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

1,2 Alva S. A. Supit, 3 Linda M. Tompodung
1 Department of Public Health, Manado State University, Tondano, Indonesia
2 Department of Biomedical Sciences, City University of Hong Kong
3 Faculty of Medicine, Sam Ratulangi University, Manado, Indonesia
e-mail correspondence: alva.supit@unima.ac.id

Submitted: February 2019; Revision: April 2019; Accepted: December 2019

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 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 peers 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 CTFR-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                          | Providing 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 atrophy              | 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   | (Mendel et al., 2017)  |
| Cerebral adrenoleukodystrophy        | 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 | Increase in functional spirometric tests, no improvement in the quality of life.             | No side effect attributable to the therapy.                                     | 2016   | (Alton et al., 2016)  |
### Earlier clinical trials

| Condition                          | Treatment Details                                                                 | Duration | Results                                                                                      | References                                      |
|------------------------------------|-----------------------------------------------------------------------------------|----------|--------------------------------------------------------------------------------------------|------------------------------------------------|
| **Severe combined immunodeficiency** | Providing adenosine deaminase gene (ADA) gene, Retrovirus containing ADA gene, ex-vivo gene transfer into CD34+ cells | 10       | Zero mortality after 4 years, ADA expression in myeloid and lymphoid cells; 8/10 discontinued enzyme-replacement therapy, normal life function. | (Aiuti et al., 2009)                           |
| **Severe combined immunodeficiency** | Providing γc cytokine receptor, Retrovirus containing γc cytokine receptor, ex-vivo gene transfer into CD34+ cells | 9        | Appearance of transduced T-cells and NK cells within 4 months, eradication of infections, normal life function. | (Bordignon et al., 1995; Hacein-Bey-Abina et al., 2002, 2003) |
| **Lipoprotein lipase deficiency**  | Providing LPL gene, AAV1-LPL<sup>S447X</sup> intravenous infusion | 14       | Reduction of plasma triglyceride                                                              | (Gaudet et al., 2013)                           |
References

Aiuti, A., Cattaneo, F., Galimberti, S., Benninghoff, U., Cassani, B., Callegaro, L., ... Roncarolo, M.-G. (2009). Gene Therapy for Immunodeficiency Due to Adenosine Deaminase Deficiency. New England Journal of Medicine, 360(5), 447–458. http://doi.org/10.1056/NEJMoa0805817

Alton, E. W., Armstrong, D. K., Ashby, D., Bayfield, K. J., Bilton, D., Bloomfield, E. V., ... Consortium. (2016). A randomised, double-blind, placebo-controlled trial of repeated nebulisation of non-viral cystic fibrosis transmembrane conductance regulator (CFTR) gene therapy in patients with cystic fibrosis. A randomised, double-blind, placebo-controlled trial of repeated nebulisation of non-viral cystic fibrosis transmembrane conductance regulator (CFTR) gene therapy in patients with cystic fibrosis. NIHR Journals Library. http://doi.org/10.3310/EME03050

Alton, E. W. F. W., Armstrong, D. K., Ashby, D., Bayfield, K. J., Bilton, D., Bloomfield, E. V., ... UK Cystic Fibrosis Gene Therapy Consortium. (2015). Randomised nebulisation of non-viral CFTR gene therapy in patients with cystic fibrosis, randomised, double-blind, placebo-controlled, phase 2b trial. The Lancet Respiratory Medicine, 3(9), 684–691. http://doi.org/10.1016/S2213-2600(15)00245-3

Anderson, W. F. (1984). Prospects for human gene therapy. Science (New York, N.Y.), 226(4673), 401–9. http://doi.org/10.1126/SCIENCE.6093246

Bharucha-Goebel, D., & Kaufmann, P. (2017). Treatment Advances in Spinal Muscular Atrophy. Current Neurology and Neuroscience Reports, 17(11), 91. http://doi.org/10.1007/s11910-017-0798-y

Bordignon, C., Notarangelo, L. D., Nobili, N., Ferrari, G., Casorati, G., Panina, P., ... Fischer, A. (1995). Gene therapy in peripheral blood lymphocytes and bone marrow for ADA-immunodeficient patients. Science (New York, N.Y.), 270(5235), 470–5. http://doi.org/10.1126/science.288.5466.669

Buckley, R. H. (2004). Molecular defects in human severe combined immunodeficiency and approaches to immune reconstitution. Annual Review of Immunology, 22(1), 625–655. http://doi.org/10.1146/annurev.immunol.22.080503.153741

