
Gene Therapy: Hopes and Problems

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Abstract

Gene therapy (GT) is one of the most fascinating consequences of the penetration of molecular biology and genetic engineering into medicine. Originally, it was assumed that monogenic genetic diseases will be the main field of its application. However, a great majority of the GT-based clinical trials in the last decade have dealt with acquired diseases. Still, its introduction into clinical practice is associated with serious problems. The main obstacle preventing a more rapid development in the field of GT is the imperfection of the vectors presently being used for gene transfer. At the present time, GT is predominantly being used in oncology where the barriers against its employment are weaker than in other medical disciplines. Among the acquired diseases, which are now in the focus of interest of GT, are also cardiovascular diseases. A number of different GT strategies have been developed. Their choice primarily depends on the disease to be treated. In addition to technical and strategic problems, ethical issues play a significant role in planning and performance of clinical studies.

Keywords

Cell therapy • DNA • Gene therapy • Oncolytic viruses • Plasmids • Transduction • Transfection • Transgene • Vectors

Introduction

Gene therapy (GT) is a modality whose therapeutic principle is the transfer of sequences of nucleic acids. It can be defined as a transfer of genetic material, which has a therapeutic effect, either because it supplements the cell with a new or missing function or because it suppresses its abnormal, pathological function. It can also be

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Table 1 Reasons for extensive use of gene therapy in oncology

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| 1. It is easier to kill the cell than to repair it. |
| 2. Transitory expression of the transgene may be sufficient for killing the cell. |
| 3. A number of different efficient strategies are available. |
| 4. Because of the nature of the disease, the barriers against its use are weaker than in other medical disciplines. |
| 5. Oncological patients are generally willing to undergo experimental therapies. |

employed for increasing the efficacy of other therapeutic modalities and for removing disturbing symptoms of various diseases, like pain. A strong support for GT development is provided by the progress of proteomics and, especially, ever-increasing understanding of the functioning of human genome. GT is definitely one of the most important and most hopeful, but also scientifically most demanding consequence of penetration of molecular biology and genetic engineering into medicine. At the same time GT, together with cell therapy (CT), which is being developed in parallel, represent two of the most controversial therapeutic modalities of contemporary medicine. Both call forth contradictory reactions in the lay and the medical communities alike.

The original aims of GT mainly comprised the treatment of monogenic hereditary diseases, but most of the clinical trials performed till now have dealt with acquired diseases. Among these dominate oncological diseases. The reasons for this are summarized in Table 1. As concerns over the number of clinical trials registered, cardiovascular diseases are on the second place.

The present problems, which GT is facing, are considerable. They can be divided into three categories: technical, methodological (strategic), and ethical. They should be dealt with, at least most of them, before GT can be raised to the level of routine therapy. The author feels that it might be useful to define some of the terms that will be used in the text below and that may not be familiar to some of the readers. A gene, which is being transferred, is a *transgene*. The carriers used for gene transfer are *vectors*, among which

an important role is played by *plasmids*; these are small, circular genetic elements of bacterial origin. The term *transfection* describes the transfer of a foreign gene or its portion by means of the “naked” DNA or RNA. The process of transfer of a gene is called *transduction*, and the genetically modified cell is a transduced cell. The genetic material transfected frequently persists in the transduced cell in the form of *episome*, which is a circular, extrachromosomal element, replicating independently of the cell DNA in the transduced cells. *Transposons* are short segments of DNA capable of moving from one genetic location to another in a genome. They can replicate and can be integrated into random sites of the cell genome. *MicroRNAs* (*miRNAs*) are endogenous, highly conserved, short, non-protein-coding RNA molecules that mediate posttranscriptional gene expression by destabilizing target transcripts. They act by annealing to partly complementary sequences in the 3'-untranslated regions of target mRNA and thereby interfere with translation. It is assumed that miRNAs fine-tune at least 30% of protein-coding genes. Thus, miRNAs play a crucial role in the regulation of biological functions such as cell differentiation [1]. *Ribozymes* are RNA molecules, which possess sequence-specific cleavage activity. They occur naturally but can be synthesized to target-specific nucleic acid sequences.

Technical Problems

When speaking about technical problems in GT, we usually have in mind problems with vectors. Vectors are of principal importance not only for the transduction efficiency and the properties and biological behavior of the transduced cell, but also for the risks associated with GT. The present imperfection of vectors is the main hindrance to rapid progress in GT.

The properties of an ideal vector are shown in Table 2. Vectors fulfilling all these requirements are not available so far. The presently used vectors are shown in Table 3. Extraordinary efforts are being devoted to the development of vectors that would approximate the ideal set down.

