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## **Development of Gene Therapy**

Memorandum by the  
Senate Commission on Genetic Research

Report 5

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**DFG**

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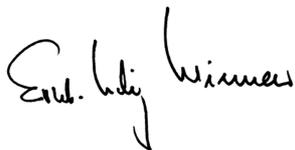
## Preface

After more than ten years, the Senate Commission on Genetic Research is issuing a second statement on gene therapy. Like hardly any other new form of treatment, gene therapy since the 1980s has repeatedly led to controversial discussions concerning its therapeutic potential and the associated health risks and ethical problems. In the meantime, however, comprehensive experimental research and initial clinical trials have significantly substantiated the until then only suspected therapeutic possibilities and their side effects. As the present statement shows, the realisation of the initially promised cures has made far less progress than expected. On the other hand, many of the sometimes exaggerated risks have also not been confirmed. Therapeutic successes as in congenital immunodeficiency diseases show that somatic gene therapy can be a valid treatment option. However, the observed and sometimes fatal side effects also show the present risks of this therapy. With regard to these problems, however, somatic gene therapy is not fundamentally different from other treatment options. The following holds: Before and during clinical testing and application, the benefits and risks must always be assessed in comparison with the alternative treatment options available and on the basis of solid information from experimental and clinical observations. In addition to being scientifically up-to-date, this requires close contact and an open exchange with scientists conducting basic research and those engaged in clinical research, as well as with the advisory ethics committees and the licensing authorities. All scientists are required to practice such an open discussion, just as the public is required to participate in this discussion in a critical but unbiased manner. Therefore, this statement is addressed both to science and to the interested public and aims to point out the current state and the further perspectives of somatic gene therapy.

Since somatic gene therapy using retroviral vectors, despite some significant advances, still remains in a rather experimental stage, the Senate Commission rightfully warns against a broad application at the present time. In addition to further optimisation of the treatment, there is a significant need for research concerning our understanding of the observed and expected side effects. Therefore, it is welcome that the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) with its current funding of a special Priority Programme to examine the cell entry and persistence of gene therapy vectors has also made a significant contribution to understanding the mechanisms that can lead to possible side effects in somatic gene therapy. As was also confirmed by foreign experts, Germany has a number of internationally leading teams and very promising young researchers in this field. Therefore, it is even more important that these scientists, in addition to financial support and the provision of suitable clinical and scientific structures, are also given clearly visible career perspectives.

At this point, I would like to thank the Senate Commission on Genetic Research and the authors of this statement for giving a comprehensive presentation of the development of somatic gene therapy and for contributing to the ongoing discussion, on the basis of the current state of scientific knowledge and the future perspectives.

Bonn, December 2006



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# 1 Summary

Since the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) first published a statement on gene therapy in 1995, this field has made enormous progress. Initial clinical successes have shown the therapeutic potential, but also the risks of this treatment option. Like all other forms of therapy, the clinical application of gene therapy requires a careful weighing of the benefits and risks. This field of research is still in a pilot stage and, like few other fields of medical research, relies on a close collaboration and a constant exchange between scientists engaged in basic research and clinicians of different disciplines. This requires an iterative optimisation process whereby, in contrast to classical clinical research, new results from the laboratory are directly transported to a clinical study and the knowledge gained from this process is then once again used to make further progress in the laboratory.

The successful further development of gene therapy over the medium-term especially requires research in the area of developing more efficient and safer vectors as well as the molecular investigation of the effects and side effects of gene therapy in the animal model and in the context of experimental clinical studies. A stronger focus has to be put on the assessment of the risk profile of potentially used therapeutic genes. The use of gene therapy is restricted to treatment and prevention. Gene doping or cosmetic application is not a justifiable use. The present legal framework is adequate; the current registration of gene transfer studies in a central registry should be continued. Overall, the past ten years have demonstrated the therapeutic potential of gene therapy with initial successes in immunodeficiency diseases and have also delivered promising treatment approaches for a series of monogenetic hereditary diseases as well as for the acquired diseases of cancer and HIV infection/AIDS.

## 2 Introduction and brief historic outline

**Gene therapy** is defined as the introduction of genes into tissues or cells with the objective of obtaining a therapeutic or preventive benefit as a result of the expression and function of these genes. The process of introducing genes into cells is called **gene transfer**. A vehicle that carries the gene, the **vector**, is required to accomplish this. Therefore, gene therapy is the medical treatment with gene transfer drugs that are defined in the Medicines Act. The desired gene transfer exclusively concerns somatic cells (somatic gene therapy). Germline gene transfer is forbidden by law in Germany.

During the last two decades, only few fields of medical research have received as much attention as somatic gene therapy. Discoveries in molecular biology and genetics which in 2001 reached their peak with the sequencing of large parts of the human genome provided the prerequisites for gene therapy. In the early phase of gene therapy at the beginning of the 1990s these facts were partially discussed in an uncritical and too euphoric manner. This led to unrealistic expectations. The methods of gene transfer have significantly improved during the past few years, and the first clinically successful gene therapies have been performed in patients. Nevertheless, it is also necessary to emphasise today that the development of mature gene therapy procedures for many diseases that are otherwise untreatable will take many years, despite the fact that the success of individual gene therapies is foreseeable over the medium-term.