Edelstein, M. L., Abedi, M. R., & Wixon, J. (2007). Gene therapy clinical trials worldwide to 2007—an update. The Journal of Gene Medicine, 9(10), 833–842. http://doi.org/10.1002/jgm.1100

Eichler, F., Duncan, C., Musolino, P. L., Orchard, P. J., De Oliveira, S., Thrasher, A. J., ... Williams, D. A. (2017). Haematopoietic Stem-Cell Gene Therapy for Cerebral Adrenoleukodystrophy. New England Journal of Medicine, 377(17), 1630–1638. http://doi.org/10.1056/NEJMoa1700554

Fletcher, J. C. (1983). Moral Problems and Ethical Issues in Prospective Human Gene Therapy. Virginia Law Review, 69(3), 515. http://doi.org/10.2307/1072768

Gabriel, R., von Kalle, C., & Schmidt, M. (2015). Mapping the precision of genome editing. Nature Biotechnology, 33(2), 150–152. http://doi.org/10.1038/nbt.3142

Gaudet, D., Méthot, J., Déry, S., Brisson, D., Essiembre, C., Tremblay, G., ... van Deventer, S. (2013). Efficacy and long-term safety of alipogene tiparvovec (AAV1-LPLS447X) gene therapy for lipoprotein lipase deficiency: an open-label trial. Gene Therapy, 20(4), 361–369. http://doi.org/10.1038/gt.2012.43

George, L. A., Sullivan, S. K., Giemzas, A., Rasko, J. E. J., Samelson-Jones, B. J., Ducore, J., ... High, K. A. (2017). Hemophilia B Gene Therapy with a High-Specific-Activity Factor IX Variant. New England Journal of Medicine, 377(23), 2215–2227. http://doi.org/10.1056/NEJMoa1708538

Ginn, S. L., Alexander, I. E., Edelstein, M. L., Abedi, M. R., & Wixon, J. (2013). Gene therapy clinical trials worldwide to 2012—an update. The Journal of Gene Medicine, 15(2), 65–77. http://doi.org/10.1002/jgm.2698

Ginn, S. L., Amaya, A. K., Alexander, I. E., Edelstein, M., & Abedi, M. R. (2018). Gene therapy clinical trials worldwide to 2017: An update. The Journal of Gene Medicine, 20(5), e3015. http://doi.org/10.1002/jgm.3015

Hacein-Bey-Abina, S., Le Deist, F., Carlier, F., Bourneaud, C., Hue, C., De Villartay, J.-P., ... Cavazzana-Calvo, M. (2002). Sustained Correction of X-Linked Severe Combined Immunodeficiency by ex Vivo Gene Therapy. New England Journal of Medicine, 346(16), 1185–1193. http://doi.org/10.1056/NEJMoa012616

Hacein-Bey-Abina, S., von Kalle, C., Schmidt, M., Le Deist, F., Wulffraat, N., McIntyre, E., ... Fischer, A. (2003). A Serious Adverse Event after Successful Gene Therapy for X-Linked Severe Combined Immunodeficiency. New England Journal of Medicine, 348(3), 255–256. http://doi.org/10.1056/NEJMoa0303163480314

Hackam, D. G., & Redelmeier, D. A. (2006). Translation of Research Evidence From Animals to Humans. Jama, 296(14), 1727. http://doi.org/10.1001/jama.296.14.1731

Henrard, S., Devleesschauwer, B., Beutels, P., Callens, M., De Smet, F., Hermans, C., & Speybroeck, N. (2014). The health and economic burden of haemophilia in Belgium: a rare, expensive and challenging disease. Orphanet Journal of Rare Diseases, 9, 39. http://doi.org/10.1186/1750-1172-9-39

Jacobson, S. G., Cideciyan, A. V., Ratnakaram, R., Heon, E., Schwartz, S. B., Roman, A. J., ... Hauswirth, W. W. (2012). Gene Therapy for Leber Congenital Amaurosis Caused by RPE65 Mutations. Archives of Ophthalmology, 130(1), 9. http://doi.org/10.1001/archophthalmol.2011.298
Kumar, S. R., Markusic, D. M., Biswas, M., High, K. A., & Herzog R.W. (2016). Clinical development of gene therapy: results and lessons from recent successes. *Molecular Therapy - Methods & Clinical Development*, 3, 16034. [http://doi.org/10.1038/mtm.2016.34](http://doi.org/10.1038/mtm.2016.34)

