



History of gene therapy



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ABSTRACT

Two decades after the initial gene therapy trials and more than 1700 approved clinical trials worldwide we not only have gained much new information and knowledge regarding gene therapy in general, but also learned to understand the concern that has persisted in society. Despite the setbacks gene therapy has faced, success stories have increasingly emerged. Examples for these are the positive recommendation for a gene therapy product (Glybera) by the EMA for approval in the European Union and the positive trials for the treatment of ADA deficiency, SCID-X1 and adrenoleukodystrophy. Nevertheless, our knowledge continues to grow and during the course of time more safety data has become available that helps us to develop better gene therapy approaches. Also, with the increased understanding of molecular medicine, we have been able to develop more specific and efficient gene transfer vectors which are now producing clinical results.

In this review, we will take a historical view and highlight some of the milestones that had an important impact on the development of gene therapy. We will also discuss briefly the safety and ethical aspects of gene therapy and address some concerns that have been connected with gene therapy as an important therapeutic modality.

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1. Introduction

The European Medicines Agency (EMA) defines that a gene therapy medicinal product is a biological medicinal product which fulfills the following two characteristics: (a) it contains an active substance which contains or consists of a recombinant nucleic acid used in or administered to human beings with a view to regulating, repairing, replacing, adding or deleting a genetic sequence; (b) its therapeutic, prophylactic or diagnostic effect relates directly to the recombinant nucleic acid sequence it contains, or to the product of genetic expression of this sequence. Gene therapy medicinal products shall not include vaccines against infectious diseases.

Abbreviations: ADA-SCID, adenosine deaminase deficiency; cDNA, complementary DNA; CHMP, Committee on Human Medicinal Products; DHFR, dihydrofolate reductase; DNA, deoxyribonucleic acid; *E1*, adenoviral gene; *E1B 55K*, adenoviral gene; EMA, European Medicines Agency; FDA, US Food and Drug Administration; GCV, Ganciclovir; GMP, Good manufacturing practise; HAT medium, hypoxanthine and thymidine containing medium; HGPRT, hypoxanthine-guanine phosphoribosyl transferase; *HSV-TK*, Herpes simplex virus thymidine kinase; *NeoR*, Neomycin resistance gene; OTC, ornithine transcarbamylase; p53, protein 53, a tumour suppressor; PEG-ADA, polyethylene glycol adenine deaminase; RAC, Recombinant DNA Advisory Committee; RNA, ribonucleic acid; RSV, Rous sarcoma virus; SFDA, China's State Food and Drug Administration; TALEN, transcription activator-like effector nuclease; TIL, tumour-infiltrating lymphocyte; UCLA, University of California, Los Angeles; ZFN, zinc finger nucleases.

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The US Food and Drug Administration (FDA) defines gene therapy as products “that mediate their effects by transcription and/or translation of transferred genetic material and/or by integrating into the host genome and that are administered as nucleic acids, viruses, or genetically engineered microorganisms. The products may be used to modify cells in vivo or transferred to cells ex vivo prior to administration to the recipient”.

Generally, gene therapy can be categorized into two categories – germ line gene therapy and somatic gene therapy. The difference between these two approaches is that in somatic gene therapy genetic material is inserted in some target cells, but the change is not passed along to the next generation, whereas in germ line gene therapy the therapeutic or modified gene will be passed on to the next generation. This difference is of importance, since current legislation allows gene therapy only on somatic cells. Fig. 1 is highlighting some of the milestones during the history of gene therapy.

2. The transforming principle

Frederick Griffith was a British bacteriologist who focused on the epidemiology and pathology of bacterial pneumonia. In 1928, he published a report (known also as “Griffith's Experiment”), wherein he describes the transformation of a non-virulent pneumococcal type into a virulent type (Griffith, 1928). In that study he mixed living bacteria of the non-virulent R form of Type I pneumococcus with heat-inactivated bacteria of the virulent S form of Type II pneumococcus and subsequently infected mice with this mixture. To his surprise, the

