A short perspective on gene therapy: Clinical experience on gene therapy of glioblastoma multiforme

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Abstract
More than two decades have passed since the first gene therapy clinical trial was conducted. During this time, we have gained much knowledge regarding gene therapy in general, but also learned to understand the fear that persists in society. We have experienced drawbacks and successes. More than 1700 clinical trials have been conducted where gene therapy is used as a means for therapy. In the very first trial, patients with advanced melanoma were treated with tumor infiltrating lymphocytes genetically modified ex-vivo to express tumor necrosis factor. Around the same time the first gene therapy trial was conducted, the ethical aspects of performing gene therapy on humans was intensively discussed. What are the risks involved with gene therapy? Can we control the technology? What is ethically acceptable and what are the indications gene therapy can be used for? Initially, gene therapy was thought to be implemented mainly for the treatment of monogenetic diseases, such as adenosine deaminase deficiency. However, other therapeutic areas have become of interest and currently cancer is the most studied therapeutic area for gene therapy based medicines. In this review I will be giving a short introduction into gene therapy and will direct the discussion to where we should go from here. Furthermore, I will focus on the use of the Herpes simplex virus-thymidine kinase for gene therapy of malignant gliomas and highlight the efficacy of gene therapy for the treatment of malignant gliomas, but other strategies will also be mentioned.

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Key words: Gene therapy; Glioblastoma multiforme; Herpes simplex virus - thymidine kinase

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GENE THERAPY
Gene therapy has experienced several ups and downs during the last few decades. The last one was particularly troublesome as many promising therapies have failed in clinical trials. However, with the increasing knowledge of epigenetics and their impact in many diseases, such as cancer and atherosclerosis, the potential of gene therapy has regained attention. Also, with the introduction of safer and more specific gene transfer vectors, the fear of gene therapy has been released, at least to some extent. According to the European Medicines Agency (EMA), a gene therapy medicinal product means “a biological medicinal product that contains an active substance which contains or consists of a recombinant nucleic acid used in or administered to human beings with a view of regulating, replacing, adding or deleting a genetic sequence,
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as well as if its therapeutic, prophylactic or diagnostic effect relates to the recombinant nucleic acid sequence it contains, or to the products of genetic expression of this sequence[9]. Gene therapy can be categorized into (1) germ line gene therapy and (2) somatic gene therapy. In somatic gene therapy, the genetic material is not passed along to the next generation, whereas in germ line gene therapy it is. This difference is of importance as to date, gene therapy is only allowed on somatic cells. Gene therapy to somatic cells is prohibited.

There are several strategies how foreign genetic material (i.e. a transgene) can be introduced to the patient. Firstly, the transgene can be introduced directly to patients (i.e. \textit{in vivo} gene therapy) (Figure 1)[3-8] or alternatively, the genetic material can be introduced \textit{ex vivo}[8-11]. In this case, either autologous or heterologous cells are transduced outside the patient (i.e. \textit{ex vivo}) and then administered to the patient (Figure 1). When heterologous cells are used, the cells need to be protected from the recipient’s immune system. This might be achieved by cell encapsulation of transduced cells into microparticles[12-14].

Different gene delivery methods have been used for introducing the genetic material into the cells. These methods can be categorized into physical, viral and non-viral methods. Examples of physical methods are electroporation, ultrasound and gene gun methods. In case of viral or non-viral gene delivery, a biological (a virus) or a synthetic (liposomes or nanoparticles) are used as gene carriers to deliver the genetic material into the cells. Of the viral vectors, adenoviruses are currently the most dominant gene delivery vectors used in gene therapy, followed by retroviral vectors (including lentiviral vectors) and plasmid DNA. Adeno associated viral vectors have also gained momentum recently and represent an interesting alternative to retroviral vectors. Other gene transfer vectors that are more or less commonly used are vaccinia viruses and poxviruses (Figure 2A). The use of bacteria as gene transfer vectors is not new but only recently has gained more attention.

