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THE DEVELOPMENT OF A SAFE AND OPTIMIZED GENE THERAPY FOR HUMAN DISEASES

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ABSTRACT

Gene therapy is the therapeutic delivery of a gene or nucleic acid into a patient's cells to cure or alleviate the symptoms of a disease that was caused by genetic malfunction, either gain- of loss-of function. Throughout the years, gene therapy has been faced with fluctuations of development before reaching its current stage. In the early stage, gene therapy was concerned to possess several problems such as toxicity, mutagenesis, and adverse immune responses which would harm the patients, instead of benefiting them. Fortunately, gene therapy has currently reached the phase where its administration can be performed in a safe, controllable manner with a good tolerability and excellent therapeutic effect. This review will recite the development of gene therapy research, highlight the vector-related safety issues, and discuss the latest updates in recent clinical trials with promising results in correcting gene defects in the cell, reducing the symptoms of the disease, as well as improving the patient's quality of life.

Keywords: ***gene therapy, clinical trial, genetic disease, safety***

Introduction

The potency of gene therapy to cure genetic diseases has been exclaimed throughout its developmental history, intertwined with optimisms of the researchers, financial supports from the stakeholders, as well as hope and wishes from the patients and their families. And that was not because of no reason. Gene therapy holds the key to treat virtually all diseases right at their genetic roots. They range from the maliciously famous cancer—which has obvious genetic bases—to infectious diseases such as AIDS and malaria. As long as a condition involves human beings, it can be handled nicely—or at least theoretically—by gene therapy. The currently incurable diseases, once the genetic basis is known, will be a nice target of gene therapy. On the parallel side of the field, the development of sequencing technology is rapidly expanding, making it easier to spot even a single polymorphism responsible for any condition reported. The bioinformatics—database of transcriptomes, metabolomes, and signaling pathways are also expanding exponentially, faster than ever happened in history. The next step is intuitively predictable: a genetic-based intervention will be designed, personalized to the patients, and delivered right into the Achilles' heels of the disease. Or will it?

Early development of gene therapy

Even during its conception stage, gene therapy had already being criticized by a wave of skepticism and opposition at that time, mostly questioning its safety and ethical appropriateness (Anderson, 1984; Fletcher, 1983), pushing gene therapy researchers to strictly design the protocol. In 1989, Rosenberg *et al* published about the first gene therapy trial in human (Rosenberg et al., 1990), and since then, the number of clinical trials had been increasing, reaching 1.340 in 2004 and 2.600 in

2018 (Edelstein, Abedi, & Wixon, 2007; Ginn, Alexander, Edelstein, Abedi, & Wixon, 2013; Ginn, Amaya, Alexander, Edelstein, & Abedi, 2018). This seemingly increasing number was not without setbacks. In 1999, a fatal case of gene therapy had severely blown the field, although the mortality was caused by an unanticipated idiopathic immune response that was fulminant against the adenovirus vector (Raper et al., 2003). The failure was made more severe because the patient actually never needed the gene therapy to survive, because such condition of ornithine transcarbamylase deficiency can be well-managed with diet and supplementations.

Nevertheless, by learning from this failure, researchers have become more aware that human trials cannot solely rely on safety data from animal research (Hackam & Redelmeier, 2006), and thus, a more careful patient selection is mandatory. The importance of patient selection was actually realized and published some years prior to the fatal case (Morsy et al., 1996). However, ironically, this publication emphasized the importance of selection mainly to increase the success rate, not the safety (Morsy et al., 1996). A personalized approach to characterize potential recipients for gene therapy is critical, because when it comes to clinical trials, one "unexpected result" will not just affect the error bars but may halt the entire research community.

Despite this unfortunate case, the field kept progressing, and as the potential adverse effects are being addressed and tackled, we are now anticipating more encouraging reports from ongoing clinical trials and basic biomedical research.

Safety issues seem to be caused more by the vector than the genes

Basic biomedical researchers who first developed the therapy in pre-clinical studies may already be very familiar with the issue of vector toxicity—which costs a lot of additional bench works and troubleshooting. This phenomenon, arguably, extends into clinical trials (Somia & Verma, 2000). To support this argument, a summary of the most recent (as per February 2019) and earlier human trials is displayed in Table 1. This list is not exhaustive but sufficient to capture the progress of the research (for more reviews, see Edelstein et al., 2007; Ginn et al., 2013; Naldini, 2015). Earlier trials mostly utilized retrovirus and adenovirus as vectors. The main adverse effect of these viruses are insertional mutagenesis and induction of strong immune response (Thomas, Ehrhardt, & Kay, 2003), which contributed to the most famous cases of adverse effects reported: the fatal case of gene therapy administration due to immune response against adenovirus vector, and the iatrogenic leukemia in SCID trial due to insertional mutagenesis (Hacein-Bey-Abina et al., 2003; Raper et al., 2003). It is important to note that among the peer subjects in the same arm of trial, improvements were achieved, and these fatal events did not occur, suggesting that the genes might exert an excellent degree of rescue *generically* without any direct adverse effect. The vector, on the other hand, would be prone to idiopathic response from the individuals. Interestingly, from Table 1, a decrease in adverse events is observed in most recent trials, mostly because of the vector selection.

