

Chapter 2

Introduction to Gene Therapy: A Clinical Aftermath

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Abstract

Despite three decades of huge progress in molecular genetics, in cloning of disease causative gene as well as technology breakthroughs in viral biotechnology, out of thousands of gene therapy clinical trials that have been initiated, only very few are now reaching regulatory approval. We shall review some of the major hurdles, and based on the current either positive or negative examples, we try to initiate drawing a learning curve from experience and possibly identify the major drivers for future successful achievement of human gene therapy trials.

Key words: Gene therapy, Clinical trials, Viral and nonviral approaches, Systemic delivery, Local delivery, Ex vivo gene therapy

1. Three Decades of Human Clinical Gene Therapy

The invention of recombinant DNA technology (1) consequently led to the immediate inception of engineered gene transfer into human cells, aiming at reversing a cellular dysfunction or creating new cellular function. The concept of direct therapeutic benefit based on a gene defect correction in human cells or on gene therapy was born.

Exactly 30 years ago, Martin Cline made a first early and certainly premature human gene therapy attempt in 1979 at treating severe thalassemia patients through an ex vivo β -globing gene transfer protocol in the bone marrow of two patients in Italy and Israel (2). As the protocol had not received any otherwise mandatory approval by regulatory bodies, the study was promptly terminated and Cline was forced to resign his department chairmanship at UCLA (University of California, Los Angeles) and lost several research grants. Subsequently, the Recombinant

DNA Advisory Committee (RAC) at the National Institute of Health (NIH) was urged in 1980 to expand its regulatory function beyond recombinant DNA experiments so as to include human gene therapy studies.

In 1982, a seminar was held at the Branbury Conference Center of Cold Spring Harbors Labs. A group of scientists, led by Ted Friedmann and Paul Berg, came together to build the foundations of gene therapy and to draw what its future might be. As an outcome, the first book on gene therapy (3) was and is still a landmark reference to this field.

In 1989, Rosenberg et al. initiated the first RAC-approved gene therapy clinical trial, which was actually a “gene-labeling” study targeting a neomycin-resistance gene transfer into tumor-infiltrating lymphocytes using a retroviral construct, for the treatment of metastatic melanoma with Interleukin-2 (4).

Effectively, a therapeutic gene clinical trial took place in 1990 to treat severe combined immunodeficiency (SCID) by transferring the adenosine deaminase (ADA) gene into T-cells using a retroviral vector. No significant clinical benefit was observed, albeit the protocol appeared to be safe for the patients (5, 6).

These pioneer clinical studies, as well as some others, land-marked the inception in the 1990s of a major burst of academic, clinical, biotechnological, and sustained financial efforts lasting for more than two decades (7). Even today, there are thousand clinical trials registered as ongoing. Among which, 65 trials that are declared in late stage (i.e., phase II–phase III) have proven to be safe and would be in the clinical benefit evaluation phase (Table 1).

Factually, one can also notice a sustained input of about a hundred new clinical trials per year since 1999 (7). This seems in

Table 1
Number of gene therapy trials worldwide (7)

Phase	Gene therapy clinical trials	
	Number	%
Phase I	928	60.4
Phase I/II	288	18.7
Phase II	254	16.5
Phase II/III	13	0.8
Phase III	52	3.4
Single subject	2	0.1
Total	1,537	

clear contrast with the commonly held opinion that gene therapy would be no longer active because of disengagement, especially from certain large pharmaceutical industries, after a “1990s golden age.”

Despite this constant entry flow into clinical trials, the quasi-absence of a registered drug after 20 years is quite compelling and worth revisiting from a pure clinical development strategy perspective.

Most of the initial failures were most probably due to very naive “science-driven” approach to clinical practice, but even today, many projects are simply blocked because of fundamental absence of translational research practice and still a strong underestimation of some key technical challenges. The rest of this book addresses the fundamentals to be considered at the molecular biology and the bioengineering level, but one should also pay attention to the most standard clinical development parameters, which sometimes are simply lacking in the project development plans.

In the late 1990s, a news & views section in a major journal was entitled: “Gene therapy has been keeping for long pretending to be 5 years from the clinics.” With more than a thousand clinical trials launched, the goal is no longer to enter man study for the sake of a nice publication. The goal is set to complete successfully human clinical trials and get to product registration, which we are closer now than ever.

2. Gene Therapy: Definition and Basic Prerequisites

As a source of major hope for many incurable human diseases, the concept of human gene therapy was immediately perceived as the highest promise for curative treatment: a therapy acting at the root of the genetic dysfunction.