**CURRENT STATUS**

So far, neither the Food and Drug Administration nor the EMA has approved any human gene therapy product for commercial use. Initially, the main targets for gene therapy were monogenic disorders, such as adenosine deaminase deficiency and adrenoleukodystrophy. Even though drawbacks have been reported, which is exemplified by the work of Hacei-Bey-Abina for example, there have also been successes[5,10-13]. Eventually gene therapy has shifted from these monogenetic diseases into other disease areas such as cancer and cardiovascular diseases[14-16]. Currently, cancer is the most common disease area that gene therapy is applied to. More than 60% of all ongoing gene therapy clinical trials are developed for the treatment of cancer (Figure 2B). Many cancer types have been targeted with gene therapy including tumors of the brain, lung, breast, pancreatic, liver, colorectal, prostate, bladder, head and neck, ovarian and renal cancer. The first clinical trial on cancer started in 1990, where patients with advanced melanoma were treated with tumor infiltrating lymphocytes genetically modified \textit{ex vivo} to express tumor necrosis factor[17].

In October 2003, China became the first country to approve the commercial production of a gene therapy product[21-23]. Shenzhen SiBiono GenTech (Shenzhen, China) obtained a drug license from the State Food and Drug Administration of China for its recombinant adenovirus-p53 gene therapy (Gendicine). Gendicine was approved for the treatment of head and neck squamous cell carcinoma (HNSCC). Two years later, in 2005, the conditionally replicating adenovirus H-101 (i.e. an adenovirus wherein the E1B-55 kDa gene has been deleted, allowing the virus to selectively replicate in and lyse p53-deficient cancer cells) gained marketing approval for HNSCC[24]. More recently, Rexin-G, a pathotropic targeted retroviral vector designed to interfere with cyclin GI gene by integrating into the host DNA, has recently been approved in the Philippines for the treatment of all solid tumors that are refractory to standard chemotherapy[25]. In addition, it is currently in clinical trials in the U.S. and has been granted Orphan Drug Status by the FDA for three cancer indications: (1) pancreatic cancer; (2) soft tissue sarcoma; and (3) osteosarcoma[26-28].