Recent development of vector engineering has led to the introduction of safer vectors, such as nanoparticles and adeno-associated viruses (AAVs). In fact, most of the successful trials reported in 2017 have utilized AAVs as their vectors, all with no fatal case, at least until the time of the publication (table 1, refs. George et al., 2017; Mendell et al., 2017; Rangarajan et al., 2017). In addition to that, the first approved human gene therapy worldwide (Glybera®) is also AAV-based which contains lipoprotein lipase gene. This encouraging phenomenon can be attributed to the safe properties of AAVs, as the immune response against them is rarely to be fulminant and the transduced gene would stay episomal, thus minimizing the risk of insertional mutagenesis. However, it is notable that Nault et al (2015) have found a trace of AAV2 genome in hepatocarcinoma (HC) cells that may contribute to the initiation or progression of the disease. A direct causal link between AAV infection and HC

cannot be drawn conclusively, since it is only found in 11 of 193 patients unrelated to AAV-based gene therapy. The using of nanoparticles as vectors are also yielding encouraging results. *CTFR*-containing liposome was administered by nebulization to cystic fibrosis patients with a marked improvement in forced-expiratory volume after 1 second (FEV1) (Alton et al., 2016; Alton et al., 2015) Inhalation administration warrants a routine administration (in this case, monthly), which is safe, without any accumulation of toxic metabolites or induction of destructive immune response.

Last but not least, systemic injection of gene therapy would demand more parameters to be considered compared to local administration. A successful case of gene therapy towards Leber congenital amaurosis (an inherited retinal disease) without any systemic effect can be attributed to the closed compartmental nature of the retina, in addition to the vector chosen for the therapy (AAV) (Jacobson et al., 2012). The same also applies for the inhalation of CTRF-containing nanoparticles mentioned above, which had no adverse systemic effect, partly due to its local application (Alton et al., 2016).

Treatment for incurable diseases and restoration of the quality of life

For hemophilia patients, a lifelong supplementation of blood clotting factors is essential, in addition to the constant threat of spontaneous bleeding, decrease of disability-adjusted life years, and high cost for disease management (Henrard et al., 2014; Siddiqi, Ebrahim, Soucie, Parker, & Atrash, 2010). Conventional therapy is by infusion of clotting factor VIII or IX for hemophilia A and B, respectively, which demands a high-level, lifelong medical intervention, let alone the limitations of physical activity burdened by the patients (Mannucci, 2003). Before the successful cloning of factor VIII and factor IX in 1980s, the source of clotting factors were from human donor, which increased the risk of transfusion-related complications for the recipients, including a higher risk of HIV infection (White, McMillan, Kingdon, & Shoemaker, 1989). After a successful cloning, recombinant clotting factors were manufactured from various cell lines, enabling the vast-scale production of clotting factors while minimizing the risk of transfusion-related infections (Pier Mannuccio Mannucci, Mancuso, & Santagostino, 2012; White et al., 1989). This success, although had revolutionized the hemophilia treatment, was not enough, because the life quality of the patients is still low due to the adherence required. The ideal therapy would release the patients to have a normal life and discontinue the painful recurring treatments. And that was where gene therapy came into the stage. The first clinical trial was first conducted in 1998, again using retrovirus as vector with a limited response and a high risk (Powell & et al, 2001). Subsequently, at least 5 trials were held with variable result and adverse effects, Mannucci, 2003) before it reached the consistent success rate and almost zero side effect as echoed recently: “a cure for hemophilia within reach” (van den Berg, 2017).

The same hope was also delivered to many other patients. For severe-combined immunodeficiency syndrome (SCID) patients, the life expectancy is less than 2 year without any treatment (Buckley, 2004). Children with spinal muscular atrophy (SMN) type 1 usually need mechanical ventilator and rarely reach beyond 2 years of age (Bharucha-Goebel & Kaufmann, 2017). Both groups can now benefit from a single injection dose of gene therapy with almost 100% success rate. And these are only a small fraction of incurable genetic diseases suffered by real people worldwide. By the success of gene therapy trials reported this year, not only the patients’ life expectancy can be lengthened, their life function is also improvable. It is interesting to note that in more recent clinical trials, the assessed positive parameters included a functional score of life quality, rather than merely laboratory values of the protein of interests. A successful gene therapy will bring the patients out of lifelong dependency into an autonomous individual that can maintain their homeostatic function independently.