The first well documented gene therapy studies were started at the beginning of the 1990s. By 2005 it was estimated that more than 1100 gene therapy studies have been conducted throughout the world; one third of these in Europe with a special focus in Germany. In 2003 as well as in November 2005 China approved the first gene therapy drugs for the treatment of certain malignant tumours. A first European application for the approval of a gene therapy drug for the treatment of an aggressive brain tumour was submitted to the European Agency for the Evaluation of Medicinal Products (EMA) in 2005.

Despite continued great difficulties in the technical implementation, the successes of gene therapy can doubtlessly be confirmed today. For example, successful causal therapies have been developed during the past five years for patients with severe hereditary immunodeficiency diseases. These treatments are visibly beneficial to these patients with life-threatening conditions (see section 3). However, in one study, three children who were treated with retroviral vectors developed a leukaemia-like disease three years after treatment. At first, this was exclusively attributed to a side effect of the vectors used in combination with the underlying disease, but may possibly also be due to the overexpression of the target gene. This and the death of a patient in the USA in 1999 as a result of a very high systemically administered dose of adenoviral vectors were tragic events that were viewed by the public as a setback for gene therapy. Nevertheless, the same principles apply to gene therapy as to other medical interventions: Effective procedures are associated with potential side effects. Side effects can be reduced by improving the procedures when the underlying mechanisms are understood. For each indication and each procedure the relationship of effect to side effects (therapeutic index) must be determined in meticulous preclinical and clinical studies. German scientists have made important contributions in this field, from basic research of the vector-host interaction to clinical studies. Among other things, in 2006 they reported on the correction of a severe immunodeficiency in adult patients through gene therapy.

### 3 Clinical application: successes and setbacks

Compared with previous approaches, gene therapy offers a new way of treatment with a high potential for innovation since genes are used as drugs, while conventional drug development uses chemical substances, products from microorganisms or proteins. However, in practice it quickly became apparent that the initial prognoses for gene therapy had clearly underestimated the developmental requirements.

The selection of suitable vectors is crucial for the efficacy of a gene therapy. Different therapeutic or prevention objectives can require different vectors. For example, the selection depends on whether the gene transfer occurs in the patient (*in vivo*) or in the cell culture dish (*ex vivo*, or *in vitro*) since these procedures place different demands on the safety and targeting precision of the vector. The development of improved vectors for gene therapy continues to be one of the central tasks for research.

Gene transfer vectors for somatic gene therapy must have the following properties:

- They must be able to efficiently modify certain human cells.
- They must be able to ensure a sufficiently strong and sustained gene expression.
- They must exhibit a risk profile that is as low as possible for the desired form of treatment.

Presently, clinical gene therapy studies often use viral vectors that are incapable of replication and that are derived from retroviruses, adenoviruses, adeno-associated viruses and pox viruses. To this purpose sections of the viral genome required for replication have been removed or inactivated and replaced by the therapeutic gene. After introducing the vectors into helper cell lines which contribute the necessary functions for virus formation, defective viruses are formed that are suitable for gene transfers, but which no longer can reproduce outside of the helper cells. Furthermore, plasmid DNA in pure form or mixed with other reagents is used as a non-viral vector. Viruses with a limited ability to reproduce are used in clinical studies in combination with therapeutic or preventive genes in cancer therapy and vaccination.

The disease groups that so far have mainly been examined in clinical studies on gene therapy are cancers, monogenic hereditary diseases, infectious diseases (especially HIV/AIDS) and cardiovascular diseases. Of these, cancers represent the largest segment (60 percent). Most clinical gene therapy studies are in very early clinical phases; only a few have reached phase III or have achieved proof of clinical efficacy. It must be assumed that many of the previously completed or presently ongoing clinical gene therapy studies of phases I and II will not yet lead to a drug that can be routinely used, since these are pilot studies for the treatment of very rare diseases.

Proof of the clinical efficacy of a gene therapy could particularly be obtained in studies on the treatment of severe immune defects. These are studies on the inherited combined immune defects ("severe combined immunodeficiency", X-SCID; the Cavazzano-Calvi and Fischer group in Paris as well as the Thrasher group in London) on adenosine deaminase deficiency (ADA-SCID; the Auiti and Bordignon group in Milan) and on chronic granulomatosis (CGD; the Grez and Hölzer group in Frankfurt/Main). First indications of the clinical efficacy of gene therapy were also obtained in the use of the CD40 ligand in chronic lymphatic leukaemia (Kipps group in San Diego), in the transfer of the GM-CSF gene in malignant melanoma (Lattime group in Philadelphia) as well as in the treatment of haemophilia B (factor IX; the McKay and High group in Stanford/Philadelphia).

Approximately ten years of intensive research and development conducted by many groups was required to treat a sufficiently large number of cells outside of the human body with gene vectors in such a manner that after returning these genetically modified cells to patients a therapeutic success could be realised. As expected, these initial successes in the treatment of monogenetic hereditary diseases were achieved with retrovirally modified blood stem or precursor cells, especially in the immune deficiency syndromes X-SCID and ADA-SCID. In these cases, *ex vivo* vectors derived from the murine leukaemia virus that are incapable of reproduction and result in the mainly accidental integration of the therapeutic gene into a chromosome of the particular host cell were used. The diseases mentioned offer especially favourable prerequisites for gene therapy: a therapeutic effect is already achieved when a limited number of target cells are modified, the genetically modified cells

have a growth advantage in the organism or such an advantage is realised by pretreating the patients, and the cells modified with the gene transfer vector are not rejected because of the immunodeficiency.

