Evolution of Gene Therapy, Historical Perspective

Harry L. Malech, MD[a,*], Elizabeth K. Garabedian, RN, MSLS[b], Matthew M. Hsieh, MD[c]

KEYWORDS

• Gamma retrovirus vector • Lentiviral vector • Gene editing
• Hematopoietic stem cells • Transduction • Apheresis • CD34+ HSC
• Insertional mutagenesis

KEY POINTS

- Gene therapy by the genetic modification of hematopoietic stem cells (HSC) has reached a stage of development that has resulted in substantial clinical benefits.
- This article explores the separate threads of knowledge, conceptual design, materials, and equipment required to reach our current era of clinically beneficial gene therapy.
- The history of gene therapy targeting hematopoietic stem cells include improvements in integrating vectors such as lentivectors and improvements in gene editing methods such as CRISPR/Cas9.
- Understanding the pathophysiology of adverse events such as insertional mutagenesis is important for seeking improvements in vector design that may enhance the safety of gene therapy.

INTRODUCTION TO THEORETIC CONCEPTS AND EARLY BACKGROUND HISTORY IMPACTING HEMATOPOIETIC STEM CELL GENE THERAPY

The history of gene therapy comprises the advance of theoretic concepts, understanding of the human genome, availability of critical materials and instruments, design of vectors and chemical tools to manipulate and change genomic DNA, improvements in the procurement and culture/maintenance of stemness of HSC in culture, improvements in myeloid conditioning, the outcomes of conduct of clinical trials, observing successes and problems occurring in clinical trials, and deep study and

Conflict of Interest: The authors declare no conflicts.

[a] Genetic Immunotherapy Section, Laboratory of Clinical Immunology and Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 10 Center Drive, MSC1456, Bldg 10, Rm 5-3750, Bethesda, MD 20892-1456, USA; [b] National Human Genome Research Institute, NIH, 10 Center Dr, MSC1611, Bldg 10, Rm 10C-103, Bethesda, MD 20892-1611, USA; [c] National Heart, Lung, and Blood Institute, NIH, 10 Center Drive, MSC1812, Bldg 10, Rm 9N119, Bethesda, MD 20892-1812, USA
* Corresponding author.
E-mail address: hmalech@niaid.nih.gov

Hematol Oncol Clin N Am 36 (2022) 627–645

elucidation of the mechanisms of problems that arise in clinical trials to seek and incorporate corrective measures. The evolution of our understanding of ethical issues impacting gene therapy, and the logistics of access to and cost of successful gene therapy treatments are also important elements of this history.[1]

In this broad brush and somewhat unconventional view of the history of gene therapy, we address general principles; key experiments, basic science, and clinical trials that illustrate some general principle; and the evolution of materials and instrumentation that make current clinical approaches to gene therapy of HSC possible. We aim to complement rather than duplicate the extensive discussion of the background studies of gene therapy and the march of the many published clinical trials in specific disorders or categories of disorders that are the subject of the other chapters in this series, as well as excellent recent reviews.[2]

The earliest experiments that laid the foundation for gene therapy began with experiments on the transforming properties of bacteria.[3] Alloway reported in 1932 that nonvirulent (R type) pneumococci became lethal by adding cell-free extracts from virulent (S type) pneumococci. When injected with these "transformed" pneumococci, the mice developed pneumonia and died.

In our view, the key conceptual background to all gene therapy emerged in the 1940s with the seminal work by Avery and colleagues on bacterial transformation (which one could perhaps very loosely call gene therapy of bacteria). They identified DNA as the transforming factor that could change the physiology of a bacterial strain,[4] and more specifically, showed that the "transforming substance" was precipitated out by alcohol and later confirmed to be DNA. This was one of the key background elements to Watson and Crick in identifying the structure of DNA,[5] postulating its role as the genomic code of all prokaryotic and eukaryotic organisms, and thus demonstrating that nucleic acid sequences, rather than proteins, carry genetic information. The next critical discovery was that of Marshall Nirenberg, who in 1961 discovered the "triplet" code by which DNA encodes for the assembly of the 20 amino acids that serve as the building blocks of proteins.[6]

In parallel with this elucidation of the biochemical basis of heredity, were emerging concepts from early transformation studies in mammalian cells, for example, the early reports that the transformation of 8-azaguanine sensitive cells with nuclei and chromosomes from 8-azaguanine resistant cells rendered the transformants resistant due to transfer of a mutated hypoxanthine-guanine phosphoribosyltransferase gene.[7,8] An early review of mammalian cell transformation studies conducted over the following 18 years was reviewed in 1980 by Shows and Sakaguchi.[9] This body of work further established that newly acquired biochemical traits from DNA transformation experiments in mammalian cells can be heritable.

