Editorial

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Novel gene therapy advances for treating primary immunodeficiency disorders – an update

Ruhul Amin, PhD[a], Ronald Darwin, PhD[b], Sandip Chakraborty, MVSc[c], Biplab K. Dey, PhD[a], Kuldeep Dhama, MVSc, PhD[d,*], Talha B. Emran, PhD[e,f,*]

Dear Editor,

Primary immunodeficiency diseases (PIDs) are a group of over 400 rare and diverse hereditary disorders that affect the immune system's ability to combat infections. PIDs are caused by genetic mutations and are present from birth, as opposed to secondary immunodeficiency disorders, which are acquired later in life as a result of infections, medicines, or other medical issues[1]. Over the past three decades, significant advancements in the technology and practices of gene therapy have been made. PIDs have been successfully treated with gene therapy on a large scale (Fig. 1). A working copy of the problematic gene is inserted or altered into the patient's own Hematopoietic Stem Cells (HSCs) in this innovative procedure. Gene therapy, in conjunction with hematopoietic cell transplantation (HCT), offers the potential to treat persons with PIDs. HCT entails supplying healthy donor HSCs that can grow into fully functioning immune cells in PID patients. During 1999–2000, was use of integrating gamma-retroviral vectors (murine derived) during the initial studies concerning gene therapy for PIDs. Clinical efficiency was shown by these studies in several instances. However, genotoxicity as well as lymphoproliferative disorders in various patients are caused by the vectors used in these studies. Thus, the most recent research use lentiviral vectors wherein enhancer elements of long terminal repeats (LTRs) could self-inactivate in reverse transcription [self-inactivating (SIN) vectors]. It has been seen that the safety profiles of these SIN vectors are excellent and any genotoxicity of clinical significance is not caused by these vectors[2]. Researchers are always working to enhance the safety and efficacy of vector systems to reduce possible dangers to gene therapy patients. The use of SIN lentiviral vectors is one method for improving the safety of gene therapy applications. However, assessing safety in clinical trials is a complicated and continuing procedure[3].

For a wide variety of PIDs, an approach of curative therapy is provided by allogenic hematopoietic stem cell transplantation (allo-HSCT) that has shown a very high success rate when there is the availability of a suitable donor. However, it is unfortunate that a very limited number of donors (human leukocyte antigen/HLA-matched) are available for a large number of patients and the results of allo-HSCT transplant are unsatisfactory when there is the use of HLA-mismatched donor. In this regard, there has been the development of gene therapy with the HSCs of the patients that can act as an alternative way helping in the elimination of the chances of graft-versus-host disease, which is primarily responsible for mortality as well as morbidity following allo-HSCT. In the HSCs of the recipient, replacement of the faulty genes and the transplantation (autologous) of cells that are corrected genetically rather than the replacement of the stem cells of the recipient with that of the donor is important in offering an alternative for various PIDs that proves to be lifesaving[4–7].

Adenosine deaminase severe combined immunodeficiency (ADA SCID) was the first condition for which gene therapy was used in humans. In this situation, a normal ADA gene introduction was targeted into peripheral blood cells. Following gene therapy, the survival of lymphocytes designated by the ADA gene lasted for over a decade, indicative of the lifetime of cells in spite of ex vivo modification[8].

In the recent past, various studies (preclinical) have been published on the use of editing of genome mediated by Cas9 and homology-directed repair (HDR) in PID models. The safety of therapy directed against X-SCID by editing of the genome has been established and its efficiency in the cells of the patients *in vitro* in preclinical models has also been confirmed. Editing of interleukin-2 receptor common γ-chain (*IL2RG*) in HSCs has been achieved efficiently by the use of very specific zinc finger nucleases or clustered regularly interspaces short palindromic repeats (CRISPR)/Cas9. Determination of the threshold proportion of editing of *IL2RG* in repopulating cells has been done by use of mouse models that are immunodeficient in nature and gene-edited HSCs of patients and has been predicted to be essential for immune reconstitution. Another study showed that in long-term (LT)-HSCs, 20% integration frequencies targeted can be achieved with a strategy based on CRISPR-Cas9/adeno-associated virus type 6 (AAV6). Such achievement is possible in

[a]Faculty of Pharmaceutical Science, Assam down town University, Panikhaiti, Gandhinagar, Guwahati, Assam, India, [b]School of Pharmaceutical Sciences, Vels Institute of Science Technology & Advanced Studies, Chennai, [c]Department of Veterinary Microbiology, College of Veterinary Sciences and Animal Husbandry, R.K. Nagar, West Tripura, Tripura, [d]Division of Pathology, ICAR-Indian Veterinary Research Institute, Bareilly, Izatnagar, Uttar Pradesh, India, [e]Department of Pharmacy, BGC Trust University Bangladesh, Chittagong, Bangladesh and [f]Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University, Dhaka, Bangladesh