Table 2 Properties of an ideal vector

1. It should be easy to prepare.
2. It should penetrate into a large number of target cells.
3. It should not be toxic either for the target cells or for other cells that might be hit unintentionally.
4. The transgene must be transferred in a transcriptionally active state.
5. It should be capable of transferring large genes.
6. The expression of transgene should be sufficiently high and must persist long enough to achieve the effect desired.
7. Its immunogenicity should be low.
8. It should not induce any serious systemic untoward reactions in the recipient.
9. Its administration should not represent any risks for the recipient's contacts.

Many investigators consider this endeavor to be the most important part of contemporary research in GT. The vectors are usually divided into non-viral and viral. The former group is nearly entirely represented by DNA in the form of non-linearized or linearized plasmids, which are introduced into the target cells by physical or chemical means or their combination. A number of different techniques have been developed for this purpose. Plasmids can be introduced into the cell directly, e.g., by microinjection, electroporation, ultrasound (s.c. sonoporation), bioballistics (by means of the gene gun), hydrodynamic method (based on an increase of intravenous pressure), or in the form of complexes. Especially popular is s.c. *lipofection*, usually based on the connection of DNA with cationic lipids. These lipoplexes fuse with the cell membrane and enter the cell. Another technique making use of the connection of anionic DNA with a variety of cationic polymers is sometimes called *polyplexion*. It is the aim of the latter manipulations to increase the stability of DNA and to facilitate the endocytosis mediated by cell receptors. The major advantage of the nonbiological methods is the relatively easy preparation of the genetic materials to be administered and their low toxicity and low immunogenicity. Among the disadvantages of the use of the plasmid-based gene transfer, the dominant are low stability in vivo, low efficiency of uptake by the target tissue, short term expression of the transgene, and a rare integration of the foreign DNA

into the cell genome. A lot of effort is being exerted to overcome the disadvantages of the present nonviral vectors. The aim is especially to increase their stability outside the cell, their internalization, modification of intracellular trafficking from endosome to lysosome, facilitation of their dissociation from the carrier, and their entry into the nucleus. A number of sophisticated approaches are being employed for this purpose [2–4]. One of the most hopeful approaches is the construction of condensed particles of size less than 100 nm. Their preparation pertains to nanotechnologies and has been made possible by the recent major progress in the field of mechanics and physics. DNA is condensed and encapsulated, making use of the electrostatic interaction between anionic phosphate groups in DNA and the cationic carrier. In this form, DNA is protected against the action of endonucleases, and the cellular uptake is increased. Coupling cell – penetrating peptides and nuclear localization signals to the particle surface – can further facilitate it. Many researchers believe that particles prepared in this way, which are designated as *synthetic virus-like particles* by some of them, might, by their properties, approximate an ideal vector, as has been defined above. Hopes are also associated with the newly introduced transposon-based vectors [5]. Since the transposons present in mammalian genome have been inactivated millions of years ago, the gene carriers used are based on a reconstruction of active elements found in fish and amphibian genomes. Two of them carrying the fairy tale names of Sleeping Beauty [6] and Frog Prince [7] are being used for gene transfer.

In most of the GT clinical studies carried out so far, viral vectors (VV) have been used. The most frequently employed VVs and their basic properties are listed in Table 4. When compared with the nonviral vectors, VVs possess three major advantages. First, their surface structures predetermine their interaction with the cell receptors and penetration into the cell. It follows that the uptake of the transgene is much higher than in the case of nonviral vectors. Second, viral genome is equipped with regulation elements easily recognizable by mammalian cells. Third, some viruses

Table 3 Vectors used

Vector	Technical demands	Efficacy of	
		Transduction	Integration
1. DNA	Low	Low	Low
2. RNA	Low	Low	-
3. Viruses	High	High	High ^a
4. Synthetic VLP ^b	High	High	?
5. Bacteria	High	High	-
6. Transposons	High	?	High

^aIn the case of some viral vectors^bVirus-like particles

(retroviruses, adeno-associated viruses (AAVs)) enable integration of the foreign genetic material into the cell genome, this ensuring a long-term expression of the transgene. On the other hand, VVs also have some disadvantages. First, the construction of VV is a challenging process practicable only in a well-advanced virus laboratory. Second, most of the viruses are strongly immunogenic. Preexisting antibodies may curtail or even block the expression of the gene delivered and the immunity developed after their administration may prevent repeated use of the same VV. Intensive investigations are under way for the preparation of VV with lowered immunogenicity. The “gutless” adenovirus can serve as an example of a significant success [8]. Third, the small size of viruses limits the size of the genetic material to be transferred. Fourth, the broad tissue tropism of some of the VVs is increasing the possibility of untoward off-target effects being induced. Therefore, considerable effort is being directed to such modification of VV as would increase their tissue specificity. There are quite a number of possible solutions [9]. One line of endeavor is to utilize specific receptors, which is attainable by adjusting the viral genome so that the requisite ligand is included in the surface structures of the virus particle. This has been termed *transductional* targeting. Another possibility is the inclusion of a promoter functional only in the target cell. Such targeting is called *transcriptional*. Fifth, there are biological risks associated with the use of VV. These risks are diminished, but not completely eliminated, by modifying the viral genetic material so as to disable the agent for replication.