Lam, B. L., Davis, J. L., Gregori, N. Z., MacLaren, R. E., Girach, A., Verriotto, J. D., ... Feuer, W. J. (2019). Choroideremia Gene Therapy Phase 2 Clinical Trial: 24-Month Results. *American Journal of Ophthalmology*, 197, 65–73. [http://doi.org/10.1016/j.ajo.2018.09.012](http://doi.org/10.1016/j.ajo.2018.09.012)

Mannucci, P. M. (2003). Hemophilia: treatment options in the twenty-first century. *Journal of Thrombosis and Haemostasis*, 1(7), 1349–1355. [http://doi.org/10.1046/j.1538-7836.2003.00262.x](http://doi.org/10.1046/j.1538-7836.2003.00262.x)

Mannucci, P. M., Mansuso, M. E., & Santagostino, E. (2012). How I treat Hemophilia. *Blood*, 119(18). [http://doi.org/10.1182/blood.2011182211](http://doi.org/10.1182/blood.2011182211)

Mendell, J. R., Al-Zaidy, S., Shell, R., Arnold, W. D., Rodino-Klapac, L. R., Prior, T. W., ... Kaspar, B. K. (2017). Single-Dose Gene-Replacement Therapy for Spinal Muscular Atrophy. *New England Journal of Medicine*, 377(18), 1713–1722. [http://doi.org/10.1056/NEJMo1706198](http://doi.org/10.1056/NEJMo1706198)

Moray, M. A., Zhao, J. Z., Ngo, T. T., Warman, A. W., O'Brien, W. E., Graham, F. L., & Caskey, C. T. (1996). Patient selection may affect gene therapy success. Dominant negative effects observed for ornithine transcarbamylase deficient patient following adenoviral gene transfer. *Molecular Genetics and Metabolism*, 80(1–2), 148–158. [http://doi.org/10.1016/J.MEMETAB.2003.08.016](http://doi.org/10.1016/J.MEMETAB.2003.08.016)

Rosenberg, S. A., Aebersold, P., Cornetta, K., Kasid, A., Morgan, R. A., Moen, R., ... Anderson, W. F. (1990). Gene Transfer into Humans — Immunotherapy of Patients with Advanced Melanoma, Using Tumor-Infiltrating Lymphocytes Modified by Retroviral Gene Transduction. *New England Journal of Medicine*, 323(9), 570–578. [http://doi.org/10.1056/NEJM199008303230904](http://doi.org/10.1056/NEJM199008303230904)

Siddiqi, A.-A., Ebrahim, S. H., Soucie, J. M., Parker, C. S., & Atrash, H. K. (2010). Burden of Disease Resulting from Hemophilia in the U.S. *American Journal of Preventive Medicine*, 39(4), S482–S488. [http://doi.org/10.1016/j.amepre.2009.12.016](http://doi.org/10.1016/j.amepre.2009.12.016)

Somnia, N., & Verma, I. M. (2000). Gene therapy: trials and tribulations. *Nature Reviews Genetics*, 1(2), 91–99. [http://doi.org/10.1038/35038533](http://doi.org/10.1038/35038533)

Supit, A. S. A. (2017). Improving the function of CRISPR-Cas9 for genome editing therapy: Editing the editor. *Journal Bioteknologi & Biosains Indonesia (JBBI)*, 4(1), 44. [http://doi.org/10.29122/jbbi.v4i1.2068](http://doi.org/10.29122/jbbi.v4i1.2068)

Thomas, C. E., Ehrhardt, A., & Kay, M. A. (2003). Progress and problems with the use of viral vectors for gene therapy. *Nature Reviews Genetics*, 4(S), 346–358. [http://doi.org/10.1038/nrg1066](http://doi.org/10.1038/nrg1066)

van den Berg, H. M. (2017). A Cure for Hemophilia within Reach. *New England Journal of Medicine*, NEJMe1713888. [http://doi.org/10.1056/NEJMe1713888](http://doi.org/10.1056/NEJMe1713888)

White, G. C., McMillan, C. W., Kingdon, H. S., & Shoemaker, C. B. (1989). Use of Recombinant Antihemophilic Factor in the Treatment of Two Patients with Classic Hemophilia. *New England Journal of Medicine*, 320(3), 166–170. [http://doi.org/10.1056/NEJM198901193200307](http://doi.org/10.1056/NEJM198901193200307)

Ylä-Herttuala, S. (2012). Endgame: Glybera Finally Recommended for Approval as the First Gene Therapy Drug in the European Union. *Molecular Therapy*, 20(10), 1831–1832. [http://doi.org/10.1038/MT.2012.194](http://doi.org/10.1038/MT.2012.194)