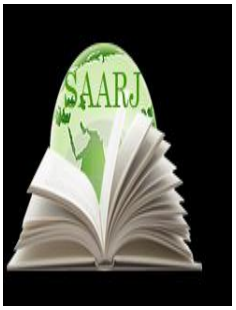

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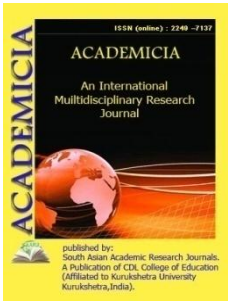

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CURING GENETIC DISEASE WITH GENE THERAPY: A REVIEW

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ABSTRACT

Development of viral vectors that enable high efficiency gene transfer into mammalian cells in the early 1980s anticipated the treatment of severe monogenic illnesses in humans. The use of gene transfer utilizing viral vectors has proven effective in disorders of the blood and immune systems, although with many curative trials also revealing severe adverse effects (SAEs) (SAEs). In children with X-linked severe combined immunodeficiency (SCID-X1), chronic granulomatous disease, and Wiskott-Aldrich syndrome, these SAEs were induced by incorrect activation of oncogenes. Subsequent investigations have identified the vector sequences responsible for these changing processes. Members of the Transatlantic Gene Therapy Consortium [TAGTC] have jointly created novel vectors that have proved safer in preclinical tests and utilized these vectors in new clinical trials in SCID-X1. These studies have showed indications of early effectiveness and preliminary integration analysis results from the SCID-X1 trial indicate a better safety profile.

KEYWORDS: *Diseases, Gene, Genetic, Therapy, Vectors.*

1. INTRODUCTION

Genetic treatments employing viral vectors have increasingly been proven to have effectiveness in monogenic disorders of children. For successful gene therapy, it is necessary to create a product that enables an efficient transduction of target cells. Currently, this is done by utilizing cloned recombinant viruses to transmit genetic sequences with great efficiency. In addition, it is essential to guarantee appropriate expression of transgenes in those cells where it is physiologically required. Increasingly, modern vectors utilize chimeric promoters of mammalian genes coupled with endogenous cis-regulatory elements. Finally, the method must allow for long-term engraftment of changed gene (transduced) cells. Some of the most effective gene

therapy methods to date utilize ex vivo modification and hematopoietic stem cell (HSC) transplantation as a clinical platform to effect genetic treatments[1].

The approach for ex vivo alteration of HSC is strikingly similar to that established in the early 1980s, with some significant advances. Generally, HSCs are acquired via either bone marrow harvest, mobilized peripheral blood collection, or, less often, autologous umbilical cord blood. An HSC-enriched population of cells is produced via CD34 isolation. The resultant CD34+ cells are next cultured ex vivo in a cocktail of cytokines and then exposed to a safety-certified viral vector supernatant produced in specialist facilities according to good manufacturing practice (GMP) standards. The transduced cell product is subsequently given as an autologous hematopoietic stem cell transplant (HSCT) to the recipient. In certain procedures, the patient is subjected to preparative conditioning utilizing chemotherapy, radiation, or both as per normal HSCT transplantation protocols. In certain procedures, no conditioning is needed and these details are disease-specific. Two main benefits are achieved utilizing this gene therapy approach: 1) there is no need to seek for a histocompatible donor; and 2) there is no danger of graft-vs-host disease (GVHD) and thus no need for GVHD prevention or treatment of the patient[2].

As mentioned above, one potential illness for genetic treatment is X-linked severe combined immunodeficiency (SCID-X1). The illness is caused by loss-of-function mutations of the interleukin (IL) – 2 common gamma (γ) chain cytokine receptor. Phenotypically, infants born with this illness lack T and natural killer (NK) lymphocytes and have poorly functioning B cells leading to highly impaired immunity. The illness is deadly if neglected, frequently from otherwise very mild viral infections. Previous clinical work has demonstrated that allogeneic HSCT utilizing either matched related or matched unrelated donors (MUDs) may cure the illness frequently without any conditioning of the recipient. However, MUD transplantations in this illness are associated by higher risk of GVHD, graft failure and overall poor results of these transplants, especially when the recipient is infected at the time of transplantation. This is often the case, since individuals are commonly diagnosed owing to severe viral infections in the first year of life[3].