This is usually achieved by deleting a portion of the virus genome [10]. Specifically genetically modified cell lines that complement the missing virus function are needed for the formation of virus particles from these defective virus particles. Such particles are capable of transferring the genetic material but are unable to replicate in the transduced cells. Still, all of the several tragic events that occurred recently (invariably cases of therapy of hereditary diseases, see below) were caused by virus particles incapable of reproducing in the cells transduced. It should be added that from the iron rule of non-infectivity of VV, two exceptions exist. The first relates to the oncolytic viruses, the effects of which are based on the replication of viruses in the tumor but not in non-tumor cells, this leading to their destruction. The other exception is represented by recombinant viruses, which are being used as experimental therapeutic vaccines.

In addition to the properties of VV shown in Table 4, they also differ by the time and duration of the expression of transgene, which influences the choice of the vector in any particular situation. In the case of adenovirus-based vectors the expression is relatively fast (within 1–4 days), which is of high advantage in situations where prompt expression is important. On the other hand, the expression of the transgene often ceases within a fortnight. If AAVs are used as vectors, the expression of transgene is not very efficient for weeks, but it may be sustained over months or even years. It should be kept in mind, however, that VVs are not the only factors responsible for the duration of transgene expression in vivo. It also depends on the tissue that is targeted and on the host response factors.

There has been an ever-increasing interest in biological vectors other than viruses [11, 12]. These include bacteria genetically modified in such a way to make them nonpathogenic without losing their capability of penetrating into the target cells to be altered. These systems, properly genetically modified, could ensure a long-term expression of transgene without the risk of potent immune reactions against the vector developing. Bactofection might also permit the regulation of the production of the protein of interest, because

Table 4 Basic properties of the viral vectors most frequently used

Virus	Titre ^a (per mL)	Stability	Maximum capacity (kb)	Risks, disadvantages
Retroviruses	10 ⁶	Low	6–7	Oncogenicity, gene silencing
Adenoviruses	>10 ¹⁰	High	7.5	Toxicity, immunogenicity
AAV ^b	>10 ¹¹	Very high	4.5	Low capacity
Herpesviruses	10 ⁸	Low	>30	Recombination ^c , activation of latent infection, immunogenicity

^aTitres easily achievable

^bAdeno-associated viruses

^cPossible recombination with wild type virus

antibiotics could abolish it. There are two other great advantages of using gene-modified bacteria for GT. Their capacity for foreign genetic material is quite large and their preparation is inexpensive. Biological vectors include also the so-called biological liposomes, which are represented by spherical fragments of erythrocytes or exosomes.

An object of extraordinary interest is the development of vectors with whose aid it would be possible to direct the transgene to a particular position in the human genome. In spite of this aspiration not having been achieved so far, there is no lack of optimism among those who work on this difficult problem, and progress is evident. The mastering of this task would change the face of contemporary GT, in particular the treatment of monogenic hereditary diseases.

Methodical (Strategic) Problems

There exist some general principles for the application of GT. They can be outlined as follows: (1) understanding of the pathogenesis of the particular disease on the molecular level, (2) identification of the causative gene and knowledge of the nature of its aberration, (3) development of a therapeutic gene, (4) development of a vector that will ensure expression of the therapeutic gene over a desired time, (5) consideration of the off-target action of the gene and/or target effects that are different from the anticipated ones. However, these general principles acquire concrete and often very distinct forms, depending on the nature of the disease that is to be treated. Let us illustrate this through the examples of oncological and

cardiovascular diseases (that together are responsible for some 80% of deaths in the developed countries), in whose treatment quite controversial interventions are sometimes involved. This is not surprising. In the case of malignant tumors the object is to destroy life-threatening tissue, while in cardiovascular diseases the aim, in great majority of cases, is to renew the functioning of an impaired organ, the regenerative capacity of which is low.