The concept of gene therapy is relying on gene intervention. From a pure pharmacokinetic point of view, nucleic acid has a poor cell penetration capacity. For the past 30 years, an incredible armada of viral and nonviral vectors has been engineered to formulate the nucleic-acid-based “active principle.” Therefore, virus-derived gene delivery vectors were thought from the beginning to be optimized biomimetic vehicles. However, since they have also evolved under a very high selection environment of infectious agents, humans are also naturally equipped with very sophisticated defense systems. These defense systems, which are often specific to higher primates, cannot be ignored in the context of a gene therapy clinical development plan, especially when it comes to use of a natural human-derived virus. Other hurdles are the active virus loads and the amount of virus particles to be used to achieve therapeutic effects, which combined with the administration

route are very difficult to predict in terms of clinical pharmacology and drug safety, imposing extremely careful clinical development protocols.

As foreign DNA cannot stay freely in a dividing cell, it does not get associated with the host DNA replication machinery. On one hand, one has engineered integrative vectors enabling the “therapeutic gene” to be integrated into the host DNA, thereby enabling long-term expression potential (e.g. use of oncoretroviral or lentiviral vectors). A major drawback is the random insertion into the host genome that can lead to serious adverse effect (SAE) (8). On the other hand, one has tailored “nonintegrative vectors,” which are mainly used to transfer DNA into quiescent cells but which will be lost after a few replication cycles in dividing cells (e.g. adenoviral or adeno-associated viral vectors).

The nature of target tissue/cell and the length of desired therapeutic effect have, therefore, to be taken into consideration in the gene therapy project charter.

In addition, the routes of administration of a therapeutic principle can have major consequences both in terms of efficacy and safety. Routinely, one classifies gene therapy protocols into three main categories: ex vivo, local in vivo, and systemic in vivo administrations (see Table 2).

In other words, the field has been facing major challenges, from novelty to translational research, which have often been complicated by specific ethical concerns (9) led by the subjective perception of gene therapy practice as a “Sorcerer’s apprentice.”

For the sake of clarity, we now focus on specific sets of examples, including dead ends, mixed successes to the most promising, clinical studies that are intended to contribute to the frame into which the field should continue to contribute to the improvement of human health.

3. Current Status: Clinical Trials and Case Studies

3.1. Systemic Delivery Has Not Been Delivered

After several years of clinical attempts, lack of clinical efficacy, major SAEs, and often unsurmounted industrial bioproduction issues, one should ask the question of clinical plausibility of systemic gene therapy protocols. The treatment of human diseases often requires systemic administration procedures, and most often oral or intraparenteral routes. Using viral or nonviral approaches via the oral route, no protocol has yet been able to achieve satisfactory results in preclinical studies; therefore, most studies have focused on parental routes. Given the classical multiplicity of infection (MOI) in the range of 10–10,000, authors are considering a routine dose ranging from 10^8 to 10^{15} viral particles per kg of body weight. This effective dose definition immediately triggers

Table 2
Routes of administration used in gene therapy protocols

Routes of administration		Ex vivo	Local in vivo	Systemic
Definition	Gene transfer is performed out of the living organism; the therapeutic agent is the “reinfused cells”		Gene transfer product is injected into a local, and possibly isolated body compartment (intramuscular (IM), intratumoral, locoregional, stereotactic)	Product is administered through oral or intraparen-teral route so that it can reach all parts of the body
Examples	SCID-ADA protocol		IM: NV1FGF Locoregional: Duchenne’s dystrophy with plasmids or AAV Stereotactic: Parkinson’s disease with AAV	AAV-FIX
Comments	Autologous cells are handled in a dedicated processing center The efficacy of treatment is related to the ability of transduced cells to perform sustainable effects		Local administration is preferred if therapeutic benefit can be achieved Local immune reaction can be specific Product leakage has to be documented	Dose-limiting rate and major reaction to large viral load are commonly encountered, generally limiting the practical translational approaches, from mouse-based experiments to human clinical development

several major technical, pharmacological, and immunological hurdles to consider. We can schematically classify them as follows:

- Mastering an industrial bioprocess that is scalable to the Good Manufacturing Practice (GMP)-compliant production of clinical and eventually commercial batches
- Defining a purification process and a formulation that is on line with the vector physicochemical properties and the desired volume to be injected
- Documenting the pharmacokinetics and ADMET (adsorption, desorption, metabolism, elimination, and toxicity) properties of vectors in human at such high doses
- Documenting, in terms of long-term potential side effects, the immunoreactivity against the vector itself or the therapeutic cells, and the fate of the product if it needs to be readministered

Below are two examples of gene therapy concepts that have emerged more than 20 years ago, for which clinical realization is desperately kept on being delayed, i.e., in cystic fibrosis (10) and Duchenne's muscular dystrophy (DMD) (11).