Previous studies have demonstrated successful gene therapy in SCID-X1. Two studies treated a total of 20 children with this illness utilizing a Moloneyleukemia virus (MoLV) – based retrovirus vector producing the IL-2Rγ cDNA from the viral long-terminal repeat (LTR) cis-regulatory region containing a strong enhancer element and a viral promoter (MFG-γC). In these prior trials, effectiveness was demonstrated in 18 of 20 children treated with this vector with a restoration of T and NK cell numbers and functioning. However, 5 of 20 children in this study developed T cell leukemia linked to the insertion of the viral vector into the genome near proto-oncogenes (7) (7) These insertions resulted to dysregulated expression in four of five instances of the LMO-2 proto-oncogene involved in certain cases of de novo pediatric T cell acute lymphoblasticleukemia of these children, 4/5 were effectively treated for their leukemia with preservation of the gene-corrected immunologic function, while one kid died from therapy-resistant leukemia. Thus, although showing efficacy, these studies were also characterized by serious adverse events (SAEs) that led to the interim cessation of a number of trials worldwide and to the United States Food and Drug Administration (FDA) restriction of the use of gene therapy in SCID-X1 to rescue protocols in which eligibility would require previous failure of an allogeneic transplant[4].

2. LITERATURE REVIEW

D. A. Williams in his study discloses about aninfectious retrovirus vector that has been used to transfer a bacterial gene encoding resistance to the neomycin analogue G418 into pluripotent haematopoietic stem cells present in explanted murine bone marrow tissue. Subsequent transplantation of the cells into lethally irradiated mice results in engraftment of the animals with donor haematopoietic tissue containing the bacterial gene. This approach affords an efficient and rapid means of re-introducing genetically modified tissue into intact organisms and provides a system whereby the expression and regulation of cloned genes can be followed within the context of a well characterized developmental programme[5].

A Joyner in another study discloses about variety that seems to emerge from the commitment and maturity of stem cells, the molecular basis for this differentiation process is unclear. The insertion of cloned DNA sequences into haematopoietic progenitor cells would offer a new method for investigating this differentiating in vivo system. One laboratory has demonstrated DNA-mediated transfer of genes into mouse bone marrow cells. However, retroviruses provide a number of benefits over DNA-mediated gene transfer methods, including high efficiency infection of a broad variety of cell types in vitro and in vivo, stable and low copy integration into the host chromosome, and a specified integrated provirus structure. For these reasons recombinant DNA methods have been used to create high efficiency retrovirus vectors expressing foreign genes. We show here, employing such a retrovirus vector, the transfer of a dominant selectable drug-resistance gene into specified classes of mouse haematopoietic progenitor cells. These findings should assist the development of molecular genetic approaches to basic and clinical issues in haematopoiesis[6].

A Fischer in yet another study discusses about naturally occurring genetic diseases of the immune system offer numerous models for the study of its development and function. In a sense, their study complements the information given by the creation of genetic abnormalities in mice produced via homologous recombination methods. In this study, the latest discoveries produced in three areas are focused upon defects in T cell development and in T lymphocyte activation, and on the regulatory mechanism of peripheral immune response[7].

3. DISCUSSION

Shortly after the emergence of leukemias in the French SCID-X1 gene therapy study, a group of scientists formed the Transatlantic Gene Therapy Consortium (TAGTC) to encourage a coordinated approach in resolving the SAEs in this trial (10). (10). This group began meeting annually in a planning retreat with goals to: 1) share expertise in addressing the SAEs of the gene therapy trial in SCID-X1; 2) collaborate on vector development and preclinical studies; 3) share the costs of GMP vector production and certification; 4) develop a common clinical protocol as a platform for a multi-institutional trial and subsequently implement this trial across multiple sites; and 5) seek funding for these efforts. Vector design was based on the molecular evidence implicating the MoLV U3 region enhancer in trans-activation of LMO2 locus. Preclinical effectiveness and safety data were produced at various locations utilizing a range of in vitro and in vivo tests using both murine and human cell lines and primary cells. These results eventually led to the preparation of regulatory papers required in the United States (US), United Kingdom, and France for initiation of a clinical phase I/II study. The criteria for each of these regulatory procedures vary according to particular governmental regulations. Vector GMP manufacturing

was achieved at one location utilizing temporary transfection techniques and has previously been reported[8].