Still, there exist some general strategies aimed at blocking or tuning the expression of genes. One of the strategies being employed is to use antisense deoxyribonucleotides [13]. These sequences bind directly to the genes to be inactivated, blocking gene transcription, or to their mRNA, blocking gene translation. The latter event results in the formation of an RNA–DNA complex. Owing to the activity of the ubiquitous ribonuclease H, the RNA component of the duplex is destroyed. The antisense molecule remains untouched and can readily bind to another mRNA molecule. A major disadvantage of antisense nucleotides is their low stability. Replacing oxygen atoms with sulfur atoms can increase it. A significant enhancement of stability has been achieved by the introduction of the so-called PNA (protein nucleic acid). PNA is an analogue of the DNA molecule. Its backbone is made up of a peptide to which the individual bases are attached in a sequence, ensuring its binding to the target molecule. Another method of gene silencing, and also its fine-tuning, is based on synthetically prepared small interfering RNAs (siRNAs) mimicking the role of the endogenous double-stranded microRNA (miRNA)

(see above). siRNAs, used as GT tools, are represented by synthetically prepared short, double-stranded, noncoding RNA molecules possessing a length of 19–22 ribonucleotides. Within the cell one of the strands is destroyed and the other one binds to complementary mRNA. This results in its degradation [14]. Much interest is devoted to ribozymes, which exhibit a strong antitranslational activity [15]. They can be introduced into cells by transfection or by means of VV.

There are a number of strategies, the value of which is markedly different in oncology and cardiology. Some of the differences will be outlined in the subsequent text.

Oncology

In the treatment of malignancies, GT has at its disposal a large number of different strategies. An attempt will be made to classify the approaches used, although we are aware that this is a task that cannot be fulfilled exactly, because a clear *fundamentum divisionis* is missing. Moreover, the individual strategies can be combined, they may overlap somewhat, and they have their say at different levels.

The strategies of the first group are based on direct modification of tumor cells. In addition to the approaches listed above, which primarily aim at inactivating the activated oncogenes, several others can be put to this group. These include, e.g., the introduction of fully functional tumor-suppressor genes [16]. Their expression may lead to the restoration of cell growth control or result in apoptosis of the tumor cells. Apoptosis can also be induced by the introduction of proapoptotic genes. All of these approaches are successful in the cell-culture systems and in some experimental models. A major problem comes in vivo. It is impossible to introduce the genetic material into all cells of the tumor being treated. The unmodified cells have a growth advantage over those whose malignant phenotype has been altered. They may soon become dominant in the tumor cell population. For successful treatment, combination with other treatment modalities is needed.

Therefore, two other direct modifications of tumor cells deserve more attention. The first one is based on the introduction of genes for immunostimulatory factors into tumor cells [17]. This ensures a high local concentration of such factors without any signs of toxicity that accompany their systemic administration. This raises the probability of a robust immune response that may have a clear therapeutic effect, without it being necessary to genetically modify all cells of the tumor. Similarly, it is not necessary to affect the whole tumor-cell population if the so-called suicide genes (SG) are used [18]. After prodrug treatment the toxic metabolites spread to neighboring cells (“bystander effect”), and the release of large amounts of tumor antigens may stimulate the development of a systemic antitumor immune reaction.

A second major group of GT strategies in the therapy of tumors is the one directed at gene modification of non-tumor cells. It includes, e.g., the introduction of the gene designated MDR-1 (multidrug-resistance-1), the product of which increases resistance of bone-marrow cells to toxic effects of chemotherapy [19] or the creation of conditions for the treatment of the life-threatening graft-versus-host disease by ex vivo introducing SG into donor T-lymphocytes [20].

Another group of GTs in oncology is procedures suppressing neoangiogenesis, a necessary precondition for tumor growth and metastasis formation. Precisely in this strategy, the difference between the GT of malignant tumors and the GT of the heart is the most marked. The introduction of, e.g., the gene for the angiogenesis-suppressing factor endostatin into tumor cells lowers their oncogenic potential and ability of metastasis formation [21]. However, an anti-angiogenetic effect can also be attained by a blockade of the functionality of important proangiogenetic factors, such as members of the families of VEGF (vascular endothelial growth factor) or FGF (fibroblast growth factor), e.g., by means of the corresponding antisense.

Oncolytic viruses, i.e., viruses replicating exclusively or predominantly in tumor cells, are also considered to be agents for tumor GT. Two groups of oncolytic viruses are distinguished: those

that are naturally oncolytic and mutants of other viruses. Taken *sensu stricto*, only the latter should be taken as GT agents, because their use was preceded by their genetic adjustment.

The last, viz. the fifth, group consists of genetic therapeutic anticancer vaccines. It is very likely that during the coming decade they will enter medical practice on a large scale. There are several distinct types of vaccines that are at the stage of development. Each of them has some advantages and some disadvantages. Considerable attention is being given to DNA vaccines [22], which are bacterial plasmids into which a gene for a specific tumor antigen has been incorporated. The gene must be in a form that ensures its expression in mammalian cells. Another type of genetic vaccines is recombinant vaccines. They are represented by recombinant proteins with the peptide carrying the immunodominant epitope of the tumor antigen inserted into another protein, known to produce a potent effect on the immune system [23]. A great endeavor is given to recombinant live viruses in which a certain gene that is not essential for replication is replaced by a gene for tumor antigen. Another type of genetic vaccines consists of cellular vaccines. They have a number of advantages. The first is that it is not necessary to know the immunodominant tumor antigens. These vaccines are prepared via modifying tumor cells by the introduction of genes for immunostimulatory factors. Both autologous and allogenic vaccines are under consideration. The development in the recent years rather favors the latter [24].