Approximately three years after the first successful treatment of ten patients with X-SCID in the study conducted at Necker Hospital in Paris, three of these patients developed acute T-cell leukemias as a side effect of gene therapy and one of these patients died of the disease. In an exemplary international collaboration in which German scientists participated, significant progress was made in clarifying the molecular causes of this side effect. It was determined that the retroviral gene vectors used activated cellular proto-oncogenes during their integration into the genome of the treated T-cells and, thereby, contributed to the development of these cancers. Furthermore, more recent investigations cast doubts on the harmlessness of the correction gene used, since lymphomas, which were not necessarily related to the vector used, occurred during long-term studies in the animal model. Corresponding side effects so far have not occurred in a similar study of X-SCID patients at Great Ormond Street Children's Hospital in London. Therefore, the overall success of these gene therapy studies is positive. However, the recognised essential disadvantage of the vectors used in the Paris study with respect to the long-term course presently cannot be estimated with certainty because of inadequate clinical experience. In the treatment, which was carried out in Frankfurt/Main and reported in 2006, of adult patients with CGD by means of retroviral vectors, the integration of the vector genome into cell cycle-activated genes was also frequently observed. Although in this case, this integration presumably contributed to the therapeutic success (by propagation of the successfully treated cells in the organism), in the meantime one of the patients treated in this study has died as a result of an infection-related complication after extensive functional loss of the treated cells. The other patients continue to benefit from the therapeutic effect. Taken together these results clearly show that a significant amount of research must still be undertaken regarding the connections between therapeutic efficiency and side effects in gene therapy.

Researchers in the field of gene therapy have from the start sought out public discussion and even in very early clinical trials have provided complete insight into the treatment risks and side effects. During this process, the public has not adequately perceived that, despite the leukemias described above, gene therapy of fatal diseases (such as monogenic hereditary immunodeficiency diseases) does not exhibit a higher rate of side effects than comparable conventional forms of treatment. So far, three of the 28 patients with X-SCID who were treated developed leukaemia as a side effect of the vectors used and one of these patients died. This corresponds to a side effect rate of about ten percent with a mortality rate of four percent. In conventional therapy of these same diseases with bone marrow or blood stem cell transplantation from HLA-identical family donors, the mortality rate is ten percent or higher. In the group of children selected for gene therapy (without HLA-identical donors from the family), the mortality rate at about 30 percent is even higher. However, it must be noted that the numbers mentioned rely on a small database. Leukaemia was already known as a possible risk before using gene therapy. However, the probability of its occurrence was unclear and was estimated as being low. After a conscientious evaluation and weighing of the risk, all participants decided to undergo therapy, because without gene therapy a long-lasting correction of the cell functions was not possible and the risk of the underlying disease significantly exceeded that of the therapeutic intervention.

In light of new information about the causes of the leukaemias, new and safer vectors have been developed in the meantime that should significantly reduce the risk of activating cellular oncogenes. Furthermore, sensitive preclinical models and diagnostic methods for toxicity determination have been described. In this way the molecular mechanisms of severe side effects in the future often can be avoided and detected earlier. Furthermore, the newest data show that the toxic effect of the newly introduced gene in the X-SCID case possibly was underestimated. Therefore, in addition to the vector also the therapeutic gene must be more significantly included in the assessment of the risk profile. Based on more recent research, it has become even clearer that the previously used vectors very rarely caused severe side effects, but relatively frequently affected the expression of cellular genes. These vectors also affect the growth and function of gene-modified cells in an otherwise healthy organism. This was also shown in the CGD study described above. The risk of severe side effects probably depends on many additional factors and on the underlying disease; this can be analysed in the experimental model, but ultimately is not clearly recognisable until clinical testing. Therefore, both vector development and clinical implementation continue to require significant additional research.

The named successes of gene therapy were made possible by a significantly improved efficiency of the gene transfer. For example, hematopoietic cells today can be genetically modified with a high efficiency (>50 percent). However, achieving effective therapeutic levels was associated with a higher

probability of symptomatic side effects. This simultaneously means that - as with other drug therapies - a further dose increase of the previously used vectors is more likely to be associated with more extensive side effects. Dose determination and toxicity determination have always been an inseparable part of developing active pharmaceutical substances and gene therapy is no exception. Through detailed investigation of the molecular causes, promising approaches have been discovered which will significantly improve the risk-benefit profile of the next generations of gene vectors. Therefore, for a series of monogenetic hereditary diseases and the acquired diseases of cancer and HIV infection/AIDS, gene therapy offers more innovative, worthwhile treatment options than ever before. It is especially the objective of HIV gene therapy to introduce protective genes (which, for example, prevent virus entry) into blood stem cells of the patient, whereby the successfully treated cells then continuously produce HIV-resistant immune cells, thereby, preserve the function of the immune system.