The institutions making up TAGTC are listed in Table 1. The vector suggested and eventually utilized in the experiment, SRS11-IL2RG, is illustrated in Figure 1. The vector is a self-inactivating (SIN) engineered γ-retrovirus in which the U3 enhancer is removed from the 3' LTR. In this SIN design, during reverse transcription of the viral genome, the 3' LTR is replicated so that the integrated provirus is devoid of both 5' and 3' U3 enhancer regions. A variety of preclinical studies were used to compare this vector to the MFG-γC vector used in the previous trial, including: 1) efficacy in restoring IL2R signaling in vitro; 2) reduced propensity for immortalization of primary murine bone marrow in vitro (12); 3) reduced transactivation of the LMO2 locus in a plasmid reporter assays in Jurkat T cells (13); 4) reduced insertions in proto-oncogenes in mice transplanted with transduced HSC (manuscript in preparation) (manuscript in preparation)[9].

In the United States, extensive reviews by the National Institutes of Health Recombinant DNA Advisory Committee, the FDA, local international review boards, and a study section of the National Institute of Allergy and Infectious Diseases (NIAID) led to multiple — often conflicting — recommendations for changes in the protocol. The resulting approved protocol "Gene transfer for SCID-X1 using a self-inactivating (SIN) gamma retroviral vector, a multi-institutional phase I/II trial evaluating the treatment of SCID-X1 patients with retrovirus-mediated gene transfer" was ultimately approved by regulatory agencies for opening in five sites internationally (Table 2) and is listed on Clinicaltrials.gov. The trial is sponsored in the United States by the NIAID.

The study began for recruitment at the US locations in January 2011 and to far has recruited ~50 percent of its accrual goal (nine participants) (nine patients). The longest followed patients are currently >3 years after infusion of gene modified cells. There have been no SAEs directly linked to the vector or to insertional events. Analysis of T cells in several individuals revealed expression of IL-2RG on surface of T cells at levels somewhat below wild-type T cells, as anticipated based on the weaker nature of the promoter. Efficacy trials are ongoing, however to far the vector seems to operate as anticipated with a number of patients exhibiting restoration of peripheral T cell counts and T cell activities as assessed by in vitro stimulation index and return of NK cell numbers. Tracking of recent thymic immigrants through T cell receptor excision circles indicate the functioning of the thymus in many individuals. Integration studies are underway[10].

TABLE 1: TRANSATLANTIC GENE THERAPY CONSORTIUM[1].

Children's Hospital Boston, Harvard Medical School (Boston)
CIEMAT (Madrid)
Cincinnati Children's Hospital, U. of Cincinnati College of Medicine (Cincinnati)
Genethon (Paris)
Georg-Speyer-Haus (Frankfurt, Germany)
German Cancer Institute (Heidelberg)
Great Ormond Street, Institute for Child Health (London)
Hannover Medical School (Hannover)

Lund University (Sweden
Mattel Children's Hospital, UCLA (Los Angeles)

Figure 1: The SRS11-IL2RG vector. The vector was derived from a murine MoloneyLeukemia virus (MoLV). The long terminal repeat (LTR) includes a deletion of the U3 region denoted by Δ, the R and U5 regions are shown. The IL-2 receptor γ cDNA (IL3RG) is expressed from an internal mammalian promoter made up of the elongation factor 1α (EF1α) sequences. Arrow denotes orientation of mRNA generated from integrated vector[2].

TABLE 2: PARTICIPATING INSTITUTION SITES IN SCID-X1 CLINICAL GENE THERAPY TRIAL[3].

Great Ormond Street, Institute for Child Health (London)
Hôpital Necker EnfantsMalades (Paris)
Children's Hospital Boston, Harvard Medical School (Boston)
Cincinnati Children's Hospital, U. of Cincinnati College of Medicine (Cincinnati
Mattel Children's Hospital, UCLA (Los Angeles)

Collaborative effort across many universities making up the TAGTC has resulted to the creation of an enhancer-deleted vector expressing the IL-2RG. In preclinical investigations, the vector demonstrated better safety profile utilizing a range of in vitro and in vivo assays. This is a unique multinational cooperation with shared expenses, developmental effort, and eventual clinical testing. The clinical study, currently continuing and accumulating more participants, indicates early effectiveness. Although the follow-up is still too early to evaluate overall safety, no SAEs have yet been found linked to the vector[4].