Cardiovascular Diseases

Interest in the use of GT in cardiology has been growing in the recent past, the reasons being several. The most important has been the gradual but rather fast recognition of the basics of physiological processes and the mechanisms that lead to the development of pathological states at the molecular level. Contributory to its development have been the quickly accruing successes in experimental systems. The growing interest in GT in cardiology has also reflected the slowdown in the development of efficient and safe new drugs.

Similarly as in other medical disciplines, a condition for the use of GT in cardiology is reliable and clinically relevant vectors, with safety aspects being more important than in oncology. The vectors most frequently used so far have been adenoviruses; however, it is probable that their place will gradually be taken over by adeno-associated viruses (AAVs) [25–27]. The recent discovery that some AAV serotypes are highly cardiotropic has been very helpful in this respect. However, a vector is still being sought that could be administered intravenously and that would have specific uptake by cardiomyocytes, with minimal off-target effects [28].

The use of a large spectrum of strategies is being considered and some are already in use. It is much more difficult to classify them in cardiology, because the efforts are less straightforward than in the field of oncology, in which the aim of the interventions is destruction of the unwanted tissue. Possibly the most marked differences are the absence of strategies influencing the immune system (with the exception of transplantations) and no use for SG. Another difference is a closer interconnection of GT and CT in cardiology. The choice of the strategy always depends on the purpose of the intervention. They are necessarily different when the therapy is meant just to serve as bridge to transplantation or bridge to recovery or whether a long-term expression of transgene is required [29]. To find optimal delivery system of the vectors is another important point. Among those which are under investigation is direct needle injection, pericardial delivery, catheter delivery into coronary arteries, and endocardial delivery.

From the literature available, it is apparent that special attention in heart GT is being paid to miRNA. This interest has been ever increasing with the gradual broadening of the recognition of the role played by the different miRNA species in the pathogenesis of cardiovascular diseases such as heart failure, cardiac hypertrophy, ischemia, arrhythmia, and atherosclerosis [30–32], and the recognition of potential approaches for miRNA-based interventions [33]. As has been summarized in a recent review, many cardiac patients can be treated by correcting their miRNA

expression [34]. The fact that the involvement of certain miRNAs in several different heart diseases has been experimentally established brings the miRNA-based strategy closer to extensive clinical application.

There have been many applications of GT, with different strategies being used. In the next section some of them will be mentioned, the object not being to cover the entire field and its problems, but rather to document, on several examples, their diversity and the possibilities they offer.

GT is trying to break the classical dogma of heart regeneration, i.e., cardiomyocytes become postmitotic soon after birth. Recent findings of the research on myocardial regeneration suggest that it is possible to induce adult cardiomyocytes to reenter division by means of genes, the products of which are involved in the regulation of the cell cycle or act as pro-mitogenic growth factors, such as VEGF or FGF [35]. However, there seems to be a long way to go before these new observations are fully translated into clinical practice. Although in animal models of ischemic myocardium the administration of plasmids carrying VEGF or FGF have resulted in an increased collateral blood flow [36], similar studies in humans did not provide consistent results. The administration of a plasmid carrying VEGF gene into inoperable heart has been reported to result in increased perfusion and reduced angina symptoms [37], and favorable results have been reported also by another group [38]. However, they have not been confirmed by a more recent study [39].

One of the main topics of GT in cardiovascular diseases is the modification of ion channels. Their aberration is central to many cardiovascular diseases, including hypertension, heart failure, ventricular arrhythmias, or atrial fibrillation [40, 41]. There are also efforts aimed at developing biological pacemakers that might serve as an alternative to electronic devices [42]. A cell therapy approach using gene-modified human mesenchymal stem cells implanted into dog heart produced encouraging results [43].

The last-cited experimental study may serve as an example of interconnection of GT and CT.

It is not the only instance. Quite a few other studies are under way that are based on the same principle, i.e., genetic manipulation of cells using cardiac stem cells, endothelial stem cells, bone-marrow stem cells, and adipose-tissue-derived stem cells, with this resulting in differentiation into cardiomyocytes. Sophisticated techniques for obtaining, for in vitro treatment, for implantation, and for in vivo activation of their growth, differentiation, and migration have been developed (for a review; see Madonna et al. [44]).