In contrast to the method presented above for diseases that are fatal if left untreated, the use of gene therapy vectors to express antigens as a vaccine requires the use of vectors and procedures with a very low risk of side effects. Also in this case, the risk-benefit analysis must refer to the presently used vaccines against infectious diseases whose risk of side effects lies in the per mill range or below. Therefore, this application uses non-viral vectors or viral vectors that are incapable of reproduction, which effect only a temporary and local cell modification, but no chromosomal integration. The risk of these vectors is estimated as being very low, but the gene transfer efficiency is also lower and the gene expression is only temporary. Before each clinical trial of gene transfer drugs, a responsible risk-benefit analysis in terms of the individual approach used must be undertaken (as is also common with other drugs).

Because of the risks of gene therapy procedures with inserting vector systems as well as the necessary costs associated with their development, the use of retrovirally modified cells in humans is in the foreseeable future only permissible for the treatment of serious diseases after carefully weighing the particular risk-benefit relationship. Vector systems which do not cause permanent changes - and thus essentially do not differ from other pharmaceutically active substances - can also be used in diseases that are not life-threatening after comprehensive safety tests have been performed on the particular vector and the transgenic product. However, even with vectors that have been proven to be safe, an application of gene therapy that is not medically indicated, to improve performance, for example in competitive sports (gene doping), cannot be justified for ethical and medical reasons.

## 4 Present situation and further research needs

Research to develop a successful gene therapy serves as an example for other areas of *translational research*, in which knowledge obtained from biomedical basic research should be directly transferred to clinical application. This medical field faces special challenges and difficulties and its success to a large degree depends on a functioning dialog between the basic sciences (for example, vector development and optimisation) and applied clinical research. Of special significance for the successful development of gene therapy research in Germany is the close collaboration of scientists engaged in basic research and physicians experienced in treating the target disease. As is apparent from the results of previous clinical studies discussed above, the further development of promising gene therapy approaches requires an iterative optimisation process which, unlike prior clinical research, transports new knowledge from the laboratory into the clinic, tests it there, substantiates or discards a hypothesis and is then followed by renewed preclinical optimisations. Naturally, this must occur according to strict ethical criteria.

In Germany it is especially the targeted funding of the Federal Ministry of Education and Research (BMBF) and project funding by the DFG in the past ten years that have established a substantial number of successful and interdisciplinary gene therapy groups. Most notably, a number of younger natural scientists and physicians could be convinced to return to Germany from the USA. Especially in the fields of vector development and genome insertion analysis of retroviral vectors, but also in the implementation of clinical gene therapy studies, German scientists have achieved an internationally recognised standing. The pioneering time of rapid successes in gene therapy research is coming to an end. Systematic basic research is increasingly required to solve the acknowledged problems. With appropriate funding the German research tradition of systematic and detailed analysis should serve as a good foundation for maintaining and expanding the achieved position over the long term. Research is especially needed in the following areas: (i) improved efficiency and safety of gene transfer vectors, (ii) optimising the specificity of viral vectors used for defined target cells in *in vivo* gene therapy, (iii) investigation of the persistence of gene-modified cells in patients and (iv) research of the molecular causes of side effects. In addition to these direct research topics, the aspect of vector production and testing also plays an important role for the development of gene therapy. Without adequate support it could prove limiting for further progress.

The first clinical successes of gene therapy when treating X-SCID were based on special preconditions: The defective bone marrow cells removed from these patients receive a selective advantage as a result of the therapeutic gene transfer; after being transplanted back into the patients, they respond to the natural signals of the body and can reproduce. These "healed cells" regenerate the immune system and even when only a relatively small number of cells are treated outside of the organism (*ex vivo*) this selection advantage results in a therapeutic success. In an analogous manner the treatment of CGD with gene therapy apparently gave rise to a selection advantage for the *ex vivo* treated cells by insertion of the vector genome into genes important for cell growth. However, in most diseases a selection advantage is not expected for the treated cells. Furthermore – as stated above – increase of the vector dose is possibly associated with an increased complication rate. As a consequence of this, additional research is needed in the development of more efficient and simultaneously safer vectors as well as in the investigation of the risk profile of therapeutically used genes, first *in vitro* and in the animal model and - if successful - in clinical studies. By better understanding the factors that can contribute to the multiplication of the viruses used, it is possible to achieve improvements, whereby over the medium-term additional optimisation can be expected by connecting viral functional complexes from different viruses as well as in combinations with non-viral systems. In this way, it may be possible to combine desired properties of different vector systems and exclude undesirable properties. Against the background of the developing field of synthetic biology, the synthetic production of a gene transfer vector appears to be an attainable goal.

In addition to the fact that only relatively few cells can be treated in *ex vivo* gene therapy, an additional disadvantage - with the exception of blood cells - is that most somatic cells cannot be removed simply for cell culture therapy. Therefore, the objective is administration of the gene transfer vector in the patient (*in vivo*). During this process, the concentration of the administered vectors is very rapidly diluted and they come in contact with many cell types that are not affected by the particular disease. To become therapeutically effective and cause as few side effects as possible in other cells the *in vivo* applied vectors must be much more specific and effective when penetrating their particular

target cells and in their therapeutic effect in these cells than in *ex vivo* gene transfer. Therefore, increasing the specificity of the vectors for therapeutically significant target cells of the organism and the efficacy of their penetration and effect in these cells is an additional research topic of central importance and an essential requirement for a broader clinical application of gene therapy.