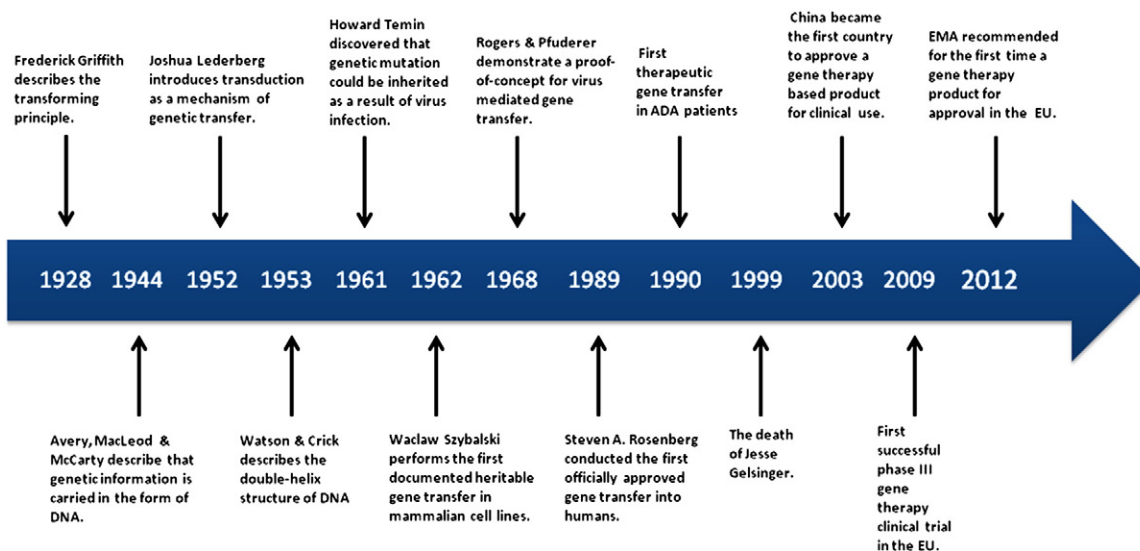


Fig. 1. Timeline highlighting some important milestones of gene therapy.

outcome was that the mice developed pneumonia infections and died. Moreover, Griffith was able to isolate colonies of the S form of Type II pneumococcus from the blood of these mice. Since the original virulent S form of Type II pneumococcus was heat-inactivated, he concluded that not only must have the R form of pneumococcus converted to the S form, but also the pneumococcal type must have transformed from Type I to Type II. This was a phenomenon never seen before. A year later, Dawson and Sia confirmed Griffith's findings and even developed a method of achieving transformation in vitro (Dawson and Sia, 1931). Soon after this, Dawson left the laboratory and the young scientist James L. Alloway continued the studies on this subject and took the pursuit one step further. What he did was to disrupt the S form of pneumococcus, thereby releasing its intracellular content and filtered it through a fine filter. Then he added this cell-free extract to growing culture of the R form of pneumococcus and what he observed was that transformation took place (Alloway, 1932). He concluded that something in the cell-free extract was responsible for the transformation of pneumococcus bacteria. This "something" he called the "transforming principle". Not knowing what it was, he performed subsequent studies and observed that it could be precipitated out of solution with alcohol (Alloway, 1933). It was only until 1941 when Avery and McCarty focused on purifying the transforming substance with the aim to identify the substance that caused transformation. Eventually, McCarty and Avery demonstrated that the transformation was caused by deoxyribonucleic acid (DNA) (Avery et al., 1944). This was in 1944 and in a time when most geneticists, including Avery himself, believed that genes must be composed of proteins. From there on scientific understanding of the molecular basis of life changed dramatically and DNA became a topic of intense research.

3. Transduction

Joshua Lederberg was a geneticist and microbiologist who received the Nobel Prize in 1958 for his work on bacterial genetics. He discovered that certain bacteria may transfer genetic material by mating (i.e. conjugation), which described another mechanism of transfer of genetic material in addition to bacterial transformation (Tatum and Lederberg, 1947). Furthermore, Lederberg uncovered a third mechanism together with Norton Zinder of genetic transfer in bacteria, termed as transduction (Zinder and Lederberg, 1952). They observed that recombination of nutritional and drug-resistant mutants of *Salmonella* with the wild type form could take place even when separated by a fine glass filter. They argued that an active "filtrate" was responsible for the transfer of hereditary traits between bacterial strains (Zinder and Lederberg, 1952). When they purified the agent, they found out that it did not

consist of pure DNA, but instead it proved to be a bacteriophage of *Salmonella typhimurium* that was responsible for carrying DNA from one bacterium to another. Zinder and Lederberg introduced the term "transduction" as to describe this mechanism. This discovery was of fundamental scientific significance as it explained how bacteria of different species could gain resistance to the same antibiotic very quickly. The basic understanding, that phages could transfer genetic materials was a phenomenon that initiated the research of its potential benefit as a tool and was soon extended also to eukaryotic viruses.