Many other key concepts that evolved into current methods of viral vector-mediated gene therapy were developed in the 1970s, during a period of the active investigation of viruses capable of transforming normal tissues into cancers. From this work, the concept emerged that perhaps these DNA and RNA tumor viruses known to insert into the genome of target cells could be modified in some way to remove the tumor causing elements, but retain their genome insertion capabilities to deliver a therapeutic payload. Some of the earliest published reviews of the history of gene therapy incorporating these essential concepts were those written in a series of reports over time by Theodore Friedmann[10–13] who shared the 2015 Japan Prize with Alain Fischer for "For the Proposal of the Concept of Gene Therapy and its Clinical Applications."

general consensus among the US Food and Drug Administration (FDA),[14] the European Medicines Agency (EMA),[15] and the American Society of Gene and Cell Therapy (ASGCT)[16] defining gene therapy as changes in gene expression, achieved by replacing or correcting a disease-causing gene, inactivating a target gene, or inserting a new or modified gene, using a vector or delivery system of genetic sequence or gene, genetically modified microorganisms, viruses, or cells.

By the late 1970s, while our understanding of the molecular basis of human diseases was advancing through cloning and sequencing of genes, there were major technical challenges to implement gene transfer. Exogenous DNA could be introduced to target cells by transformation or transfection, but the overall efficiency was low. Additionally, if the introduced gene(s) did not provide a survival advantage, the durability of gene transfer was also low. The resulting gene transfer efficiency at that time was about one in 100,000 cells, but nonetheless was proposed as a method to achieve genetic correction.[17]

Intense interest in inherited hemoglobinopathies such as sickle cell disease and beta-thalassemia fueled work on beta-globin, one of the first genes to be cloned and then studied with the intent of gene transfer for clinical application. Mulligan and colleagues replaced the viral capsid protein (VP1) of the SV40 genome with complementary DNA of rabbit beta-globin in a monkey kidney cell line, which produced large quantities of rabbit beta-globin mRNA and protein.[18] As there was no inherent advantage for beta-globin gene transformed cells, several laboratories worked on selectable genes to be cotransferred. Pellicer and colleagues successfully inserted beta-globin and thymidine kinase (TK) genes into murine teratocarcinoma cells.[19] The Cline laboratory inserted dihydrofolate reductase (DHFR) or TK in murine marrow cells.[20]

Cline and colleagues from UCLA then applied these results and tested them clinically.[21–23] An experimental protocol to insert genetically modified marrow cells from patients with beta-thalassemia, inject the cells in the femur after local irradiation, and treat with a selecting agent was submitted to the human research review committee at their home institution. Because the first 2 patients to be treated were receiving their care in other countries (in a hospital in Naples, Italy and at Hadassah Hospital, Jerusalem, Israel), not covered by the UCLA review committee, the team sought in parallel and secured permission in Naples and Hadassah for the clinical study. Both patients were informed of the experimental nature and the low likelihood of success in this approach. After femur irradiation and infusion of modified marrow cells, the patients reported no adverse events, and selective agents were not used. Three months later, there was no demonstrable clinical benefit in both patients. Although safety of this clinical gene transfer was undebated, many controversial issues were brought forth.[24–26] Can a clinical protocol proceed with permission from some but not all institutions? How many preclinical experiments (in vitro or animal), and what degree of “success” are needed to garner approval? While the responses to these issues are much more straightforward today, various review committees at that time were caught off guard and the consensus was that this was a rather premature and in retrospect problematic initial attempt at the clinical application of gene therapy.[27]

These first 2 attempts at human gene therapy generated much media attention and scrutiny by regulatory committees. The remainder of the decade into the early 1990s, scientists was quietly working on recombinant DNA methods, in vitro and animal models for testing, and strategies to enhance transgene expression. It quickly became

the gene transfer experiments then focused on vector optimization and design, and brought this background discussion into the early modern era of gene therapy.

The following sections of this review will provide a historical background of a number of parallel developments that provided the laboratory and clinical tools and materials that facilitate our current approaches to gene therapy targeting blood cells including HSC.

DESIGN OF INTEGRATING VECTORS USED FOR HEMATOPOIETIC STEM CELLS GENE THERAPY

Vectors engineered from gamma retroviruses,[28] long under study as the cause of a variety of cancers in mice, had the desired property of efficient insertion into the genome of target cells. Murine gamma retroviruses and their derivatives were the first of the genome integrating vectors to be applied to T lymphocytes and HSC in the clinical setting.