Research continues to be required in investigating the molecular causes of the side effects of gene therapy. It is precisely this topic that has become extremely pertinent when three cases of leukaemia occurred in 28 successfully treated patients with X-SCID. The risk of side effects that can be caused by the mainly undirected integration of the retroviral vector genome into the genome of the host cell was originally estimated as being very small. Integration by chance can disturb the function of genes that normally regulate cell growth; undirected growth and tumours may result. Despite significant progress, the causes of the unexpectedly frequent occurrence of this side effect in X-SCID patients have not yet been fully clarified. Explaining the causes of side effects will not only lead to an understanding of the underlying molecular mechanisms, but also to more effective and safer gene transfer protocols and vectors for clinical application. More recent studies show that a change in cell proliferation through vector insertion possibly occurs more frequently, but that this does not necessarily lead to tumours. Investigating the underlying causes and conditions is of decisive importance for the further development of this approach in gene therapy. In addition to the untargeted integration of the vector, the therapeutic gene itself, can play a role in tumour development. Initial studies have shown that these effects occur with some delay and therefore do not become manifest in short-term experiments. Therefore, long-term preclinical observations must be performed and animal models must be developed that show corresponding side effects as early as possible.

A crucial factor for the clinical application of gene therapy is producing the required quantity of the gene transfer drug in accordance with the regulatory guidelines ("Good Manufacturing Practice" in manufacturing the investigational drug (GMP), "Good Laboratory Practice" in pharmacological/toxicological testing (GLP), "Good Clinical Practice" in clinical studies (GCP)). The development of gene therapy presently is in the hands of academic research groups and small biotechnology companies. Without question, university groups usually are not in a position to achieve vector production in accordance with GMP guidelines. Other countries have gone different ways with regard to GMP production of gene therapy vectors or genetically modified cells. In the USA university institutions have become involved with biotechnology companies and the NIH. From 1997 to 2003 the French foundation "l'Association Française contre les Myopathies" established a "Gene Vector Production Network" to make it easier to use and modify gene therapy vectors for research purposes. One of the ideas was to develop a European network from this for GMP production. However, this was not realised. In Great Britain the Department of Health has made available four million GBP for the time period 2003 to 2008 to be used in the production of gene therapy vectors for gene therapy studies within the NHS (National Health Systems). In Germany possibilities of GMP-consistent production of vectors for gene therapy are presently available at biotechnology companies, several pharmaceutical companies and at the Helmholtz Centre for Infection Research in Braunschweig. Fundamentally, vector production and testing can be realised either by establishing common production facilities in the public sector or through specialised small to medium-size businesses (frequently as spin-offs of university groups) as service providers. In each case, adequate financing is required. Presently, these costs can only rarely be covered by project funding of the interdisciplinary academic research groups composed of physicians and natural scientists. In consideration of the fact that gene therapy can only continue to develop successfully in interaction between research and development, on the one hand, and in clinical studies, on the other hand, the aspect of vector production and testing is an important location factor for this field of research.

In summary, fundamental progress has been achieved in the successful application of gene therapy only as a result of intensive, basic science-oriented, preclinical and clinical research and testing as well as in constant communication between these disciplines. This need for research is acknowledged by the DFG, among other means, through the establishment of the Priority Programme "Mechanisms of Cell Entry and the Persistence of Gene Vectors" in 2005. The objective of this Priority Programme is the interdisciplinary investigation of the biological safety of entry and the persistence of viral and non-viral gene transfer vectors with a scientific focus on the cells of the hematopoietic and lymphatic system. This DFG Priority Programme which is more basic research-oriented complements other national ("innovative therapies" of the BMBF) and international (CLINIGEN of the EU) funding initiatives that transition to clinical application. Since these funding initiatives in each case only partially cover the necessary spectrum of basic and clinical research that is needed for translational research, close cooperation between the different funding organisations is essential to offer the interdisciplinary groups of scientists and clinicians funding that is of sufficient breadth. The programme for funding of

clinical studies jointly conducted by the BMBF and DFG stands as an example for successful cooperation in this field. An alternative path is opened by the programme of the Clinical Research Units of the DFG in which, based on a clearly defined thematic focus, clinically relevant research fields are established in the clinics on a priority basis, also with the collaboration of scientists engaged in basic research. The close interaction between basic research scientists and clinicians within such an association guarantees optimal conditions for the requirements of translational research. An example of this is the Clinical Research Unit "Stem Cell Therapy" at the medical school in Hannover, in which gene therapy approaches are also transferred to the clinic.

## 5 Legal and ethical considerations

Because of the associated unforeseeable risk potential, germline gene transfer in Germany is forbidden by the Embryo Protection Act for good reasons. Therefore, this will not be discussed in detail.

In comparison with other innovative therapies, somatic gene therapy does not pose any fundamentally different or new ethical or legal problems. Before a first application in humans, the associated risks must be clarified by means of an animal experiment. Furthermore, the Commission for Somatic Gene Therapy of the Scientific Advisory Board of the German Medical Council has also advised against individual treatments with gene transfer drugs, because the development of a therapy rationally only seems possible as a result of information obtained from its use in a series of subjects or patients during a clinical trial.

