European Journal of Internal Medicine 25 (2014) 31–36

Contents lists available at ScienceDirect

European Journal of Internal Medicine

journal homepage: www.elsevier.com/locate/ejim

Review article

Gene therapy as a new treatment option for inherited monogenic diseases

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ARTICLE INFO

Article history:
Received 28 August 2013
Received in revised form 15 September 2013
Accepted 18 September 2013
Available online 12 October 2013

Keywords:
Gene therapy
Inherited monogenic disorders
SCID
Hemophilia B
Lipoprotein lipase
Deficiency

ABSTRACT

Background: Gene therapy, replacing a defective gene by a functional copy, has been in development for more than 40 years. Initial efforts involved engineering viral vectors to deliver genes to the appropriate cells. Early successes in severe combined immunodeficiency (SCID) were later derailed by safety issues including host reaction to the vector and gene insertion near promoters that favored secondary leukemia.
Methods: Systematic review of the literature using PubMed.gov with key word gene therapy from 1972 to March 2013. Google search with key word gene therapy.
Results: Despite early setbacks, progresses for monogenic diseases continued unabated. Patients with SCIDs have been cured and the first gene therapy has been approved for lipoprotein lipase deficiency. Many clinical research studies are ongoing as part of systematic clinical development program with a view to have more gene therapies approved.
Conclusion: Our review highlights progresses and questions that remain to be answered to make gene therapy an integral part of our therapeutic arsenal.

1. Introduction

Physicians are now able to correct the genetic defect of patients affected with rare inheritable diseases. This review focuses on some remarkable clinical results obtained with gene therapy for monogenic disorders and highlights new directions that could have major impacts on medical practice.

1.1. Early developments

The transfer of genes for a therapeutic benefit has been tried for more than 40 years [1]. The principle is simple: a defective gene is replaced by a functional copy that corrects the problem. Gene therapy focuses on three components: the therapeutic gene, the vector that delivers it, and the mode of administration.

In the mid-1980s, the concept came closer to reality when the first "cure" was reported in *little* mice. These mice, a model for human pituitary dwarfism, have reduced levels of growth hormone. Scientists succeeded in inserting a rat growth hormone gene into the pro-nucleus of mice egg and the deficiency was corrected [2]. However, the gene was not controlled and gigantism resulted, an early indication that transferred genes needed to be regulated.

Despite this first success, gene therapy turned out to be more challenging than anticipated [3].

Because a gene's transfer can also be done by modifying a cell that is re-introduced in the patient's body, monogenic diseases of blood cells, such as sickle cell disease or β-thalassemia, were initially considered [3]. In these diseases, the molecular defects were understood and the target cell, the hematopoietic stem cell (HSC) was easily accessible. It could be genetically corrected *ex-vivo* and transplanted back. Hence, gene therapy had a major advantage over the conventional transplantation of HSCs from compatible donors: it was available for all patients and avoided graft rejection [4]. Unfortunately, the regulation of the different globins chains was more complex than anticipated and it was not possible to transfer the β-globin gene in a sufficient number of HSCs to obtain an appropriate erythrocyte precursor expression [5].

Thus, in the mid-1980s, scientists turned to a rare disorder that was thought simpler to address, severe combined immunodeficiency disease (SCID) due to deficiency of the enzyme adenosine deaminase (ADA-SCID) [3,6]. SCIDs include multiple genetic defects, all leading to impaired differentiation of T lymphocytes with, for some, additional blocks in the differentiation of B lymphocytes and/or natural killer (NK) lymphocytes [7]. SCID, in its X-linked form (SCID-X1), is the most common and also known as the bubble boy disease. Infant boys have chronic diarrhea, severe opportunistic infections and fail to thrive. In the absence of immune reconstitution by allogeneic bone marrow transplantation (BMT), kids generally die within the first two years of life. ADA-SCID, the second most common form, was the first for which the genetic and molecular defects were identified [8].

The first ADA-SCID clinical trials were published in the mid-90s. The gene transfer was attempted *ex vivo* into umbilical cord blood cells or autologous T lymphocytes using a murine retroviral vector [6,9,10]. Retroviral vectors require cell proliferation for efficient transduction and thus, can be used for disorders of blood cells. Unfortunately, in these studies, not enough cells could be transduced for a sufficient time.

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0953-6205/$ – see front matter
http://dx.doi.org/10.1016/j.ejim.2013.09.009

Other attempts where pursued in non-hematologic genetic diseases such as cystic fibrosis [11], Duchenne muscular dystrophy [12] or familial hypercholesterolemia [13]. Adenoviruses were used as vectors. Adenoviruses are easy to produce but can induce local inflammation as well as immune reaction that limit the expression of transgene expression. Again, the results were disappointing [14].

Despite these difficulties, efforts continued unabated and in the late 1990s better gene transfer protocols and vectors were developed. Successful treatments in animal models were reported [15]. With the new millennium, the first successful gene therapy was reported in boys with SCID-X1 [16]. SCID-X1 is characterized by an early block in T cell and NK cell differentiation caused by mutations of the gene encoding the γc cytokine receptor subunit of interleukin-2, -4, -7, -9, and -15 receptors, which deliver growth and differentiation signals to lymphoid progenitors. In this landmark study, boys who lacked HLA-identical donors had their HSC transduced *ex-vivo* with a retrovirus containing the γc gene and transferred back to them. The genetically modified cells, replete with a functional γc gene, were expected to populate patients' marrow. Indeed, after 10 months of follow-up these boys appeared cured [16] and with more than one year of follow-up for some, eight of the nine patients were doing well and living in a normal environment [17].