4. Hypoxanthine-guanine phosphoribosyl transferase – the first heritable gene transfer

Waclaw Szybalski had started pioneering studies on lambda phages at the McArldle Laboratory for Cancer Research, University of Wisconsin–Madison Medical School. His interest lay upon how genes are transferred, modified and regulated. Szybalski knew that cells are able to take up foreign DNA. However, no one had been successful in demonstrating heritable transformation of a biochemical trait, until 1962, when Szybalski published his study "DNA-mediated heritable transformation of a biochemical trait" (Szybalska and Szybalski, 1962). In that study, Szybalski described a technique, wherein cells that had been genetically modified could be selected based on their phenotype (Fig. 2). The basis for his concept was that cells require dihydrofolate reductase (DHFR) for the de novo synthesis of nucleic acids, particularly purine. When DHFR is inhibited the cell is left with no other option, but to use an alternate salvage pathway, which utilizes the enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT). As the name already indicates, HGPRT is a transferase that catalyzes the conversion of hypoxanthine to inosine monophosphate and guanine to guanosine monophosphate, which can be used for purine synthesis. Based on this knowledge, Szybalski established derivatives of the human bone marrow cell line D98S, whereof some were HGPRT⁽⁺⁾ and some HGPRT⁽⁻⁾. Aminopterin is a compound that inhibits de novo purine synthesis by inhibiting DHFR. In order for a cell to survive in the presence of aminopterin it needs to synthesise purines through the alternate salvage pathway. Consequently, when cells are grown in a cocktail of aminopterin, hypoxanthine and thymidine (i.e. the HAT medium), only cells which are HGPRT⁽⁺⁾ are able to synthesise DNA which is needed for the cell to survive or to proliferate (Fig. 2). What Szybalski did next is that he isolated DNA from HGPRT⁽⁺⁾ cells, which he used to transform HGPRT⁽⁻⁾ recipient cells. What he observed was that the cells did not die in the presence of the HAT medium, but he could rescue them (Szybalska and Szybalski, 1962) (Fig. 2). To put it in other words,

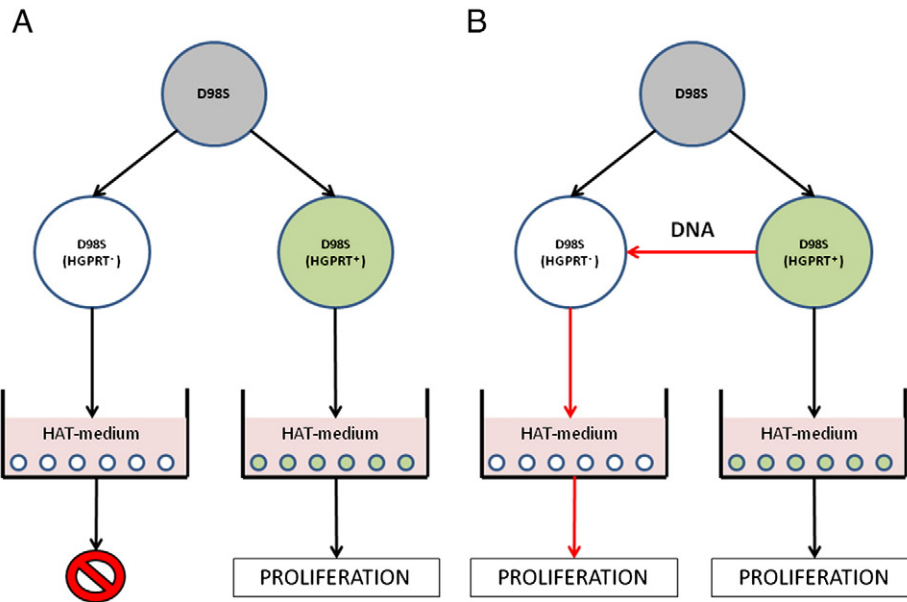


Fig. 2. Principle of the HAT-selection. Dihydrofolate reductase (DHFR) is required for the de novo synthesis of nucleic acids (essential in DNA synthesis during cell proliferation). Aminopterin on the other hand is a compound, present in the HAT-medium that inhibits de novo purine synthesis by inhibiting DHFR. Purines can be provided by the alternate salvage pathway through the enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT). However, cells lacking HGPRT activity will die in the presence of aminopterin, as they cannot synthesise DNA (A). HGPRT⁽⁻⁾ cells, however, can be rescued by isolating the DNA of HGPRT⁽⁺⁾ cells and transferring it to HGPRT⁽⁻⁾ cells (B), i.e. the HAT-selection.

Szybalski demonstrated that a genetic defect could be rescued by transferring functional DNA from another (foreign) source. Moreover, he demonstrated that the rescued gene could be inherited, as the daughter cells bore the same phenotype, as the transformed parent cells. The results of his study became the first documented evidence of heritable gene transfer in mammalian cells. A decade later the same method became key to a Nobel-winning invention describing the generation of monoclonal antibodies.

5. First steps of gene therapy

A decade after the initial finding that phages could transfer genetic material from one bacterium to another, Howard Temin discovered that in a similar fashion specific genetic mutations could be inherited as a result of virus infection (Temin, 1961). Based on his experimental observations he concluded that chicken cells infected with the Rous sarcoma virus (RSV) stably inherited viral specific gene mutations that contained the information for the generation of RSV progenies. This observation became of great significance, as it unveiled the conundrum that genetic information could flow only from DNA to RNA. As the Rous sarcoma virus is a RNA virus, Temin's study showed that information could also flow from RNA to DNA, which subsequently led to the discovery of RNA-dependent DNA polymerases. Furthermore, it was realized that the acquisition of the new characteristic was stably inherited through chromosomal insertion of the foreign genetic material (Sambrook et al., 1968).