The production of organisms altered by genetic engineering in the laboratory as well as the establishment and operation of genetic engineering facilities are subject to compulsory registration or official approval in accordance with §§ 8ff. of the Genetic Engineering Act (GenTG). In contrast, the Genetic Engineering Act does not include the use of genetically altered organisms in humans. Therefore, the Medicines Act (AMG) should be applied primarily to a clinical study and the EU guideline 726/2004 should be applied to a subsequent approval. However, the treatment room used in a clinical study can be considered a genetic engineering facility in accordance with the Genetic Engineering Act.

As in the case of any other drug therapy that is still in the experimental stage, clinical studies that use gene transfer drugs require a voluntary and self-determined consent of the study participants. This consent must be preceded by adequate information provided by the physician which especially also must include indications of the measure's novelty and the expected or feared risks. Before and during the implementation of the clinical study, a risk-benefit analysis is required in which the risks of using the drug and the need to protect the target group (patients or healthy subjects), on the one hand, must be weighed against the possible benefit to the target group and the importance of the drug for medicine, on the other hand. This consideration, for example, leads to the use of gene transfer drugs with a low risk as prophylactic vaccines against infectious diseases in healthy subjects, while other gene transfer drugs for the treatment of fatal disease such as certain brain tumours are only tested on patients who have exploited all available means of conventional therapy and have a life expectancy of not more than a few months.

The coming into force of the 12th amendment of the Medicines Act (AMG) in 2004, by means of which the European GCP directive (Directive 2001/20/EU) was implemented, specifically establishes which guidelines and regulations should be applied to the manufacturing and development through market approval of gene therapy or gene transfer drugs (both terms are essentially synonymous) and defines the certain state of scientific knowledge. According to § 4 Par. 9 of the Medicines Act, this drug group on the one hand includes viral and non-viral gene transfer vectors, plasmid DNA and oncolytic viruses for *in vivo* gene transfer and, on the other hand, *ex vivo* genetically modified cells.

On the one hand, approval must be obtained in gene therapy for industrial or commercially produced individual formulations. For example, this affects gene transfer drugs that are produced by companies or also blood banks and which contain genetically modified cells according to a standard model while using the same gene vector and the same therapeutic gene. These drugs are intended for distribution to physicians for use in prevention, treatment or *in vivo* diagnostics in a specific patient. On the other hand, approval must be obtained for gene transfer drugs produced in advance for *in vivo* administration in many patients, such as, for example, viral vectors. Approval can be applied for on the basis of results from clinical studies of phases I to III at the European Agency for the Evaluation of Medicinal Products (EMA).

Before starting a clinical study, the positive evaluation of the competent ethics committee and the authorisation of the Paul Ehrlich Institute (PEI) are necessary. Insofar as none of the members have any relevant expertise, ethics committees will consult external experts in the evaluation of applications for gene therapy studies. The consultation that ethics committees in 1994 to 2005 usually obtained from the Commission on Somatic Gene Therapy of the Scientific Advisory Board of the German Medical Council has been discontinued until further notice by the Executive Board of the German Medical Association.

The 12th amendment of the AMG legally stipulates the approval or evaluation time limits of the Paul Ehrlich Institute and the competent ethics committees which expedites the procedures. In clinical studies of gene transfer drugs that contain genetically altered organisms (for example, viral vectors

and viruses that to a limited degree are capable of reproduction or microorganisms), approval of the clinical study by the Paul Ehrlich Institute also includes the required approval to release. The same holds for the approval of gene therapy drugs by the EMEA.

Since gene transfer drugs are a new class of drugs that are subject to an ongoing development process, regulatory guidelines usually only can provide general information. Studies to confirm the quality, safety and efficacy of a given gene transfer drug usually must be decided on an individual basis. During this process, small biotechnology companies or academic research facilities are often confronted with new questions. In such a case, the applicant can make use of a consultation at the Paul Ehrlich Institute before submitting an application for a clinical study. The secrecy of the data and the confidentiality of the contents of the consultations are guaranteed by law.

Since 2004 a European database (EudraCT) has been established at EMEA which provides the competent authorities, the European Commission and the EMEA with necessary information about clinical studies in all European member states. However, this registry is not accessible to the public. In Germany there is an additional "German Registry for Somatic Gene Transfer Studies" (DeReG). This registry was established in 2001 on the initiative of the German Society of Gene Therapy (DG-GT) and the Commission for Somatic Gene Therapy in Freiburg and was funded by the BMBF. It includes information that presently cannot be obtained from any other available international study registry. For example, the Freiburg registry contains the side effects of individual patients from small phase I studies. Furthermore, this registry can be used to quickly and reliably inform the public, as needed (occurring side effects or successes). Therefore, the maintenance of such a publicly accessible registry appears to make sense to increase clarity and comprehensibility in the field of gene therapy. Therefore, the DFG continues to require that applicants show proof of having registered the gene therapy study in the DeReG before a clinical research project is approved. Whether the planned national study registry can fulfil the specified tasks in an analogous manner is presently still open and will not be known until its further implementation.

