

Chapter 2

History of Gene Therapy

It is difficult to pinpoint the beginning of gene therapy, but 1967 can be considered as the beginning of at least the discussion about gene therapy. Marshall Nirenberg, who won the Nobel Prize in physiology in 1968, wrote in a 1967 paper about “programming cells with synthetic messages,” recognized the usefulness of this procedure but also discussed its potential pitfalls and dangers [22].

Between 1970 and 1973, the American physician Stanfield Rogers collaborated with a German physician to develop a treatment for hyperargininemia. Two sisters suffering from this disease were chosen to be treated, in an experimental procedure, with Shope papilloma virus (SPV). Although Rogers called it a “wart virus,” this virus can cause malignant transformation of cells in humans. Rogers believed that the virus would cause expression of the gene that regulated the production of arginine. This could later be demonstrated to be a false assumption. A final paper in 1975 reported the gene therapy experiment failed [23].

In the 1970s, research into recombinant DNA was moving forward. Extreme foresight has to be credited to the National Institutes of Health (NIH) in 1974 when it took the lead in regulating recombinant DNA research. A regulatory oversight body was created, which was called “the Recombinant DNA Advisory Committee (RAC) to the NIH Director,” with RAC members initially being experts in mainly recombinant DNA technology. Over time membership was expanded to individuals coming from a wide range of scientific and medical disciplines, including ethicists and members of patient and other lay communities.

The RAC was initially put in charge of approving research projects involving recombinant DNA in NIH-funded laboratories in the United States. The RAC then got involved in regulating gene marking research projects and finally started to review gene therapy protocols together with the United States Food and Drug Administration (FDA). While the RAC would review the soundness and merit of the scientific aspect of the recombinant DNA technology applied, the FDA would focus on the safety and efficacy of the genetically modified products, including their manufacturing processes. Regulations that both the RAC and the FDA apply are based on the guidelines on human experimentation that stem from the work of the National

Commission for the Protection of Human Subjects, as documented in the Belmont Report from 1978. Specific regulations were established that stipulated that recombinant DNA research proposals had to go through several review processes (US Office of Science and Technology Policy 1991). For a clinical trial involving recombinant DNA, approval was first required by the home institution's Institutional Biosafety Committee (IBC) and the Institutional Review Board (IRB); final approval was then required by the RAC. These regulations applied to all NIH-funded institutions involved in recombinant DNA research, even if the specific project in question was not funded through NIH money and even if the research was not taking place in the United States.

A first attempt of applying human gene therapy was conducted in 1980 under rather questionable circumstances by Martin Cline at the University of California, Los Angeles (UCLA). Without obtaining approval from the UCLA IRB and the other regulatory bodies, Cline performed a recombinant DNA transfer of the beta-thalassemia gene into bone marrow cells of two patients with beta-thalassemia, in Italy and Israel. At the time, IRBs had not yet been established in Italy; additionally, the principal investigator did not fully disclose the exact nature of the gene transfer clinical trial he was planning to conduct to the IRB in Israel. In October of 1980, the Los Angeles Times received information about these studies and published the details of Cline's recombinant DNA treatments [24]. Cline suffered grave consequences. He was forced to resign his department chairmanship and lost some grants, and for a period of 3 years, all of his applications for grant support were accompanied by a report of the investigations into his activities from 1979 to 1980.

In light of Dr. Cline's experiment, and at the prompting of the National Council of Churches, the Synagogue Council of America, and the United States Catholic Conference, the President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research, a congressionally mandated group founded in 1978 and working independently from 1980 to 1983, became involved with the issue of gene therapy. The group released a landmark study called "Splicing Life" in 1982 [25] which defended the continuation of gene therapy research strongly. In this study, the laboratory risks associated with gene therapy research were reviewed carefully, it responded to concerns that scientists were "playing God," and it was concluded that a distinction can be made between acceptable and unacceptable consequences of gene therapy research. The President's Commission also suggested that the RAC should broaden the scope of its reviews to include ethical and social implications of gene therapy.

In 1984 the RAC created a new committee, called the Human Gene Therapy Working Group (later called the Human Gene Therapy Subcommittee (HGTS)), specifically to review clinical gene therapy protocols [26]. The first task of the Working Group was to produce a reference document, "Points to Consider for Protocols for the Transfer of Recombinant DNA into the Genome of Human Subjects." The intention of this document was to guide investigators applying for RAC approval of clinical gene therapy protocols [27].