It became apparent that viruses possessed properties that could be very useful in delivering genes into cells of interest. Accumulating evidence of successful cell transformation studies gave rise to the thought that genetic engineering may become a new approach for treating genetic diseases. In 1966, Edward Tatum published a paper evoking the effectiveness of viruses to be used in somatic-cell genetics and possibly in genetic therapy (Tatum, 1966). Of course, it was also clear that it would be necessary to strip those viruses from their pathology causing genes and replace them with a therapeutic gene or genes. Unfortunately, at that time appropriate tools for recombinant DNA technologies were not yet established. However, a couple of years after Edward Tatum's critical paper, Rogers et al. demonstrated an initial proof-of-concept of virus mediated gene transfer.

In that study, the tobacco mosaic virus was used as a vector vehicle to introduce a polyadenylate stretch to the viral RNA (Rogers and Pfuderer, 1968). Motivated by the results, they went even further and some years later they performed the first direct human gene therapy trial. In that study, the wild-type Shope papilloma virus was used with the intention to introduce the gene for arginase into two girls suffering from a urea cycle disorder (Rogers et al., 1973; Terheggen et al., 1975). They believed that the Shope papilloma virus encoded the gene for arginase activity and that the gene could be transferred by introducing the virus to the patients. Unfortunately, the outcome of the trial was negative. There was neither a change in the arginine levels, nor in the clinical course of the hyperargininemia. Later on, after sequencing of the Shope papilloma virus genome, it was revealed that the Shope papilloma virus genome actually does not encode an arginase.

In 1990, Martin Cline became the first to attempt gene therapy using recombinant DNA. Before that, Cline had already succeeded experimentally in inserting foreign genes (i.e. dihydrofolate reductase and herpes simplex virus thymidine kinase) into mouse bone marrow stem cells (Mercola et al., 1982). Furthermore, he was able to demonstrate that these modified cells were able to partially repopulate the bone marrow of other mice (Mercola et al., 1982). Encouraged by these results, Cline aimed at testing this therapeutic approach in humans. He applied to the UCLA Human Subjects committee for permission to carry out the same approach for treating patients suffering from β -thalassemia. This condition invariably results in severe and life-threatening anaemia due to a deficiency in the production of the beta-globulin portion of haemoglobin protein (due to a genetic defect/absence of the beta-globulin gene), for which the only treatment relies on frequent blood transfusion. Cline initiated the study and extracted bone marrow cells from two β -thalassemia patients. One patient was treated in Italy and one in Israel. However, he did so without having received permission to perform those studies from the UCLA Institutional Review Board. Furthermore, the Board had clear concerns about the efficacy of this therapy (Beutler, 2001; Mercola et al., 1980).

The first officially approved clinical protocol to introduce a foreign gene into humans was approved by the Recombinant DNA Advisory Committee (RAC) in December 1988. In that, no actual therapy was

proposed, but instead, S.A. Rosenberg aimed at using gene marking techniques to track the movements of tumour-infiltrating blood cells in cancer patients (Rosenberg et al., 1990). His proposal was based on previous findings demonstrating that treatment of metastatic melanoma with tumour-infiltrating lymphocytes (TILs) concomitant with interleukin-2 treatment resulted in regression of the disease in some patients (Rosenberg et al., 1988). Initially, Rosenberg tested the feasibility of genetically modified TILs by studying the distribution and possible long-term survival of TILs in the circulation, lymph nodes or at tumour sites. For that, he extracted the TILs from metastatic melanoma patients and performed retroviral gene transfer to introduce a marker gene (bacterial NeoR gene, which leads to neomycin resistance) to these cells after which he re-administered them back to the patients (Rosenberg, 1992; Rosenberg et al., 1990, 1993). The aim was to clarify, whether there is a clinical correlation between the infiltration of TILs and their effectiveness against tumours. Based on this initial trial he obtained subsequently permission to treat two patients with advanced melanoma with ex vivo modified TILs expressing tumour necrosis factor (Rosenberg, 1992). The results of this study revealed that the tumours did not grow at the injection site (Rosenberg et al., 1993). Furthermore, there was no evidence of viable tumour cells when these sites were surgically resected approximately 3 weeks after injection (Rosenberg et al., 1993).

Between the initial trial by Rosenberg (transduction of the neomycin resistance gene into TILs) and the therapeutic trial (transduction of tumour necrosis factor into TILs), Michael R. Blaese was the first to conduct a trial using a therapeutic gene (Blaese et al., 1995). In September 14th 1990 the FDA approved the first time a gene therapy trial with a therapeutic attempt in humans. Two children suffering from adenosine deaminase deficiency (ADA-SCID), a monogenetic disease leading to severe immunodeficiency, were treated with white blood cells taken from the blood of these patients and modified ex vivo to express the normal gene for making adenosine deaminase. One patient, Ashanti DeSilva, exhibited a temporary response, whereas the response in the second patient was far less (Blaese et al., 1995). However, there was a debate about the effect of Ashanti's gene therapy as she still received simultaneously enzyme replacement therapy with polyethylene glycol adenine deaminase (PEG-ADA), which she had to take alongside with the gene therapy. A little bit later ADA-SCID trial was also started in the EU (Bordignon et al., 1995) and further gene transfer trials were started for several diseases. The first gene transfer in Scandinavia was done in 1995 with the first clear results that efficient gene transfer can be achieved in human brain after direct in vivo gene delivery (Puumalainen et al., 1998).