2. Safety concerns

While successes were reported in children with SCID-X1, a major safety issue was derailing another gene therapy trial. In September 1999, four days after receiving gene therapy with an adenovirus vector infused into his liver, an 18-year-old boy died [18]. He was suffering from ornithine transcarbamylase deficiency (OTCD), an inherited liver disease which causes ammonia build up in the blood. The boy died of multiple-organ failure attributed to an immune reaction to the adenovirus.

The case, widely publicized [19], alarmed the public and many medical institutions as, at that time, 30% of all gene therapy studies were using adenovirus vectors [20]. The FDA put the OTCD trial on hold and halted two other trials that infused adenoviruses into patients' liver [21].

With more information about the death of this patient, serious breaches of good clinical practices (GCPs) were highlighted. They included a failure to report to the FDA and to the Recombinant DNA Advisory Committee (RAC) a change in the way the virus was to be delivered. The RAC was established in 1974 by the National Institute of Health (NIH) to address public concerns regarding the safety of genetic material. The RAC reviews human gene transfer research for institutions receiving NIH funding [22]. Even more troubling, patient volunteers who participated in the OTCD program before the boy's death–but who were given lower doses of virus–suffered significant liver toxicity that was not reported, as mandated, to the FDA. With this critical information, the FDA could have prevented this tragedy [23].

These 'dark days' for the field raised multiple scientific and regulatory issues [19].

Also troubling was a new set of safety problems, this time developing in boys with SCID-X1, the very same boys that were previously considered possibly cured from their disease [17]. During the follow up of the previously mentioned study [16], initially two [24,25] and then four of 10 children in this French trial [26] and 1 of 10 children from a similar trial in the UK [27] developed a secondary leukemia. So much hope was raised that this was immensely disappointing. Luckily, the academic teams did not give up and worked harder to understand what happened. The leukemic T-cell clones from boys showed integration of the replacement gene near the LMO2 T cell oncogene [24]. The growth advantage of the gene-corrected T cells combined with the activation of LMO2 explained the leukemia. Retroviral vectors, while integrating randomly into the host genome, show a preference for transcriptionally active genes and contain sequences that are prone to activating nearby genes [26]. It was thought that the likelihood of such an event could be reduced [28,29]. However, a similar insertion of a retroviral vector near the EVI1-MDS1 proto-oncogenes led to the clonal expansion of myeloid cells in two patients with chronic granulomatous disease (CGD) [30]. The level of marked neutrophil rose because of oligoclonal outgrowth of transduced cells with vector inserted in the proto-oncogene. After 2 years, both patients developed myelodysplastic syndromes, with one requiring BMT and the other dying of sepsis [31].

3. Recent advances

Despite these issues, clinical trials continued unabated. Between 1990 and 2007, more than 1500 studies utilizing viral and non-viral vectors were approved [32]. Since 2007 approximately 100 are approved each year [33]. We report here on the most significant developments (Table 1).

3.1. SCIDs and murine γ retroviral vectors

For boys enrolled in the SCID-X1 study, after eleven years of follow-up, data were encouraging. While leukemia developed within 2 to 5 years in 5 children with one dying as a consequence, 18 of 20 treated boys were alive. The immunodeficiency was corrected in 17 and, for most, the correction of the T cell immunodeficiency was nearly complete, notably in four of the five boys who underwent chemotherapy for secondary leukemia [34].

Unlike SCID-X1 and GCD, the efficacy and safety in ADA-SCID are remarkable. ADA-SCID is a fatal autosomal recessive form of SCID characterized by impaired immunity, recurrent infections and failure to thrive. Because of the ADA deficiency toxic levels of purine metabolites accumulate and cause hepatic, skeletal, and neurologic problems. While a hematopoietic stem-cell transplant from an HLA-identical sibling is the treatment of choice, it is only available to few [35]. Enzyme

Table 1
Recent important clinical progresses with gene therapy for monogenic disorders.

Name	*Gene*/protein	Vector	Delivery	Indication	Results
Glybera®	Lipoprotein lipase	AAV1	Intra-muscular	LPD, approved EU	Decreased pancreatitis. No major safety issue.
NA	Adenosine deaminase	Murine retrovirus	HSC infusion	ADA-SCID	Immunodeficiency corrected. No major safety issue.
NA	γc	Murine retrovirus	HSC infusion	SCID-X1	Immunodeficiency corrected. Safety: secondary leukemia.
Lenti-D™	*ABCD1*	Lentivirus	HSC infusion	X-ALD	Disease stabilized. No major safety issue.
NA	*RPE65*	AAV2	Subretinal injection	Leber's amaurosis	Vision improved. No major safety issue.
Lentiglobin®	β-Globin	Lentivirus	HSC infusion	β-Thalassemia	Anemia corrected. No major safety issue.

AAV: adeno-associated virus; LPD: lipoprotein-lipase deficiency; NA: not applicable; HSC: hematopoietic stem cells; ADA-SCID: Severe combined immunodeficiency due to adenosine deaminase deficiency; SCID-X1: X-linked severe combined immunodeficiency; X-ALD: X-linked adrenoleukodystrophy.