In fall of 1985 the RAC Subcommittee had finished and published its "Points to Consider" document and was waiting to receive clinical gene therapy protocols for

review. It took almost 3 years until the first protocol that was presented to the RAC in 1988 was a gene marking study by Steven Rosenberg. He proposed to use gene marking techniques to track trafficking of tumor-infiltrating blood cells in cancer patients. However, no actual “gene therapy” was proposed. After several months of discussion among the HGTS members and requests for additional information from the investigator, the protocol was finally approved in December of 1988 via mail ballot. The gene marking study was initially off to a bad start, since a lawsuit filed by the Foundation on Economic Trends questioning the validity of the review process halted it. Eventually, Rosenberg was allowed to continue and performed the gene marking in humans with fruitful results [28].

We will now mainly focus on the history of retroviral- and lentiviral-mediated clinical gene transfer studies. It should be pointed out, however, that many other gene therapy clinical trials utilizing nonviral vector gene transfer, adenoviral vector, and adeno-associated viral vectors have been carried out to this day with the majority of them testing novel gene therapy approaches for cancer, metabolic diseases, or hemophilia.

In 1990 the HGTS received two protocols to review. The first protocol was from Michael Blaese and W. French Anderson for T lymphocyte-directed gene therapy for ADA SCID. Severe combined immunodeficiency (SCID) caused by ADA deficiency is a monogenic disease, which means that just one gene in the human genome is defective; in this particular disease, it is the gene responsible for the production of the enzyme adenosine deaminase (ADA). ADA is needed as a detoxifying agent in the maturation process of T cells. If ADA is lacking, T cell function is severely impaired, leading to the absence of a cellular immune response, leaving patients vulnerable to recurrent opportunistic infections and even death from these infections. Initially it was thought that the gene should be inserted into autologous hematopoietic stem cells (HSCs) to improve upon allogeneic bone marrow transplantation which can cure ADA deficiency but is also associated with high morbidity and mortality in ADA patients. Disappointing gene transduction and engraftment results in nonhuman primates, however, convincing the researchers not to use autologous HSCs, since retroviral vectors could not transduce them efficiently. HSCs are mainly resting cells, and retroviral vectors can only integrate into dividing cells. It was decided that autologous peripheral blood T cells would be a better target, as they can be stimulated in culture to divide. It was possible to obtain enough peripheral T cells from ADA patients using an apheresis procedure. The HGTS reviewed the protocol, which was really the first human gene therapy protocol, and approved it. Two children with ADA SCID were then treated with T cell gene therapy for ADA SCID in 1990. Transduced autologous T cells were infused, and the children were followed for the survival of transduced T cells and any signs of clinical benefit. It could be demonstrated that transduced peripheral blood T cells do persist *in vivo* over years and produce ADA. However, the level of ADA in the patient remained too low to contribute to a clinical benefit. Both patients did not show any adverse reactions to the treatment [29].

The other clinical gene therapy protocol was received again from Steven Rosenberg. He wanted to use the same tumor-infiltrating blood cells he had

previously investigated in his gene marking study as delivery vehicles for a tumor necrosis factor gene designed to kill tumor cells. His protocol was also approved [30].

At this point it should be mentioned that every clinical gene therapy study presented to the RAC for approval also has to be reviewed and approved by the local Institutional Review Board (IRB), by the Institutional Biosafety Committee (IBC), and by the FDA, which is the final authority for approval of such a study through an Investigational New Drug (IND) application.