Even though the first studies did not come up with the results that were expected, gene therapy experienced a boom, until the tragic death of Jesse Gelsinger (Stolberg, 1999). In 1999 the worst case scenario for gene therapy became a reality, when 18-year old Jesse Gelsinger took part in a gene therapy clinical trial at the University of Pennsylvania in Philadelphia. He suffered from a partial deficiency of ornithine transcarbamylase (OTC), a liver enzyme that is required for the removal of excessive nitrogen from amino acids and proteins. Gelsinger's immune system responded immediately after a very high dose adenovirus administration and he died four days later because of multiorgan failure (Stolberg, 1999). Important in this case was that he became the first patient in whom death could be directly linked to the viral vector used for the treatment.

6. Current position of gene therapy

Up to date, cancer is by far the most common disease treated by gene therapy. It composes over 60% of all ongoing clinical gene therapy trials worldwide, followed by monogenetic and cardiovascular diseases (Fig. 3).

Currently, more than 1800 approved gene therapy clinical trials worldwide have been conducted or are still ongoing. Adenoviral vectors, retroviral vectors and naked plasmid have been the most commonly used gene transfer vectors in clinical trials (Fig. 4).

In 2003, China became the first country to approve a gene therapy based product for clinical use. Gendicine™, developed by SiBiono Gene Tech Co. is an adenoviral vector, wherein the E1 gene is replaced with a human p53 cDNA. Gendicine™ is a non-replicative virus and received approval for the treatment of head- and neck squamous cell carcinoma (Peng, 2005; Wilson, 2005). Noteworthy in this case is the fact that the China's State Food and Drug Administration (SFDA) approved Gendicine without data from a standard phase III clinical trial (Xin, 2006). Consequently, soon after the approval of Gendicine™, there was discussion about the efficacy of the treatment (Guo and Xin, 2006; Xin, 2006).

Despite this, two years after approving Gendicine™, the Chinese SFDA granted approval of another gene therapy product, Oncorine™. In contrast to Gendicine™, Oncorine™ is a conditionally replicative adenovirus. It was developed by Sunway Biotech Co. Ltd and gained marketing approval in China in 2005 in combination with chemotherapy for the treatment of late-stage refractory nasopharyngeal cancer. Oncorine™ contains a deletion in E1B 55K region, which restricts the virus to bind and inactivate wild-type p53 protein (Bischoff et al., 1996). Inactivation of the host cell p53 is essential for wild-type

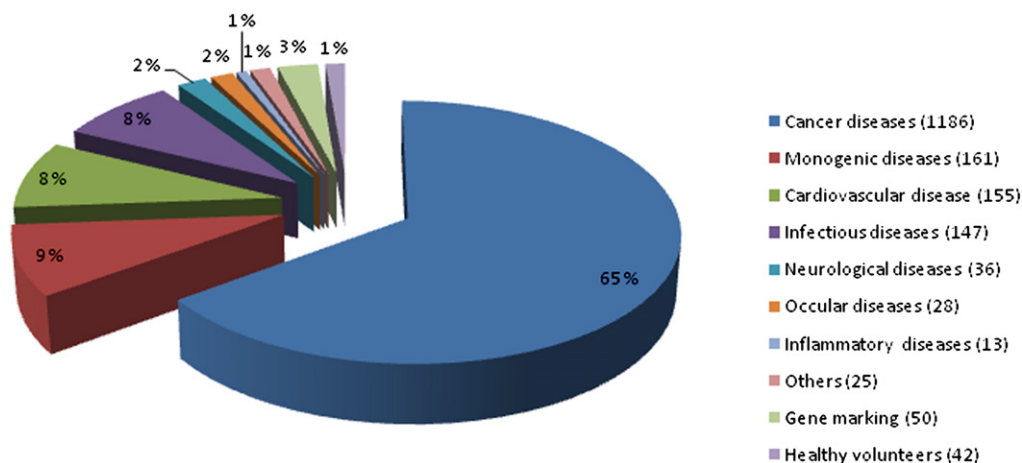


Fig. 3. Graphical presentation of different indications that have been addressed by gene therapy in clinical trials. Initial studies were on monogenetic diseases, but cancer became soon the major interest.

Source: The Journal of Gene Medicine, Wiley and Sons (<http://www.abedia.com/wiley/index.html>).