Another task for GT in cardiology is the prevention of rejection of heart transplant. In GT the aim is to inactivate genes that code for cytokines and adhesion molecules, the products of which are involved in rejection (for a review; see Suzuki et al. [45]).

To summarize, in spite of considerable efforts having been exerted and in spite of GT clinical trials representing the second largest group (after oncology) of clinical trials registered, the recent progress of GT in cardiology has been rather modest, more modest than anticipated 10 years ago. In their recent fine review Katz et al. [46] summarized the results of the recent experimental studies, described the advantages and disadvantages of the different approaches, and then defined the conditions that would lead to an optimization of the methods to be used. Notwithstanding the existing shortcomings, their conclusion is optimistic “the outlook remains promising.”

Ethical Problems

Ethical issues are of paramount importance for GT. Their importance is stressed by some serious events that took place in the past 10 years in the treatment of some genetic diseases. When a group of French scientists reported the successful treatment of children suffering from severe combined immunodeficiency (SCID) with a retrovirus carrying a therapeutic gene, a surge of enthusiasm followed. Unfortunately, 4 of the 11 children treated developed acute T-cell leukemia [47]. One of them died of leukemia. Another case of leukemia was reported in similarly treated British children [48]. The subsequent molecular analysis

Table 5 Guidelines for gene therapy clinical studies

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1. Every preparation must be produced in accordance with good manufacturing practice. Its quality and safety must be verified by all the methods specified by the law and must respect the newest achievements of biomedical sciences.
 2. The researchers must do their best to inform the patient about both the benefits and possible risks of the therapy. In these interviews they have to respect the education and the intelligence of the patient.
 3. Every clinical undertaking should unconditionally respect the rules of good clinical practice. The research group must have extensive experience in testing new pharmaceuticals.
 4. The protocol approved must be strictly adhered to. Patients who do not fulfill the criteria specified in the protocol must not be included.
 5. Any untoward or unexpected reaction must be reported without delay and thoroughly analyzed.
 6. The supervising authority should have enough resources for constant control of the undertaking.
 7. All undertakings should be double blind. There are two strong reasons for this. (1) The interest of the researchers in a positive outcome of the study, which may be subconsciously reflected in the process of evaluation. (2) The placebo effect, which is known to be strong in seriously ill patients.
 8. At this stage of knowledge, gene therapy should not be performed in patients suffering from diseases which can be successfully treated by other means. On the other hand, gene therapy should not be limited to patients in the terminal phase of their disease.
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revealed that in at least four of these children, a similar pathogenic mechanism was involved. The retroviral vector was integrated in close neighborhood of the promoter of LMO2 gene, coding for a transcription factor whose overexpression was apparently involved in the pathogenesis of the disease. The phenomenon, called insertional mutagenesis, resulted in uncontrollable cell proliferation. Such risk is particularly associated with retrovirus integration. Theoretically, a similar risk may be coupled with AAV, the genomes of which are also readily integrated into cell genomes. However, their safety profile seems to be much higher than in the case of retroviruses because AAV DNA preferentially integrates into a certain locus of chromosome 19. Another death was reported from a trial aimed at GT treatment of ornithine-decarboxylase deficiency. It was caused by the use of a disproportionately high concentration of a recombinant adenovirus which produced a deadly toxic shock [49].

Another problem may be caused by the toxicity of siRNAs arising from competition with cellular miRNA processing [50] or from its off-target effects. Yet another possible source of untoward reactions may be chronic overexpression of the gene products, with uncertain consequences [51]. There is also a theoretical possibility that the VV used can recombine with a wild-type

strain. The properties of such a recombinant cannot be anticipated.

The warning events call for carefulness in the use of GT and have stimulated a new ethical debate on GT. The result has been considerable toughening of the conditions for performing clinical studies.

Table 5 summarizes the principles that should be respected in all clinical GT studies.

Conclusions

The ongoing development of GT and its gradual introduction into clinical practice embodies some serious problems, which I tried to characterize in the preceding parts of this brief review. However, their existence does not mean that GT research and applications should be calmed down. On the contrary, the breadth of the GT potential – its utility in combating not only genetic diseases but also acquired conditions that are beyond the possibilities of conventional cure – is a great promise for future medicine. Nevertheless, the up-to-now experience signifies that the road from the laboratory bench to the bedside should not be unidirectional. In the years ahead, researchers will be repeatedly returning from clinical studies to the laboratory to clarify the causes of unexpected events. Only in

this way will it be possible to fill up the vacancies in our knowledge, reduce the risks involved, and raise the effectiveness of the operations being performed. In the light of what we know at present, it might be expected that in the next decade the progress in the clinical utilization of GT will be faster in oncology than in cardiology.