In 1993 Andrew Gobeia was born with ADA SCID; prior to his birth, genetic screening had already shown that he had SCID. By that time, research conducted by Donald Kohn and Jan Nolta at Children's Hospital Los Angeles suggested the possibility that novel HSC culture methods allowed for normally resting HSCs to be driven into cell cycle. This made gene transduction with retroviral vectors a possibility. A clinical trial for stem cell gene therapy for ADA SCID was presented to the RAC, to the FDA, and to the other regulatory agencies and was approved. Umbilical cord blood, which contains hematopoietic stem cells, was collected from Andrew's umbilical cord immediately after birth. The HSCs were selected using the first clinical grade CD34+ selecting device, made by CellPro, a company which has since gone out of business over the CD34+ antibody patent fight. Selected CD34+ cells were cultured and transduced with a retroviral vector transferring the ADA gene. After transduction, the cultured cells were washed and infused into the patient. Gene marking in the peripheral blood T cells appeared and was stable for years. Three other children with ADA SCID were also treated with the same stem cell gene therapy approach, and similar results were obtained. As standard of care, all children that had undergone stem cell gene therapy were still kept on injectable PEG-ADA enzyme to maintain functional T cells and to prevent opportunistic infections. However, after stable gene expression, although low, could be measured consistently, withdrawal of injectable PEG-ADA was tried. Gene marking and ADA expression in the peripheral blood increased, as a selective survival advantage allowed for the transduced, gene-expressing cells to expand, but the numbers of gene-expressing T cells did not reach therapeutic levels. The children had to be put back on injectable PEG-ADA. In spite of not achieving the wanted clinical outcome, this first stem cell gene therapy study still paved the way for all other stem cell gene therapies to follow [31].

Similar results were obtained in initial attempts to correct chronic granulomatous disease and Gaucher disease. The therapeutic genes were again inserted into hematopoietic stem cells; however, those genes did not confer a selective advantage upon gene-transduced cells. In a Phase I clinical trial for X-linked chronic granulomatous disease (X-CGD), the investigators added flt3-ligand and granulocyte-macrophage colony-stimulating factor into the transduction culture medium and utilized fibronectin as a cell attachment matrix in their 4-day transduction protocol. The patients underwent multiple cycles of mobilization and infusion of transduced cells without preconditioning. Nine months after transplantation, the levels of gene-corrected neutrophils were only 0.06–0.2 %, which was well below the desired 5–10 % required for therapeutic effects [32]. In patients with Gaucher disease, there was also very low gene marking, little or no gene expression, and no clinical benefit [33].

In the early to mid-1990s, gene marking trials were carried out in patients undergoing autologous HSC transplantation for cancer. These trials had a dual purpose: first, to investigate the source of cancer relapse and, second, to explore gene transfer efficiency into hematopoietic stem cells and gene marking persistence. Even with full bone marrow ablation, patients showed levels of marked blood cells in the periphery well below 1 %. Sometimes there was not even any gene marking [34–36]. However, taken together these studies were able to demonstrate that autologous mobilized peripheral blood CD34+ cells contained long-term repopulating stem cells which could be transduced, to some degree, with retroviral vectors. They also clearly demonstrated that better gene therapy vectors and better transduction conditions for HSCs had to be developed.

In 1997, the first child in the world was treated with stem cell gene therapy for HIV at Children's Hospital Los Angeles. An HIV-1 RRE decoy gene was transferred into CD34+ cells from the bone marrow of HIV-1-infected pediatric patients in a Phase I feasibility and safety study to evaluate potential adverse effects from such a gene transfer procedure. Feasibility was defined as the ability to obtain an adequately large bone marrow aspirate from which enough CD34+ cells could be isolated using a closed system magnetic cell separator. It was estimated that at a minimum, 1×10^6 CD34+ cells per kg body weight would be needed. Efficacy was assessed by determining whether the cultured, gene-transduced bone marrow cells would engraft and produce white cells in the peripheral blood expressing the transferred anti-HIV gene. To test the hypothesis that anti-HIV-1 gene-expressing cells would have a selective survival advantage in a patient with HIV infection, a comparative marking approach was used. Half of the cells were transduced with a control vector transferring neomycin resistance, while the other half was transduced with a vector transferring the anti-HIV gene in conjunction with neomycin resistance. It should be pointed out that at that time, the transfer of neomycin resistance was allowed by the regulatory bodies and could be used as a marker for the detection of transduced cells. If there were a selective survival advantage of anti-HIV gene-expressing peripheral cells, these would accumulate preferentially as compared to neomycin gene-expressing cells. Sufficient numbers of CD34+ cells from the bone marrow of this child were isolated successfully and could be transduced with the retroviral vector. The transduced CD34+ cells were administered to the child without preconditioning. Gene marking in the peripheral blood developed over the following weeks and could be detected at low levels, which was expected as no preconditioning of the bone marrow was used. However, gene marking declined significantly and disappeared after several months. A selective survival advantage of the gene-transduced cells in the face of a viral load did not develop, since the patient had to take antiretroviral therapy (ART) and had no viral load, as this was demanded by the FDA due to ethical reasons [37]. This and other stem cell gene therapy clinical trials for HIV will be discussed in greater depths in the following chapters.

