemia gene therapy trials. Another possibility is to use non-genotoxic conditioning with the inflammatory cytokines (for example, interferon gamma), which has been shown to provide a mild ablation in mice with a genetically engineered deficiency in Fanconi anemia proteins.⁴⁴

The Challenges That Lie Ahead

The ultimate goal of our effort in stem cell gene therapy for Fanconi anemia is to reduce the off-target effects and to fine-tune expression of Fanconi anemia genes. In addition to the means mentioned above, the vector design and treatment of the cells may translate into a large reduction in the off-target side effects. This will likely require 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. Micro RNA is a new gene therapy tool and can be of huge importance in accurately targeting of gene expression to the desired cell population and away from the cells that should not be targeted by the gene therapy vector (e.g., antigen presenting cells that can mediate immune response to the vector).⁴⁵ As mentioned above, most of the efforts from now on will probably require minimization of oxidative stress on these cells. It is likely that combined modalities will be explored as well. For example, correction of hematopoietic stem cells and mesenchymal stem cells from the same patient, and co-infusion of these cells, may provide a better environment for engraftment of the gene-corrected hematopoietic stem cells.

Summary

Gene therapy, stem cell therapy, and stem cell gene therapy are powerful tools that will improve care for Fanconi anemia patients in the future. We have learned from Fanconi anemia gene therapy trials that they will not be successful unless they are changed. The limitations relate to the sensitivity and paucity of hematopoietic stem cells available for correction.

We should remain optimistic that the collective knowledge and unique enthusiasm of Fanconi anemia researchers and clinicians will provide a winning combination of ideas and well-designed experiments that will translate into improved care for Fanconi anemia transplant patients in the near future.

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