While the specific legal framework for gene therapy can be viewed as being adequate, the fundamental regulatory and structural problems for gene therapy research in a clinical environment are identical to those generally present in German academic clinical research. Basic conditions for clinical research in Germany that are more favourable overall, therefore, would also significantly improve the situation of scientists researching the clinical implementation of gene therapy. These problems and the corresponding solution proposals are described in the DFG White Paper on Clinical Research from 1999 as well as in the "Ten Key Points for Clinical Research" from 2004, which to a large extent are still relevant.

## 6 Conclusions and recommendations

- Since the first memorandum of the DFG on gene therapy in 1995, somatic gene therapy has achieved notable therapeutic successes, especially in monogenetically caused immunodeficiency diseases. In other potential applications it is still in early stages of development.
- Like every medical treatment, gene therapy also is associated with risks that must be carefully monitored and clarified with regard to their molecular causes. Clinical application requires a careful estimation of the benefits and risks for the specific indications. During this process, the public discussion of the successes and setbacks must also consider the prognosis of the underlying disease and alternative treatment options.
- The use of gene therapy is restricted to treatment and prevention. Use in gene doping or for cosmetic purposes is rejected.
- As long as safe retroviral vectors are unavailable, treatment with retroviral vectors should be limited to diseases without alternative treatment options.
- Furthermore, gene therapy requires a great deal of research. Basic research should be conducted in a direct, interdisciplinary manner that combines experiments in the animal model with clinical studies. Funding programmes such as the Clinical Research Units offer a basis for doing so and should increasingly be supplemented by funds that support translational research by the faculties. Furthermore, the necessary financing of expensive vector production in suitable facilities that have been approved in accordance with GMP guidelines must be ensured or be available through commercial suppliers.
- Current research should focus on the development of efficient and safe vectors for *in vitro* and *in vivo* application, including increased specificity for defined target cells as well as the molecular investigation of the effects and side effects. The risk profile of clinically used therapeutic genes must be considered.
- The existing basic legal conditions for gene therapy are sufficient.
- The registration of phase I and phase II gene transfer studies in a central registry has proven useful and continues to make sense. Registration should continue to be a requirement for funding by the DFG.

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### Internet addresses

German Registry for Somatic Gene Transfer Studies (DeReG)  
[www.dereg.de](http://www.dereg.de)

German Society of Gene Therapy e.V. (DG-GT)  
[www99.mh-hannover.de/kliniken/zellth/dggt](http://www99.mh-hannover.de/kliniken/zellth/dggt)

European Society for Gene Therapy (ESGT)  
[www.esgt.org](http://www.esgt.org)

Paul Ehrlich Institute  
[www.pei.de](http://www.pei.de)

## 8 Glossary

**AAV vectors:** Adeno-associated viruses that are used in gene therapy. They usually are not associated with human diseases, form stable particles and also infect resting cells, in which stable integration into the genome can occur. However, AAV particles only have a very limited capacity for taking up foreign genes. To propagate the AAV requires a second virus (a so-called helper virus, usually an adenovirus or parvovirus).

**ADA-SCID:** A hereditary, severe combined immune disease (SCID, severe combined immunodeficiency) in which the enzyme adenosine deaminase (ADA) is missing due to a gene defect. As a result, the body cannot degrade a protein that is poisonous to white blood cells and the T lymphocytes, which are important for the immune response, do not mature in the bone marrow or do so only in small numbers. Children affected by this disease are without any protection whatsoever and are almost completely at the mercy of all pathogens. Despite treatment and a life only under sterile conditions, they rarely survive their childhood.

**Adenosine deaminase deficiency:** See ADA-SCID.

**Adenoviral vectors:** Adenoviruses can be responsible, among other things, for the common cold in humans. Vectors with replication defects have a relatively high capacity for taking up genetic material. However, in higher doses they can lead to strong immune responses after administration.

**AMG:** Act governing the production and distribution of medicines (Medicines Act). A current version can be found at [www.gesetze-im-internet.de/amg\\_1976/index.html](http://www.gesetze-im-internet.de/amg_1976/index.html).

**BMBF:** Federal Ministry of Education and Research.

**CGD:** Chronic granulomatous disease, see chronic granulomatosis.

**Chromosomal integration:** Permanent integration of viral or introduced foreign genes into the chromosomes of the recipient.

**Chronic granulomatosis:** Genetically caused disorder of oxygen radical formation by phagocytes. As a result of the disturbed phagocyte function, the patients are strongly susceptible to infections and suffer from inflammatory diseases.

**Clinical study of phases I, II, III and IV:** Studies on the efficacy and toxicity of drugs in humans. These studies are subject to strict regulations. In phase I the toxicity or tolerance of new active substances is tested on a small number of healthy subjects. Based on the results of phase I, in phase II a larger number of study participants are used to determine the optimal dose. In phase III the actual effect is determined by using a sufficiently large number of patients with certain inclusion and exclusion criteria in order to obtain a statistically valid analysis. As required, this includes a comparison with a dummy medication without any active ingredients (placebo). Only based on a successful phase III study is the approval of a new drug possible. Thereafter, it is possible to further investigate or observe the effects of a new therapy in its approved form. This is a so-called phase IV study.