In 1998, the first adult was treated with stem cell gene therapy for HIV at City of Hope National Medical Center. A ribozyme directed against the *tat/rev* region of the HIV transcript was used as the anti-HIV gene. CD34+ cells were collected through

apheresis and a CD34+ cell isolation was performed. In this Phase I clinical trial, five adult patients were enrolled. No marrow conditioning was applied. Low gene marking in the peripheral blood was detected and persisted over several months [38].

In 1999, the first marrow-ablated adult with HIV-induced B cell lymphoma was treated with stem cell gene therapy for HIV and autologous bone marrow stem cell transplantation. The same anti-HIV gene was applied as in the previous City of Hope clinical trial. After cell infusion, high gene marking could be detected in the peripheral blood, with excellent expression of the anti-HIV gene in the transduced peripheral blood cells. However, gene marking decreased and almost completely disappeared after several months. In neither clinical trial, withdrawal of ART was allowed; therefore, a selective survival advantage of anti-HIV gene-expressing cells could be demonstrated [38].

Also in 1999, another pediatric clinical trial of stem cell gene therapy for HIV was conducted at Children's Hospital Los Angeles. Children with a low HIV viral load while on ART were the target population. The anti-HIV gene was a transdominant negative Rev protein, the best anti-HIV gene at that time. One child was treated with gene-transduced CD34+ cells isolated from a bone marrow aspirate. No marrow conditioning was used. Low-level gene marking developed in the peripheral blood after several weeks and again disappeared after a few months. The patient, however, stopped the antiretroviral medication at around 12 months after cell infusion, due to side effects of the medication. The patient was still followed at Children's Hospital for the gene therapy study. Interestingly, the HIV viral load had increased significantly, and gene marked peripheral blood T cells again appeared. The numbers of gene marked peripheral blood cells correlated with the increase in viral load. This demonstrated, although only anecdotally, as this was a single patient, the predicted selective survival advantage of anti-HIV gene-expressing HIV target cells in the face of a viral load [39].

In October 1999, the death of 18-year-old Jesse Gelsinger was reported [40]. Gelsinger had participated in a gene therapy clinical trial using adenoviral vector for the treatment of ornithine transcarbamylase (OTC) deficiency. A very high dose of adenoviral vector that was known to the PI having caused fatal adverse events in a primate study was administered to the patient intrahepatically. Gelsinger died 4 days after the infusion from a massive immune reaction. Gelsinger was not a patient that absolutely needed gene therapy since his condition was not as severe and could be treated by diet restriction and medication. It was the PI who persuaded Gelsinger to participate. Clearly, the PI had neglected to inform the FDA, the RAC, and all other regulatory bodies about the adverse reactions in the primate experiment and had neglected good clinical practice by persuading Gelsinger to participate in the clinical trial. Gelsinger's death therefore raised questions about researcher entrepreneurial activities and conflict of interest and about government oversight procedures. The United States Senate held hearings on this topic on February 2, 2000, and the FDA sent out the "March 6 letter" to all investigators conducting gene therapy clinical trials announcing new and heightened scrutiny in gene therapy oversight. This has resulted in increased reporting of adverse effects and renewed control by both the NIH RAC and the FDA. Gelsinger's death also resulted in federal charges

being brought against the principal investigator and the university who conducted this trial [41]. Finally, a settlement with the US Office of the Attorney General was reached in February of 2005.

The success of a multicenter clinical trial of stem cell gene therapy for children with X-linked SCID conducted from 2000 to 2002 in France was put into question when two of the ten children treated at the site in Paris developed a leukemia-like condition caused by clonal expansion of gene-expressing T cells. All clinical trials of stem cell gene therapy were halted but were allowed to continue after re-reviews of the affected gene therapy protocols in the United States, the United Kingdom, France, Italy, and Germany [42].

In 2007, a death in a gene therapy trial for arthritis was investigated and found to be caused by factors not related to the gene therapy clinical application. This case prompted discussion of including procedures for investigating other illnesses that might occur during a gene therapy study in the clinical trial protocol [43].



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