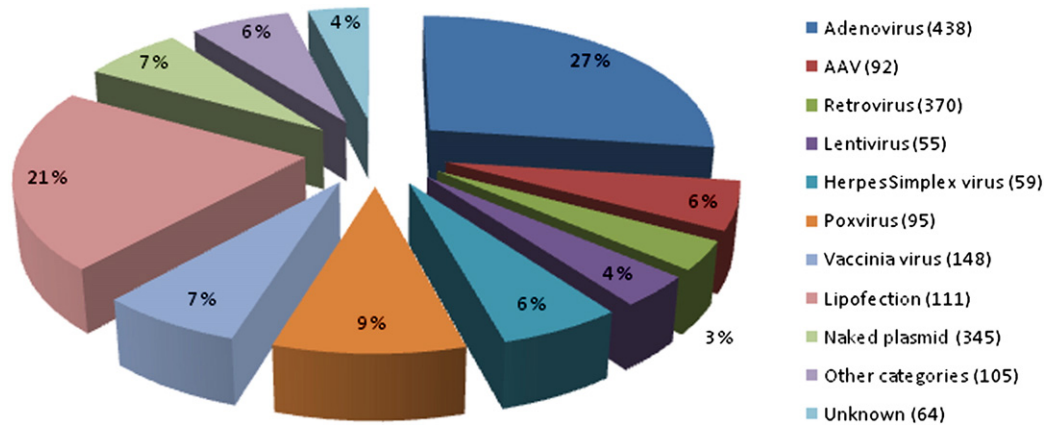


Fig. 4. Different gene transfer vectors used in clinical settings. Adenoviral, retroviral and naked plasmid/DNA have been the most commonly used gene transfer vectors. Source: The Journal of Gene Medicine, Wiley and Sons (<http://www.abedia.com/wiley/index.html>).

adenoviruses to disable the activation of apoptotic pathway when host cell shifts to S phase in the lytic infection. When E1B 55K activity is removed, the replication in normal cells is blocked, allowing only replication in p53-deficient cells. In malignant cells the viral proliferation leads to oncolysis, which is used as a cancer therapy to treat solid tumours (Fig. 5).

In 2004, Ark Therapeutics Group plc received the first commercial GMP Certification in the EU for the manufacture of commercial supplies of gene-based medicines (Cerepro®). Cerepro® is an adenoviral vector harbouring the gene for the Herpes simplex virus thymidine kinase (HSV-tk), developed by Ark Therapeutics Group plc and intended for the treatment of malignant brain tumours (Wirth et al., 2009). HSV-tk is a so called pro-drug activating enzyme that converts the nucleotide analogue Ganciclovir (GCV) to GCV-monophosphate. GCV-monophosphate is further converted by cells own kinases to GCV-diphosphate and finally to its toxic metabolite GCV-triphosphate (Moolten and Wells, 1990; Wirth et al., 2009). GCV-triphosphate is cytotoxic and results in the inhibition of the DNA polymerase thus preventing DNA replication. The clinical efficacy of Cerepro® was evaluated in two separate phase II clinical trials; a phase IIa trial, and a phase IIb trial (Immonen et al., 2004; Sandmair et al., 2000). In 2008, Cerepro® became the first and so far the only adenoviral vector that has completed a phase III clinical trial (Wirth et al., 2009). Therein, the HSV-tk adenoviral vector was

injected into the walls of the tumour cavity of glioma patients after surgical resection of the tumour (Fig. 6).

In addition, promising results have also been shown in recent gene therapy clinical trials including Leber's congenital amaurosis (Maguire et al., 2009), β -thalassemia (Cavazzana-Calvo et al., 2010; Jessup et al., 2011), X-linked severe combined immunodeficiency (SCID-X1) (Hacein-Bey-Abina et al., 2010) and ADA-SCID (Aiuti et al., 2009), haemophilia B (Jessup et al., 2011) and Wiskott-Aldrich syndrome (Boztug et al., 2010).

Finally, in July 19th 2012, the EMA recommended for the first time a gene therapy product (Glybera) for approval in the European Union. Glybera, originally developed by Amsterdam Molecular Therapeutics and now marketed by UniQure, is an adeno-associated viral vector engineered to express lipoprotein lipase in the muscle tissue for the treatment of severe lipoprotein lipase deficiency. Interestingly, Glybera had failed three times to receive a positive recommendation for approval by the Committee on Human Medicinal Products (CHMP), the institution which gives the final recommendations for marketing authorization in the EU, before the latest positive decision. This case exemplifies that gene therapy based medicines have been demanding products to develop, not only technically, but also from the regulatory perspective. A recent editorial describes some of the challenges that have existed in the regulatory process of gene therapy products (Yla-Herttuala, 2012).