These investigations are an example of so-called two-way translational research in which fundamental science first leads to clinical research studies, the findings of which then lead to fresh rounds of basic studies and future trials. The area of genetic treatments has continued to develop over ~25 years in a manner similar to many new technologies in which early triumphs are followed by a focus on side effects with successive new breakthroughs happening before widespread acceptance and uses. In this instance, the use of viral vectors for gene therapy applications demonstrated first results in the late 1990s and early 2000s. Vector insertional mutagenesis led to a tempering of excitement followed by studies to enhance safety, as reported in this article. The field has lately witnessed several breakthroughs in treating uncommon, monogenic illnesses utilizing novel vector technology. Ongoing study will be focused at expanding the indications and extending the uses outside highly specialized university research institutions[5].

In a Phase 1–2a trial (NCT02519036) supported by Ionis Pharmaceuticals and F Hoffmann–La Roche, published on June 13 in the New England Journal of Medicine, Sarah Tabrizi and colleagues from University College London (UK) tested an antisense oligonucleotide (IONISHTTRX) in adults with early Huntington's disease and found that the treatment reduced the concentration of mutant huntingtin without serious adverse effects. In another Phase 1–2a clinical trial (NCT01512888), published on April 18 in the same journal, researchers from St Jude Children's Research Hospital in Memphis (TN, USA) tested a lentiviral gene therapy for babies with X-linked severe combination immunodeficiency. The findings indicated that the gene therapy combined with low-exposure, targeted busulfan conditioning had minimal acute \stoxic effects and resulted in enhanced immunity in each of the eight treated individuals.

These remarkable findings are giving fresh hope to individuals with rare and severe genetic disorders such as these. The US Food and Drug Administration (FDA) authorized three gene therapy products in 2017, including voretigeneneparvovec-rzyl, the first licensed gene therapy treatment for individuals with proven biallelic RPE65 mutation that causes retinal degeneration. More than 25 gene treatments are presently in phase 3 or have shown therapeutic effectiveness in phase 1–2 studies in 2019. The FDA expects approving 10–20 cell and gene therapy products per year by 2025, which will most definitely include gene therapies targeting the illnesses listed above, as well as sickle cell anemia, heart disease, and cystic fibrosis[2].

4. CONCLUSION

Although we have reasons to be hopeful, obstacles remain before gene therapy can leap from lab to bedside for several reasons. To begin with, on-target delivery of the gene to the appropriate cells and tissues that are afflicted by the illness is essential to the success of gene therapy.

Most therapies currently being developed utilize inactivated viral vectors, such as AAV or lentiviruses, to transmit repaired genes or genomeediting machinery to fix the faulty gene. However, those vectors typically concentrate in the liver, possibly limiting the range of easily targetable illnesses. Remarkable efforts have been made to improve gene delivery vectors to transport transgenes to targeted cell populations. In their study published on March 6 in Molecular Therapy, Suh and colleagues from Rice University in Houston (TX, USA) created a protease-activatable AAV vector, called provector, that reacts to increased extracellular protease activity frequently observed in tissue microenvironments of heart disease. In an in-vivo model of myocardial infarction, provector may transport transgenes preferentially to areas of the injured heart with high matrix-metalloproteinases activity, with a corresponding decrease in delivery to numerous off-target organs, including the liver.

In addition, dangers of cutting-edge technology and the rarity of gene therapy for specific hereditary illnesses are the cause of many bioethical and economical problems. Off-target consequences of existing genomeediting technologies remain a significant worry and barrier to bring it into a clinical setting with acceptable and regulated safety. Even with authorized gene therapy, in the long term, treatment may produce adverse symptoms or organ damage and other side effects that haven't been documented yet in patients. Therefore, both health-care professionals and patients must weigh these dangers with the health benefit that gene therapy offers, particularly when it comes to treating a rare genetic condition that may have the potential to create serious difficulties over many decades. Furthermore, the high expenses of creating

treatments tailored to a limited number of patients may make certain gene therapies prohibitively costly, and insurance coverage could be difficult to obtain.

To address these difficulties, all stakeholders—policymakers, pharmaceutical firms, scientific researchers, and health providers— must work together to guarantee that safe, effective, and inexpensive gene therapies become accessible to people in need. For scientific researchers, the development of the best delivery systems and enhancement of the genome-editing technologies will lead to safer and more effective and cheaper gene treatments. EBioMedicine looks forward to publishing high-quality translational research on this front. For those who are suffering from severe genetic illnesses and in urgent need of efficient therapies, gene therapy is still one of the greatest possibilities for the ultimate cure.

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