**GENE THERAPY FOR MALIGNANT GLIOMAS**

Brain tumors bear several features that make them amenable to gene therapy, particularly for suicide gene therapy. First of all, brain tumors are, in most cases, single, localized lesions of dividing cells in a background of non-dividing cells. Secondly, primary brain tumors rarely metastasize outside the central nervous system and if recurrence occurs, it typically happens in the close vicinity of the original lesion[18-20]. Different approaches have been utilized for the treatment of malignant gliomas using different gene transfer vectors. Among these, prodrug activation/suicide gene therapy, anti-angiogenic gene therapy, oncolytic virotherapy and immune modulation are the most commonly used strategies[21,22,34-46]. The very first clinical gene therapy trial against brain cancer was registered in 1992[47]. In that trial, autologous tumor cells were modified \textit{ex vivo} with retrovirus to express \textit{interleukin}-2 gene in neuroblastoma. In the following year, brain cancer patients were treated with herpes simplex virus thymidine kinase suicide gene therapy using retrovirus vectors producing cells and concomitant administration of ganciclovir. However, transduction efficiency was a major problem in these trials, resulting in poor therapeutic efficacy. In 1996, Eck et al[48] published the first phase I clinical trial where adenovirus Herpes simplex virus-thymidine kinase was used with intention in patients with recurrent gliomas. In 2000, Sandmair et al[49] published a study wherein 21 patients were enrolled to compare the efficacy of retrovirus-packaging cells to adenovirus mediated gene transfer. In this study,
the therapeutic efficacy of the Herpes simplex virus-thymidine kinase, using these two approaches, was compared in context of the treatment of primary or recurrent gliomas. The mean survival time in the adenovirus Herpes simplex virus-thymidine kinase group was 15 mo and significantly longer, when compared to the survival time of the retrovirus-packaging-cells group, which was 7.4 mo. The control group, which received adenovirus LacZ had a mean survival time of 8.3 mo. Although the retrovirus-packaging-cells approaches were found safe, no efficacy was observed in malignant glioma patients. The low gene transfer efficacy with retrovirus and the lack of the treatment response indicated that retroviral Herpes simplex virus-thymidine kinase gene therapy may not be efficient enough in human clinical settings. This was further confirmed by the results from the first randomized, open-label, parallel group phase III clinical trial of 248 patients, where Herpes simplex virus-thymidine kinase produced by retroviral producing cells did not result in an improvement of survival[2]. Some years later, in 2003, a phase I clinical trial described the use of an adenoviral vector encoding for the tumor suppressor gene TP53 for the treatment of patients with recurrent malignant gliomas[47]. In that study, 15 patients underwent
intratumoral stereotactic injection of the adenoviral vector via an implanted catheter, followed by en bloc resection of the tumor and treatment of the post-resection cavity. Due to the design of the study, tumor response could not be assessed but it proved to be safe, demonstrating minimal toxicity. No systemic viral dissemination was observed and a maximum tolerated dose was not reached in this study. Analysis of tumor specimens demonstrated restricted transgene expression close to the injection site. Chiocca et al. published a phase I dose-escalation trial of the oncolytic adenovirus ONYX-015, which preferentially replicates and thereby lysed p53-deficient cells (a common feature in tumor cells). In that trial, 24 patients with recurrent malignant glioma were injected with ONYX-015 with doses ranging from $10^7$ to $10^{10}$ pfu (plaque forming units) in a total of 10 injections into 10 different sites of the cavity of resected tumors. None of the patients experienced serious adverse events related to the virus. However, in that trial, the maximum tolerated dose was not reached. All patients showed tumor progression with a median time of 46 d and a median survival time of 6.2 mo. One patient with anaplastic astrocytoma had stable disease and two patients who underwent a second resection had lymphocytic and plasmacytoid cell infiltration at the site of injection. Nevertheless, despite a good safety profile, the overall therapeutic efficacy was poor. In another study performed by Chiocca et al. 11 patients were injected with different doses of interferon-β-expressing adenoviruses ranging from $2 \times 10^9$ to $2 \times 10^{11}$ viral particles stereotactically into the tumor. This was followed by surgical removal of the tumor 4-8 d later with additional injections of the adenovirus into the tumor bed. Generally, the treatment was well tolerated with only one patient experiencing a dose-limiting side effect after post-operative injection with the highest dose. However, all patients had disease progression and/or recurrence within 4 mo after the treatment. The median time to tumor progression was 9.3 wk and the median overall survival was 17.9 wk.

The clinical efficacy of sitimagene ceradenovec was evaluated first in two separate phase II clinical trials; a phase II a trial and a phase II b trial[50-53]. Sitimagene ceradenovec is an adenoviral vector encoding for the Herpes simplex virus-thymidine kinase, which is injected into the tumor cavity of resected gliomas, followed by the administration of the pro-drug ganciclovir (Figure 3). In the randomized and controlled phase II b trial published by Immonen, carried out in 36 patients, seventeen patients with operable or recurrent malignant gliomas receiving sitimagene ceradenovec implicated a survival advantage over control patients who did not receive gene therapy. The mean survival of the patients in the sitimagene ceradenovec group (70.6 wk) was significantly longer ($P < 0.00095$) when compared to the standard care group (39.0 wk) or a historical control group ($P < 0.0017$). This study was also historically the first randomized, controlled trial with sitimagene ceradenovec where increased survival of the patients was shown when compared to standard therapy. The results from the study were very encouraging and it was concluded that sitimagene ceradenovec could provide an effective adjuvant treatment for patients with operable primary or recurrent malignant glioma. Therefore a multicenter, standard care controlled, randomized clinical phase III trial was commenced. However, the results from that trial were not as significant as those from the previous II b trial. As a result, suggestions by the EMA were given for further clinical evaluation as they concluded that the data did not provide sufficient evidence of significant clinical benefit compared to current standard treatment.