**DeReG:** German Registry for Somatic Gene Transfer Studies ([www.dereg.de](http://www.dereg.de)). This registry is publicly accessible.

**DG-GT:** German Society of Gene Therapy e.V.

**EMA:** European Agency for the Evaluation of Medicinal Products (European Medicines Agency), drug approval authority of the EU.

**EudraCT:** EU-wide registry for clinical studies of the EMA. This registry is not accessible to the public.

**Ex vivo gene transfer:** Gene transfer procedure in which the target cells (usually of the hematopoietic system) are initially isolated outside of the body and are then genetically altered with the vector and, if necessary, concentrated. Then these cells are once again administered to the body.

**GCP:** See Good Clinical Practice.

**Gene expression:** Implementation of the genetic information, usually in the form of proteins, to form cell structures and signals.

**Gene therapy:** Treatment involving the introduction of genes into the tissues or cells with the objective of obtaining a therapeutic or preventive benefit as a result of the expression and function of these genes.

**Gene transfer:** The methodical procedure of introducing genes into cells.

**GenTG:** Genetic Engineering Act. A current version can be found at [www.gesetze-im-internet.de/gentg/index.html](http://www.gesetze-im-internet.de/gentg/index.html).

**Germline gene transfer:** Gene transfer in germ cells (egg or sperm cells or their precursors). Alterations of the genotype would also be passed on to successor generations. Germline transfer is legally prohibited in Germany.

**GLP:** See Good Laboratory Practice.

**GM-CSF:** Granulocyte-macrophage colony-stimulating factor. A so-called cytokine which stimulates the growth of macrophages and, thereby, can induce an immune response against certain types of skin cancer.

**GMP:** See Good Manufacturing Practice.

**Good Clinical Practice:** International rules for preparing and conducting clinical studies in accordance with ethical and practical considerations based on the present state of scientific knowledge. Additional details can be found at [www.emea.eu.int/pdfs/human/ich/013595en.pdf](http://www.emea.eu.int/pdfs/human/ich/013595en.pdf).

**Good Laboratory Practice:** International rules and standards for quality assurance of the organisational processes and conditions of non-clinical health and environmental tests. Additional details can be found at [http://ec.europa.eu/enterprise/chemicals/legislation/glp/index\\_en.htm](http://ec.europa.eu/enterprise/chemicals/legislation/glp/index_en.htm).

**Good Manufacturing Practice:** International rules and standards for quality assurance in the manufacturing of medical devices and active substances. Additional details can be found at [www.emea.eu.int/Inspections/GMPHome.html](http://www.emea.eu.int/Inspections/GMPHome.html).

**In vivo gene transfer:** In contrast to *ex vivo* gene transfer (see above), the gene vectors in this case are directly introduced into the body of the patient. Depending on the cell specificity of the vector used, the infection or the integration of the foreign gene then occurs in a more or less targeted manner in certain cell types.

**Monogenic hereditary diseases:** Diseases that are caused by the alteration of a single gene.

**Oncogene:** A gene that usually plays a role in cell cycle regulation and whose activation through mutation contributes to or causes the development of cancer.

**Oncolytic viruses:** Viruses that can infect and switch off tumour cells in a targeted manner.

**Plasmid DNA:** DNA that is not integrated in a genome, but instead is present as an independent, ring-shaped structure in a cell. This is usually not duplicated in cell division and thus dissipates after several cell divisions - unless the plasmid DNA is permanently integrated into the genome.

**Proto-oncogene:** A gene which through a mutation can be altered to an oncogene (see above).

**Retroviral vectors:** Gene vectors that originate from retroviruses. Retroviruses are RNA viruses. However, their RNA genome is transcribed to DNA and permanently integrated into the genome of a cell. Retroviral vectors based on murine leukaemia viruses infect many different cell types, partially with a very high efficiency. But they cannot infect cells that are not actively dividing (for example, nerve cells). However, this can be achieved by using HIV-based lentiviral vectors.

**Somatic cells:** Body cells whose genetic information cannot be inherited by successor generations. They form the majority of human cells; only germ cells (egg and sperm cells) can transfer genetic information to the next generation and form the so-called germline (see above).

**Somatic gene therapy:** Application of gene transfer to somatic cells (see below). Genetic alterations are not passed on to offspring.

**T-cell leukaemia:** Blood cancer in which the regulation of white blood cell (T cells) reproduction is out of control and leads to a flooding of the blood and lymphatic system with degenerated cells.

**Therapeutic index:** The therapeutic index (also therapeutic window or therapeutic quotient) of a drug describes the ratio of its therapeutic to its toxic dose. The larger the therapeutic index, the less dangerous is the drug.

**Vector:** A vehicle that transports a therapeutic gene into the cells of the patient. In addition to different viruses that are mostly incapable of reproduction, plasmid DNA (see above) is used, as a non-viral vector, either in pure form or mixed with other reagents.

**X-SCID:** A hereditary, severe, combined immune disease (SCID, severe combined immunodeficiency). Due to the mutation of a gene for a common building block of several different types of interleukin receptors, no defence cells of the immune system can be formed. Affected patients - usually children - are highly susceptible to infections. The underlying gene is located on the X chromosome which explains the designation X-SCID.

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