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*Corresponding author. Address: Department of Pharmacy, BGC Trust University Bangladesh, Chittagong 4381, Bangladesh. E-mail: talhabmb@bgctub.ac.bd (T.B. Emran); Division of Pathology, Indian Veterinary Research Institute, Izatnagar 243122, Bareilly, Uttar Pradesh, India. E-mail: kdhama@rediffmail.com (K. Dhama).

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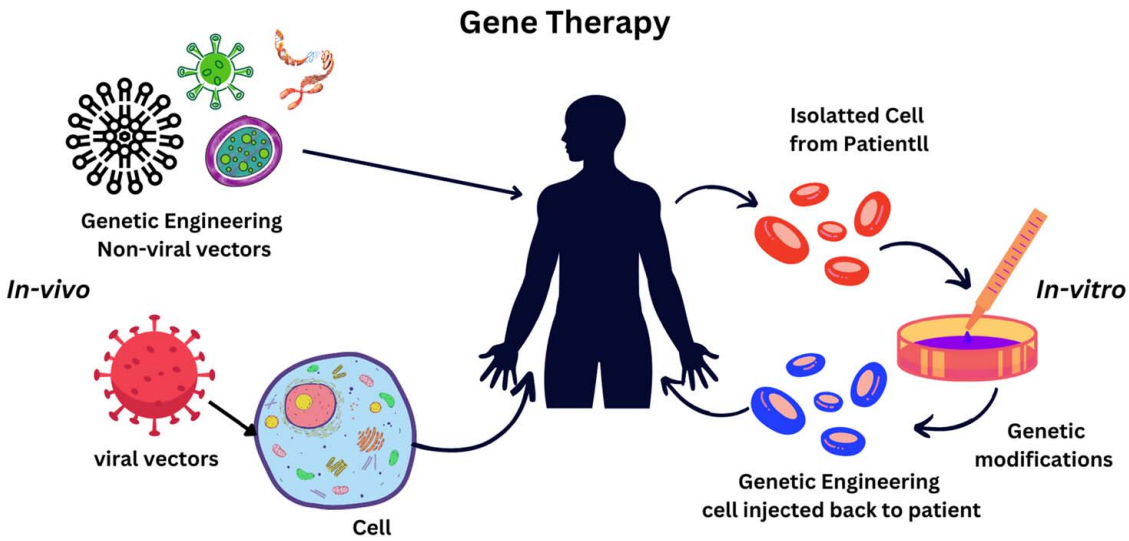

Figure 1. Overview of gene therapy in in-vivo and in-vitro preparations.

preclinical models without any trace of toxicity or abnormal hematopoiesis. This strategy has led to the rescue of lymphopoietic defects in HSCs derived from patients that are gene-corrected. Encouraging proofs have been obtained with other preclinical studies for various other PIDs that include X-linked hyper-IgM (X-HIGM) syndrome as well as chronic granulomatous disease (CGD)[9]. For Fabry disease, a lysosomal storage disorder due to a mutation in gene responsible for alpha-galactosidase A (-Gal A) enzyme production, FLT190 as a synthetic capsid (AAVS3) containing a genetic construct made up of a codon-optimized human GLA cDNA controlled by liver-specific promoter FRE1 (AAV2/S3-FRE1-GLAco) has been investigated for use in gene therapy. Animal research using pseudo-typed AAV8 vectors to effectively transport FLT190 produced positive results, with -Gal A expression levels in NHPs equivalent to hGLA mRNA levels in the liver. Importantly, any FLT190-related toxicities or adverse effects were not observed, signifying preclinical potential of FLT190-directed gene therapy for treating Fabry disease[10].

Wiskott–Aldrich syndrome (WAS), an X-linked disorder linked to WAS gene failure, affects the immunohematologist system, resulting in microthrombocytopenia and malfunctioning lymphoid and myeloid cells. Early gene therapy experiments using gamma-retroviral vectors has shown therapeutic advantages but were accompanied with high rates of insertional mutagenesis and the development of hematologic malignancies in the majority of patients later on. Researchers have focused on lentiviral-based gene therapy for patients without a properly matched donor. These investigations found positive results, such as improved thrombocytopenia, decreased susceptibility to infections, remission of eczema symptoms, and no evidence of lymphoproliferative diseases[11].