Gamma retroviruses are RNA viruses, that on entry into a cell, are "reverse transcribed" (hence "retro"virus) into a DNA sequence. It is the DNA virus sequence that ultimately inserts itself into the host cell's genomic DNA, becoming a "provirus" that in turn generates RNA virus sequences and viral mRNAs encoding virus proteins required for the replication phase of the virus life cycle. The critical issue was how to turn these viruses that efficiently insert provirus DNA genomic sequence into mammalian cell genomes, but are also efficient at causing tumors, into safe tools for gene therapy. The solution was to remove and/or inactivate as many elements of the virus genome as possible, while still retaining the ability of the highly engineered provirus sequence to insert efficiently into the mammalian cell genome. The goal was a functioning single-cycle virus capable of cell entry, uncoating, reverse transcription into provirus DNA, and insertion into the genome, but incapable of generating infectious virus. The solution involved separating the key elements required to generate replication-incompetent viral vector into 3 separate "production plasmids": (1) an envelope (env) producing element (the vector virus coat also serving the purpose of binding to target cell and facilitating virus payload entry); (2) a gag-pol producing element (gag protein important for vector RNA packaging and polymerase for reverse transcribing the RNA); and (3) the vector sequence (retaining the psi element needed for packaging and the long terminal repeat (LTR) sequences at both ends of the vector sequence, which serves both as the internal strong promoter driving the production of a therapeutic protein and containing initiation elements binding the 2 ends of the vector for the circle formation required for reverse transcription). Where possible the env and gag-pol codons were changed to avoid recombination events that could reconstruct a replication-competent virus. To simplify the process of making different gamma retrovirus vectors, permanent packaging lines were devised that constitutively produce env and gag-pol, and when a specific vector sequence is added, clones could be assessed and chosen that constitutively produced vector in adequate titers. Many laboratories contributed to this technology and created a large array of different "flavors" of therapeutic gene therapy gamma retrovirus vectors. Many of these continue to be used for the production of some CAR-T lymphocytes or therapeutic cloned T cell receptors. This tour-de-force of engineering involving the contribution of many laboratories has served as the core technology used in the first generation of gene therapy targeting HSC or lymphocytes.

The LTRs of gamma retroviruses were retained in the engineered vectors as conve-

strong enhancer elements that can activate nearby genes. The engineered vectors by design retained the insertion targeting elements of the parent virus required to insert the DNA provirus into the mammalian genome. While the insertion of vector seems to be random, it is actually stochastic in that the mechanism used by the vector couples to cellular elements, resulting in preferred sites of insertion into the genome. These preferred sites (also known as integration sites) are often located near the start of genes and in enhancer elements, and may in turn strongly interact with enhancer elements in the LTR.[29,30] While the odds of any one insert occurring in a sensitive site are very low, gene therapy for a human subject may involve tens to hundreds of millions of insertions. Depending on the vector, the LTR and the host human subject disease substrate, we now know from adverse leukemic insertional mutagenesis events occurring in a number of clinical trials, that gamma retroviral vectors can transactivate oncogenes such as *LMO2*, the *MECOM* complex, and other oncogene targets to initiate the development of leukemia. These insertional mutagenesis events will be further discussed in greater detail in the last section of this historical review. Curiously, insertional mutagenesis leading to leukemic events has not been observed when the target of gamma retroviral vector gene therapy is T lymphocytes.

Well before the first insertional mutagenesis, oncogenic events were observed in clinical trials of gene therapy using gamma retroviral vectors, certain limitations of this class of vectors (eg, limits of therapeutic payload size, limits on the use of alternate promoter elements instead of the LTR, absolute requirement for cell division for vector insertion into the genome) encouraged the development of gene therapy vectors derived from human immunodeficiency virus (HIV). HIV is part of a different group of retroviruses called lentiviruses and the vectors engineered from HIV are referred to here as "lentivectors." HIV and other lentiviruses have a more complex structure, and have a number of required functional elements not present in gamma retroviruses, such as rev, that needed to be considered while engineering HIV into a safe gene therapy tool.[31,32] As with gamma retroviruses, determining how much could be removed from the virus and whether the addition of elements from other viruses might enhance function and efficiency of the vector was an iterative discovery process. From a historical perspective, some key advantages of lentivector function and engineering, and the insertional mutagenesis oncogenic events noted above have resulted for the most part in the abandonment of gamma retroviral vectors for the transduction of HSC for clinical trials.

As with gamma retrovirus vectors, the production of lentivectors that are functional, but replication incompetent, required the separation of packaging elements into plasmids separate from the transfer vector. Almost all lentivector production for clinical application uses the membrane fusion G protein derived from vesicular stomatitis virus (VSV-G) as the vector envelope element, rather than the natural env component of HIV. The cell membrane target of the VSV-G protein is ubiquitous to all cells with high efficiency of binding and vector membrane fusion. Almost from the start, lentivector engineering strategies incorporated a self-inactivating (SIN) feature, modifying the LTR element that contains strong enhancers with transactivating potential and using safer promotors with little enhancer activity instead. This was accomplished by creating a deletion in the 3′ LTR of the vector production plasmid. During vector production, the intact 5′ LTR assists in the important packaging biochemistry needed to produce infective but replication-incompetent lentivirus vector. During transduction, the SIN 3′ LTR binds to the 5′ LTR in the circularization and priming step that retrotranscribes the insertional provirus DNA from the lentivector RNA, and is incorporated into the 5′ end

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