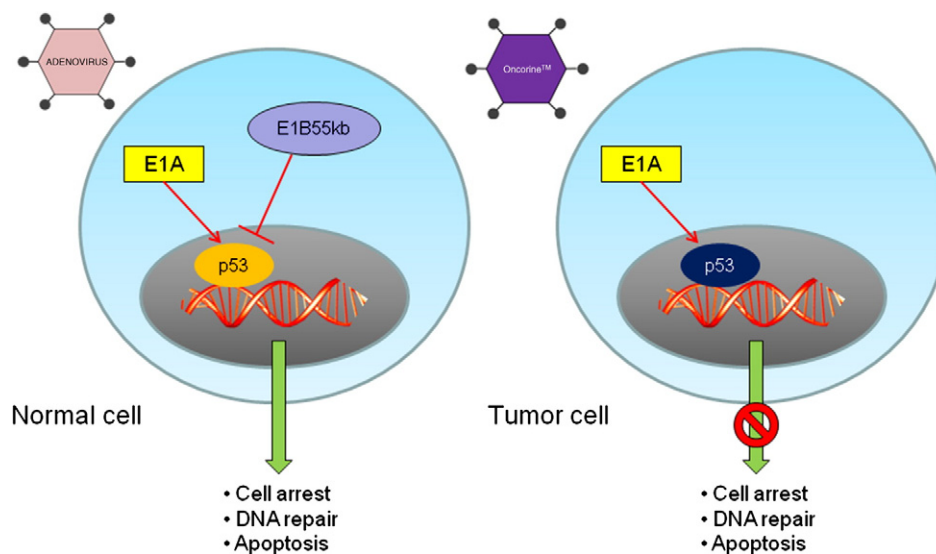


Fig. 5. Mechanism of action of Oncorine™. A deletion in E1B 55K region restricts the virus to bind and inactivate wild-type p53 protein resulting in the replication in p53-deficient cells only.

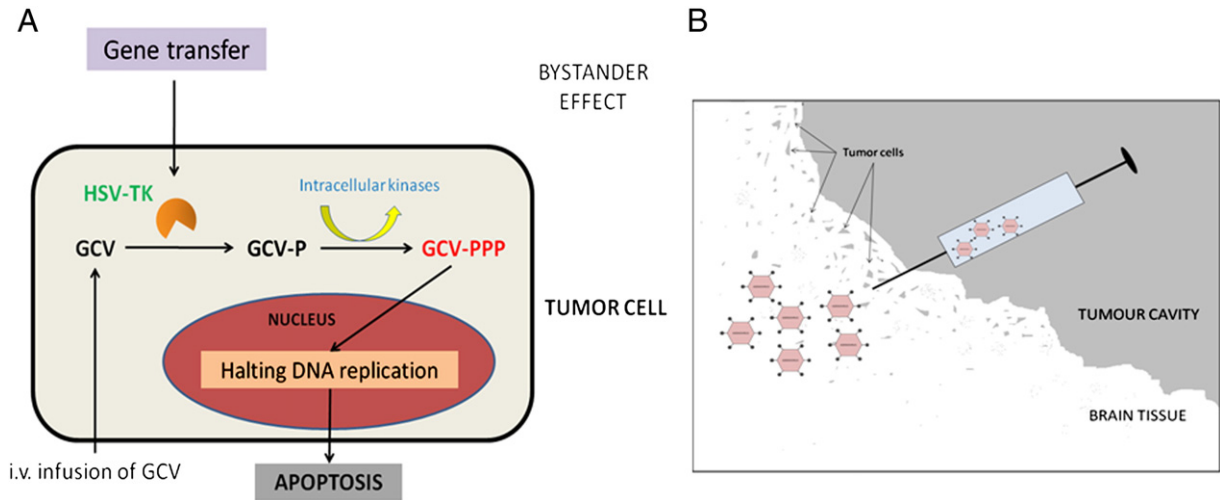


Fig. 6. (A) A schematic presentation of HSV-tk suicide gene therapy. First, the suicide gene is introduced into the target cells via local gene transfer, after which it will be expressed by the transduced cell. The second step is the administration of the pro-drug GCV, which will be first converted into a GCV-monophosphate by the HSV-tk enzyme. GCV-monophosphate again is converted by the cell's own kinases into GCV-triphosphate, which is the active toxic metabolite. Importantly, the toxic metabolite can further diffuse into neighbouring tumour cells and induce cell death. (B) Cerepro is administered into the cavity of the tumour after resection.

The 2012 approval of Glybera has demonstrated those challenges have now been overcome.

7. Safety and ethical aspects

Gene therapy is an intriguing therapeutic modality and will sooner or later be part of the standard care for a variety of different diseases. Arguable, it raises many questions, which is a clear response to uncertainty and fear towards gene therapy or its possible consequences. There are genuine concerns, regarding the safety of gene transfer in humans and potential effects of germ line approaches on offspring. Currently, legislation allows only gene therapy into somatic cells.