Gene therapy is also being explored as a prospective choice for treating Gaucher disease type 1 (GD1), which is characterized by mutations in GBA1 gene that result in glucocerebrosidase (GCase) enzyme deficiency. FLT201 is an experimental gene therapy that utilizes AAVS3 containing a unique variant of GCase (GCase-85). FLT201 has shown promising preclinical results, GCase-85 demonstrating improved stability at physiological pH as compared to wild-type GCase along with efficient transport to tissues targeted[12].

In the case of familial hemophagocytic lymphohistiocytosis (FHLH) 2, the mutated gene is *PRF1*, which encodes for perforin. Perforin is released into the immunological synapse in healthy people, producing a hole in target cells. This permits granzymes to enter the cytoplasm, causing apoptosis to begin. In a mouse model, mixed chimerism experiments have revealed that wild-type cells at low levels can cause restoration of the immune regulation, which is indicative of the fact that a suitable approach can be gene therapy. Construction of lenti virus (LV) vectors has been done. A PGK promoter (constitutive) or a portion of the PRF1 promoter was used to drive transgenic expression. In mouse models, gene expression was restored, as was cytotoxicity to natural killer (NK) and T cells. In addition, a T cell method (gene-corrected) has been investigated, and this technique may give a bridge-to-transplant therapy in patients[13,14].

FHLH3 is also a target for gene therapy. The cause of this condition is a mutation in *UNC13D* that encodes unc-13 homolog D protein that is required to prime vesicles that contain perforin for the process of exocytosis. Cells that lack the functional protein cannot undergo proper degranulation, which gives rise to cytotoxic defects. Gene correction has been investigated by several groups by the use of self-inactivating gamma retrovirus (SIN γRV), SIN alpha retrovirus or LV vectors. Such gene modifications employing these vectors have aided in the functional restoration of degranulation activity in mouse models both *in vitro* and *in vivo*. As an alternative therapeutic approach, investigations regarding gene-corrected T cells are encouraging[15]. Hemophilia A and B are X-linked recessive diseases caused by gene abnormalities affecting blood clotting factors VIII (FVIII) and IX (FIX). A small increase in blood factor (5% of normal levels) may alleviate bleeding phenotype in severely ill individuals, wherein gene therapy is a feasible

therapeutic option for these illnesses. In patients with severe hemophilia B, St. Jude/UCL phase 1/2 experiment found a dose-dependent rise in FIX levels after a single delivery of AAV vectors. Over a 7-year period, persistent expression of transgenic FIX at 5% of normal levels in the high-dose group resulted in a substantial reduction in spontaneous bleeding and a reduced necessity for anticoagulation. For the FIX protein without causing any harm, following advancements in gene therapy for hemophilia A and B, the clotting factor actions returned to normal or near to normal, resulting in 'zero bleed rates' in formerly seriously afflicted patients. These amazing advances in AAV gene treatments are projected to transform the therapy landscape for hemophilia A and B[16]. Both hemophilia and PIDs affect the blood clotting and immune systems; however, they are treated differently. Both diseases result from changes in genes, and there may be some shared genetic underpinnings between them even though they are not causally linked[17,18].

In certain PIDs, restriction of the defect may be there in the T cell compartment or lymphocyte subsets, viz., regulatory T cells. Thus, it may become possible that the autologous T cells (gene-corrected), when transferred, may become enough to control the manifestations of the disease condition clinically, thereby leading to benefit significantly. Investigations of such an approach have been done in preclinical models for various PIDs that include immune dysregulation, Polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome, X-linked lymphoproliferative disease (XLP), deficiency of perforin, Mammalian Unc-13-4, and CD-40 ligand. The safety profile of T cell gene therapy has been established with several patients being treated to date for malignancies of the hematopoietic system in immunotherapy trials. Interestingly, transformational events have not been reported in such instances[19,20].

With the introduction of graft manipulation techniques that are more sophisticated in nature, there has been an improvement in the outcomes following hematopietic stem cell treatment (HSCT), even in the haploidentical as well as mismatched donor setting. The clinical trials involving gene therapy are generating more efficacy data (long-term). Thus, it will be relatively easier for physicians to know about the type of patients to benefit from the various options of treatment available.

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Talha Bin Emran, PhD, Associate Professor, Department of Pharmacy, BGC Trust University Bangladesh, Chittagong 4381, Bangladesh; Tel: +88 030 3356193, fax: +88 031 2550224, Cell: +88 01819 942214; https://orcid.org/0000-0003-3188-2272; E-mail: talhabmb@bgctub.ac.bd.

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