Concluding remarks

This review does not cover extensively the successful clinical trials for sickle cell anemia, thalassemia major, lymphoma (Kumar, Markusic, Biswas, High, & Herzog, 2016), nor the potential future for genome-editing therapy (Gabriel, von Kalle, & Schmidt, 2015; Supit, 2017) which will broaden the scope of gene therapy for the benefits of humanity. As more genes are being identified and their ontologies are being refined, future direction of gene therapy would be more interesting, sophisticated, yet euphorically challenging. The criticisms around gene therapy that we are concerned about are actually the potential adverse effects that we are afraid we cannot control. However, our knowledge and technique are advancing rapidly to address these issues. Once the delivery has been optimized, a secure and firm scientific basis that this gene will be delivered righteously will be established to cure diseases from their roots. ***

Table 1. Summary of most recent and most early clinical trials in gene therapy

Disease	Strategy	Vector and methods	Number of patients	Follow-up (months)	Outcome	Adverse effects	Year	Ref.
Recent clinical trials								
Choroidemia	Provoding *CHM* gene	AAV2-REP1-WPRE, subretinal administration intraoperative, high dose	6	24	Sustained improvement of best-corrected visual acuity	No major systemic side effects, adverse effects due to the surgery instead of the gene therapy	2019	(Lam et al., 2019)
Hemophilia A	Providing factor VIII gene	AAV5-hfVIII-SQ, single intravenous dose, liver-specific promoter, three dose groups (low, intermediate, high)	9	12	Dose-dependent-increase of fVIII plasma level, 7 out of 9 reached normal value; 94% decrease of bleeding incident; no more FVIII infusion needed after 22 weeks;	No major side effects; mild elevation of liver enzymes, recovered by prednisone treatment	2017	(Rangarajan et al., 2017)
Hemophilia B	Providing factor IX gene	AAV-fIX-R338L, single intravenous dose, liver-specific promoter	10	Range: 7-20	Increase of fIX plasma level, no spontaneous bleeding in 9/10 patients, no fIX infusion needed anymore in 8/10 patients.	No major side effects; mild elevation of liver enzymes, recovered by prednisone treatment	2017	(George et al., 2017)
Spinal muscular athropy	Providing *SMN1* gene	AAV9-SMN, single intravenous dose, two dose group (high vs low)	15	20	100% survival rate (vs 8% in historical cohort), increase in a neuromuscular assessment score, "11 sat unassisted, 9 rolled over, 11 fed orally and could speak, and 2 walked independently".	No major side effects; mild elevation of liver enzymes, recovered by prednisone treatment	2017	(Mendell et al., 2017)
Cerebral adrenoleuko-dystrophy	Providing *ALD* gene	Lenti-D-ABCD1, ex-vivo gene transfer into CD34+ cells	17	24	Expression of ALD protein; 15/17 patients alive and functional, minimal clinical manifestation; no clonal-outgrowth.	No major side effects; 1 not-surviving patient withdrew from the study, and 1 died due to the progression of the disease.	2017	(Eichler et al., 2017)
Cystic fibrosis	Providing *CTFR* gene	CTFR-containing liposome, monthly inhalation of nebulized particles	54 placebo vs 62 treatment	12	Increase in functional spirometric tests, no improvement in the quality of life.	No side effect attributable to the therapy.	2016	(Alton et al., 2016)

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Earlier clinical trials								
Severe combined immunodeficiency	Providing adenosine deaminase gene (*ADA*) gene	Retrovirus containing *ADA* gene, ex-vivo gene transfer into CD34+ cells	10	Range: 16-96	Zero mortality after 4 years, ADA expression in myeloid and lymphoid cells; 8/10 discontinued enzyme-replacement therapy, normal life function.	Prolonged neutropenia, hypertension, infection of catheterization site, EBV reactivation, autoimmune hepatitis.	2009	(Aiuti et al., 2009)
Severe combined immunodeficiency	Providing γc cytokine receptor	Retrovirus containing γc cytokine receptor, ex-vivo gene transfer into CD34+ cells	9	30	Appearance of transduced T-cells and NK cells within 4 months, eradication of infections, normal life function.	No adverse effect at 2.5 years. However, after 3.6 years, a lymphocytosis due to clonal expansion was detected. Insertional mutagenesis was suspected.	2002, 2009	(Bordignon et al., 1995; Hacein-Bey-Abina et al., 2002, 2003)
Lipoprotein lipase deficiency	Providing *LPL* gene	AAV1-LPL^{S447X} intravenous infusion	14	24	Reduction of plasma triglyceride	No "emerging safety concerns". This is the first approved gene therapy to be on the market. (Ylä-Herttuala, 2012)	2013	(Gaudet et al., 2013)

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