**IMPROVING GENE DELIVERY**

One of the major hurdles has been how to get the relevant genetic material into a sufficient number of target cells and how to avoid the transduction of non-target cells (i.e. how to target the gene transfer vector to cells of interest). Obviously, as gene therapy has matured from clinical trials to the first commercial products, understanding of the mechanisms of gene delivery has increased notably. This is also reflected in the progress we have made in the development of viral vectors[54-58]. A number of improvements have been achieved in order to tackle issues of transduction efficiency, biodistribution and safety[55,56,57]. Ideally, a gene transfer vector should be able to efficiently and specifically transduce the target cells (dividing and non-dividing) and result in the expression of the transgene for a sufficient duration of time. The vector should not have any limitations in the transgene insertion capacity and it should be able to be manufactured easily and cost effectively in high concentrations. Furthermore, the vector should not induce any immune responses within the host, enabling repeated, safe vector administrations without adverse effects. In order to fulfill these criteria, different strategies have been exploited. However, none of the strategies are without limitations. For example, to improve gene transfer efficiency, specificity and thereby patient safety, target cells
may be removed from the patient, transduced with viral vectors and re-introduced back into the patient\cite{32,65,67}. This approach has shown to be effective, but is limited to the cells which are available (i.e. either by extraction or by growing from the stem cells in vitro\cite{58}). When the vector is delivered directly to the patient (in vivo), either locally (for example intratumoral) or systemically (i.e. into the blood circulation), a limiting factor can be the size and shape of the gene transfer vector, resulting in poor distribution within the tissue (when administered locally into the tissue) or the lack of specificity when administered systemically. To improve specificity and hence also safety of systemically administered gene delivery vectors, the surface of these vectors has been modified\cite{52,59,62}. For example, retrovirus and lentivirus have been frequently pseudotyped to widen their tropism, increase their yield in production and improve their safety, most often with the Vesicular Stomatitis virus G-protein\cite{63,64}.

CONCLUSION

Gene therapy is an intriguing therapeutic modality and sooner or later will be part of the standard care for a variety of different diseases. However, at the same time, when the first patients were treated utilizing gene therapy based technology, debates about the ethical aspects of gene therapy started\cite{66-69}. It is important that we acknowledge and understand the differences in human beings and in what they believe in. Obviously, there are concerns when it comes to the use of gene therapy. We have to ask ourselves several questions before we can justify gene therapy in humans: What is our current understanding regarding gene therapy? For example, what are the technical details of the DNA and vector to be used? The technical aspects involved, risks endeavored 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. Are we able to control this technology? In which diseases is gene therapy is ethically acceptable and what are the costs for this type of therapy? Why is gene therapy more tolerated for life-threatening diseases (e.g. diseases like cancer or AIDS) than in the correction of learning disorders? Also, somatic gene therapy appears to be more tolerated than germ line gene therapy. Where do we draw a line when dealing with genetic or chromosomal disorders? Would it be ethically acceptable to practice gene therapy on people with Dawn syndrome? What would be the justification of using gene therapy in the enhancement of some individual physical or mental properties? The use of viral vectors, such as lentiviruses and adeno-associated viruses, raises skepticism because of their ability to integrate into the genome\cite{11,65,70,71}. Understandably, this raises concerns about the safety of these vectors. This is furthermore supported by the somewhat contradictory data available in the literature\cite{52,73}. Non-viral vectors are not efficient enough yet but have gained better acceptance in the society. Despite the progress in gene therapy research, we are still at the beginning of the era of gene therapy based medicines. Emphasis should be put on the development of targeted and regulated gene transfer vectors. Especially in case of integrating vectors (such as retroviral vectors), emphasis should be laid on the development of vectors, where the integration of the transgene can be controlled, in order to avoid insertional mutagenesis. In either case, it is of utmost importance that the normal principles of good clinical research apply in the conduct of the ethical evaluation of gene therapy protocols. The safety of an individual must be the first concern of the treatment protocols and, last but not least, the integrity and free will of a patient should be respected.

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