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References

- Shivdasani RA. MicroRNAs: regulators of gene expression and cell differentiation. *Blood*. 2006;108:3646–53.
- Li S-D, Huang L. Gene therapy progress and prospects: non-viral gene therapy by systemic delivery. *Gene Ther*. 2006;13:1313–9.
- Hattori Y. Development of non-viral vector for cancer gene therapy. *Yakugaku Zasshi*. 2010;130:917–23.
- Van den Berg JH, Nuijen B, Schumacher TN, et al. Synthetic vehicles for DNA vaccination. *J Drug Target*. 2010;18:1–14.
- Ivics Z, Izsvak Z. Transposons for gene therapy. *Curr Gene Ther*. 2006;6:493–607.
- Izsvak Z, Ivics Z. Sleeping beauty transposition: biology: application for molecular therapy. *Mol Ther*. 2004;9:147–56.
- Miskey C, Izsvak Z, Plasterk RH, Ivics Z. The frog prince: a reconstructed transposon from *Rana pipiens* with high transpositional activity in vertebrate cells. *Nucleic Acids Res*. 2003;31:6873–81.
- Alba R, Bosch A, Cillon M. Gutless adenovirus: last generation adenovirus for gene therapy. *Gene Ther*. 2005;12:S18–27.
- Wachler R, Russeli SJ, Curiel DT. Engineering targeted viral vectors for gene therapy. *Nat Rev Genet*. 2007;8:273–587.
- Miyoshi H, Biomer U, Takahashi M, Gage FH, Verma IM. Development of self-inactivating lentivirus vector. *J Virol*. 1998;72:8150–7.
- Palffy R, Gardlik R, Hodosy J, et al. Bacteria in gene therapy: bactofection versus alternative. *Gene Ther*. 2006;13:101–5.
- Seow Y, Wood MJ. Biological gene delivery vehicles: beyond viral vectors. *Mol Ther*. 2009;17:767–77.
- Baker BF, Monia B. Novel mechanism for antisense-mediated regulation of gene expression. *Biochim Biophys Acta*. 1999;1449:2–18.
- Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*. 2004;116:281–97.
- Welch PJ, Barber JR, Wong-Staal F. Expression of ribozymes in gene transfer systems to modulate target RNA levels. *Curr Opin Biotechnol*. 1998;9:486–96.
- Anderson SC, Johnson DE, Harris MP, et al. p53 gene therapy in a rat model of hepatocellular carcinoma: intraarterial delivery of recombinant adenovirus. *Clin Cancer Res*. 1998;4:1649–59.
- Bubenik J. Gene therapy of cancer by vaccines carrying inserted immunostimulatory genes. *Folia Biol*. 2007;53:71–3.
- Altaner C. Prodrug cancer gene therapy. *Cancer Lett*. 2008;270:191–201.
- Schiedlmeier B, Schilz AJ, Kuhlcke K, et al. Multidrug resistance 1 gene transfer can confer chemoprotection to human peripheral blood progenitor cells engrafted in immunodeficient mice. *Hum Gene Ther*. 2002;13:233–42.
- Bonini C, Ferrari G, Verzeletti S, et al. HSV-TK gene transfer into donor lymphocytes for control of allogeneic graft-versus-leukemia. *Science*. 1997;276:1719–24.
- Lakatosova-Andelova M, Duskova M, Lucansky V, Paral P, Vonka V. Effects of endostatin production on oncogenicity and neoplastic activity of HPV16-transformed mouse cells: role of interleukin 1alpha. *Int J Oncol*. 2009;35:213–22.
- Signori E, Iurescia S, Massi E, et al. DNA vaccination strategies for anti-tumor effective gene therapy protocols. *Cancer Immunol Immunother*. 2010;59:1583–91.
- Macková J, Stašíková J, Kutinová L, et al. Prime-boost immunotherapy of HPV16-induced tumors with E7 protein delivered by Bordetella adenylate cyclase and modified vaccinia virus Ankara. *Cancer Immunol Immunother*. 2006;55:39–46.
- Vonka V. Immunotherapy of chronic myeloid leukemia: present state and future prospects. *Immunotherapy*. 2010;2:227–41.
- Wang Z, Zhu T, Qiao C, et al. Adeno-associated virus, serotype 8 efficiently delivers genes to muscle and heart. *Nat Biotechnol*. 2005;23:321–8.
- Pacak CA, Mah CS, Thattaliyath BD, et al. Recombinant adeno-associated virus serotype 9 leads to preferential cardiac transduction in vivo. *Circ Res*. 2006;99:e3–9.
- Asokan A, Conway JC, Phillips JL, et al. Reengineering a receptor footprint of adeno-associated virus enables selective and systemic gene transfer to muscle. *Nat Biotechnol*. 2010;28:79–82.
- Grey SJ, Samulski RJ. Optimizing gene delivery vectors for the treatment of heart disease. *Expert Opin Biol Ther*. 2008;6:911–22.
- Poller W, Hajjar R, Schultheiss H-P, Fechner H. Cardiac-targeted delivery of regulatory molecules and genes for the treatment of heart failure. *Cardiovasc Res*. 2010;86:353–64.
- Divakaran V, Mann DL. The merging role of microRNAs in cardiac remodeling and heart failure. *Circ Res*. 2008;103:1072–83.
- Schroen B, Heymans S. MicroRNA and beyond: the hearth reveals its treasure. *Hypertension*. 2009;54:1189–94.