There are also technical issues in terms of the quality and stability of the transgene expression that has provoked concerns about the justification of gene therapy. For example, what are the technical details of the DNA and vector to be used? The technical aspects involved, risks endeavoured by the patient and the fear of human genetic engineering are some of the major reasons why human gene therapy trials have long been difficult to conduct. The use of viral gene transfer vectors, such as retroviruses has raised scepticism about their safety, as it was shown that integration of the transgene may occur in an actively expressed site, presenting a possible threat to patients. In April 2000 the journal *Science* published an article, wherein they reported the first definitive cure of X-linked severe combined immunodeficiency (SCID-X1) by gene therapy (Cavazzana-Calvo et al., 2000). Unfortunately, soon after the initial success, leukemias occurred in follow-up trials triggered by insertional mutagenesis. Also, similar problems aroused after gene therapy of chronic granulomatous disease as a result to the gene transfer vectors used (Fehse and Roeder, 2008). However, later trials, wherein integrating vectors were used did not result in insertional mutagenesis in any of the patients (Aiuti et al., 2009; Hacein-Bey-Abina et al., 2010). Nevertheless, the fear of insertional mutagenesis is still one of the major hurdles of integrating vectors, which has had implications for their use as gene delivery vehicles in the clinics (Donsante et al., 2001; Li et al., 2011). The main risks arise from the characteristics of these vectors to integrate into gene regulatory areas or into transcriptionally active areas, respectively, which potentially can adversely result in insertional mutagenesis and oncogenesis. In order to circumvent these problems, targeted integration of transgenes to predetermined genomic sites has been one of the most important topics in current vector development. One of the most efficient methods to achieve targeted integration into human cells is based on DNA double-strand break-enhanced homologous recombination (Urnov et al., 2010). The site-specific cleavage of genomic

DNA is catalysed using zinc finger nucleases (ZFNs), meganucleases or transcription activator-like effector nucleases (TALENs) (Marcaida et al., 2010; Mussolino and Cathomen, 2012; Urnov et al., 2010).

Also, adenoviruses are generally considered to be immunogenic. However, safety data of adenoviral mediated gene therapy, collected from human trials of cardiovascular diseases and malignant gliomas, where the vector has been locally administered to the target tissue, has been uniformly very good. (Hedman et al., 2009; Immonen et al., 2004; Muona et al., 2012; Wirth et al., 2006). Neutralizing antibody response to adenoviral vector administration is extremely rapid and they are rapidly cleared from the body. Consequently, there are less data about the long-term safety of adenoviral vectors in humans. Nevertheless, there are several meta-analyses that demonstrate adenoviruses have an adequate safety profile in humans. (Hedman et al., 2009; Muona et al., 2012). In terms of safety, one of the main arguments against human gene therapy is the risk of uncontrolled genetic changes produced in an individual by gene therapy, which in worst case would be passed also onto the offspring. The fact, that other therapies including many approved and extensively used agents also can cause genetic alterations is often disregarded. For example, many different mutagenic drugs (e.g. those often used in cancer treatment), as well as radiation therapy may cause genetic alterations and if this mutation happens in germline, it will be passed onto future generations.

Notably, because of the mode of action of gene therapy products, there have been genuine ethical concerns regarding the use of gene therapy products for the treatment of human diseases, which are also discussed in the medical fields (Friedmann, 2000). Already 1972, close to 20 years before the first human gene therapy clinical trials, Theodore Friedman proposed that a complete set of ethicoscientific criteria should be implemented that would guide the development and clinical application of gene therapy techniques in the future (Friedmann and Roblin, 1972). Understandably, gene therapy readily triggers the emotions in humans, which is exemplified by the studies of Rogers and Cline (Beutler, 2001; Rogers et al., 1973). What actually happens when things go wrong? The study of Cline initiated passionate discussions about the ethical aspects and rationality of gene therapy (Beutler, 2001). It is obvious that human gene therapy as a treatment modality has been more complex than expected. We do live in a global world and it is important that we acknowledge and value the differences of human beings in respect to beliefs and concerns when it comes to gene therapy. Transparency is key to the acceptance of gene therapy. Information and the knowledge resulting from it should be accessible. We have to allow ourselves to be asked difficult questions,

whether it is concerning the safety of gene therapy or simply its justification. Do we have enough knowledge to make the right decisions? Are we able to control gene therapy? Are there situations, when gene therapy is ethically more acceptable? What are the costs for this type of therapy and who is paying for it? Obviously, somatic gene therapy appears to be more accepted than germline gene therapy. Also, gene therapy seems to be more acceptable for the treatment of deadly diseases (e.g. cancer or SCID) than using it for example for the treatment of mental disorders. We could argue whether gene therapy on people with Down's syndrome is ethically acceptable? If yes, what would be the justification for it? Moreover, what would be the justification, for example, of using gene therapy in the enhancement of some individual physical or mental properties? Gene therapy based products are most likely going to be expensive whilst the trials burden remains high. What is the socioeconomic impact of gene therapy in our society? Will it initially be available only for those who can pay for it? Without doubt, the ethical aspects regarding gene therapy need to be addressed the same way as the question about their safety.

8. Concluding remarks

Currently, the first gene based products have entered the market and it is very likely that gene therapy will find its place in specific areas of clinical medicine where there is unmet need. We believe that the development of gene medicine products should emphasize the importance of appropriate pre-clinical models, include the use of bigger, non-rodent, animal models that would support the evaluation of the efficacy and safety of gene therapy products. Furthermore, we need to acknowledge the fear of some patients in respect to gene therapy. Ultimately it is the patient who decides, whether she/he wants to be treated with gene therapy.

Conflict of interests

No conflict of interests to declare.

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