32. Mishra PK, Tyagi N, Kumar M, Tyagi SC. MicroRNA as a therapeutic target for cardiovascular diseases. *J Cell Mol Ther.* 2009;13:778–89.
33. Van Rooij E, Liu N, Olson EN. MicroRNA flex their muscles. *Trends Genet.* 2008;24:159–66.
34. Pan Z, Lu Y, Yang B. Micro RNAs: a novel class of potential therapeutic targets for cardiovascular diseases. *Acta Pharmacol Sin.* 2010;31:1–9.
35. Laguens RP, Crottogini AL. Cardiac regeneration: the gene therapy approach. *Expert Opin Biol Ther.* 2009;9:411–25.
36. Mack CA, Patel SR, Schwarz EA, et al. Biological bypass with the use of adenovirus-mediated gene transfer of the complementary deoxyribonucleic acid for vascular endothelial growth factor 121 improves myocardial perfusion and function in the ischemic porcine heart. *J Thorac Cardiovasc Surg.* 1998;115:168–76.
37. Symes JF, Losordo DW, Vale PR, et al. Gene therapy with vascular endothelial growth factor for inoperable coronary artery disease. *Ann Thorac Surg.* 1999;68:830–6.
38. Shintani S, Kusano K, Ii M, et al. Synergistic effect of combined intramyocardial CD34+ cells and VEGF2 gene therapy after MI. *Nat Clin Pract Cardiovasc Med.* 2006;3(suppl I):S123–8.
39. Stewart DJ, Kutryk MJ, Fitchett D, et al. VEGF therapy fails to improve perfusion of ischemic myocardium in patients with advanced coronary results of the NORTHER Trial. *Med Ther.* 2009;17:1109–15.
40. Cingolani E, Ramirez-Correra GA, Kizana E, Murata M, Cho HC, Marban E. Gene therapy to inhibit the calcium channel β -subunit: physiological consequences and pathophysiological effects in models of cardiac hypertrophy. *Circ Res.* 2007;101:166–75.
41. Telemaque S, Marsh JD. Modification of cardiovascular ion channels by gene therapy. *Expert Rev Cardiovasc Ther.* 2009;7:939–53.
42. Robinson RB, Brink PR, Cohen IS, Rosen MR. I(f) and the biological pacemaker. *Pharmacol Res.* 2006;53:407–15.
43. Potapova I, Plotnikov A, Lu Z, et al. Human mesenchymal stem cells as a gene delivery system to create cardiac pacemaker. *Circ Res.* 2004;94:952–9.
44. Madonna R, Rokosh G, De Caterina R, Bolli R. Hepatocyte growth factor/Met gene transfer in cardiac stem cells-potential for cardiac repair. *Basic Res Cardiol.* 2010;105:443–52.
45. Suzuki J, Isobe M, Morishita R, Nagai R. Nucleic acid drugs for prevention of cardiac rejection. *J Biomed Biotechnol.* 2009;2009:916514.
46. Katz MG, Swain JD, White JD, Low D, Stedman H, Bridges CR. Cardiac gene therapy: optimization of gene delivery technique in vivo. *Hum Gene Ther.* 2010;21:371–80.
47. Cavazzana-Calvo M, Fischer A. Gene therapy for severe combined immunodeficiency: are we there yet? *J Clin Invest.* 2007;117:1456–65.
48. Porteus MH, Connelly JP, Pruett SM. A look to future direction in gene therapy research for monogenic diseases. *PLoS Genet.* 2006;2:e133.
49. Raper SE, Chirmule N, Lee FS, et al. Fatal systemic inflammatory response syndrome in an ornithin-decarboxylase deficient patient following adenoviral gene transfer. *Mol Genet Metab.* 2003;80:148–58.
50. Grimm D, Steetz KL, Jopling CL, et al. Fatality in mice due to oversaturation cellular microRNA/short hairpin RNA pathways. *Nature.* 2006;441:537–41.
51. Fishbein I, Chorny M, Levy RJ. Site-specific gene therapy for cardiovascular diseases. *Curr Opin Drug Discov Devel.* 2010;13:203–13.



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