3.1.1. Cystic Fibrosis

Although predominantly used in the pioneering days of CF gene therapy, adenovirus-based vector usage has dropped in the last decade due to poor transduction efficiency in human airway epithelial cells and the inability for readministration. In addition, a study by Tosi et al. raised concerns that antiadenovirus immune responses, in particular cytotoxic T-lymphocyte-mediated (CTL) responses and major histocompatibility complex class I antigen (MHC-I) presentation, may be further enhanced if the host has a preexisting *Pseudomonas* infection (12). These data highlighted potential problems for adenovirus-based vectors in CF gene therapy and definitely confined the use of adenovirus-based vectors for CF gene transfer to upstream research studies.

As a potential alternative to adenovirus, adeno-associated virus (AAV) (13) was assessed for lung transduction in clinical cystic fibrosis gene therapy trials. However, the feasibility of repeated AAV administration is still unresolved, and the limited capacity of AAV to carry the full-length cystic fibrosis transmembrane conductance regulator (CFTR) gene and a suitably strong promoter remains a significant problem. However, Lai et al. (14) have recently shown that the efficiency of AAV *trans*-splicing can be greatly improved through rational vector design and may, therefore, allow the CFTR cDNA to be split between two viral vectors.

So far, two human gene therapy phase I/II protocols have been undertaken with incremental and repeat doses of AAV, up to 2×10^{12} and 2×10^{13} DNase-resistant particles, respectively (13, 15).

In both studies, viral shedding and increases in neutralizing antibodies were observed, but no serious adverse event (8) was associated to the virus administration. Importantly, a significant reduction in sputum IL-8 and some improvement in lung function were noted after the first administration, but not after the second or third administration.

On the basis of these studies, Targeted Genetics Corporation initiated a large repeat-administration multicentric phase IIb study (100 subjects), sufficiently powered to detect significant changes in lung function. Eligible subjects were randomized to two aerosolized doses of either AAV-CF or placebo 30 days apart. The subjects underwent pulmonary function testing every 2 weeks during the active portion of the study (3 months) and were followed for safety for a total of 7 months. No publication is available 4 years after the study was completed, but the company announced that the trial had not met its primary end point and, therefore, the CF program has been discontinued (16).

There may be several reasons for these new disappointing outcomes: (1) As for adenoviral vector, AAV-2 was still too inefficient in reaching airway epithelial cells via the apical membrane, (2) the inverted terminal repeat (ITR) promoter used to drive CFTR expression was not strong enough, and (3) repeat administration of AAV-2 to the lung was actually not possible owing to the mounting of an antiviral immune response. Finally, on the back of previously published AAV-2 aerosolization studies, Croteau et al. (17) evaluated the effects of exposure of healthy volunteers to AAV2. Based on airborne vector particle calculations, the authors estimated exposure to 0.0006% of the administered dose. At such an infradose, no deleterious health effects were detectable, but this underlies the strong requirement in improving the general ADMET properties of the vector system and the necessity to perform these studies even before going into phase I.

Studies are currently underway to assess the feasibility of repeated administration of lentivirus-based vectors into airways by several groups (18, 19), and further data will be needed before the relevance of such viruses for CF gene therapy can be decided. In addition, the safety profile of virus insertion into the genome of airway epithelial cells will have to be carefully monitored.

With the concept that bone marrow-derived hematopoietic or mesenchymal stem cells may have the capacity to differentiate into airway epithelial cells (20), some groups have entered this very challenging and controversial approach for the treatment of CF (21, 22).

On the nonviral side, parallel work had been made regarding the formulation of vectors (23), and the United Kingdom (UK) CF Gene Therapy Consortium clinical trial program has been carefully comparing these agents and is now assessing whether the

most efficient currently available nonviral gene transfer agent is able to alter CF lung disease. As the extension of gene transfer achieved is still too small and transient to drive any clear therapeutic benefit, most research for CF gene therapy has returned to the laboratory. In UK, there are no more trials ongoing at present, but it remains the goal of the UK Consortium to work together to meet the challenges and enhance progress to a phase III (large-scale) study this year.

Finally, electroporation and some emerging physical delivery methods such as ultrasound and magnetofection have shown encouraging results in vitro and in rodent models, and again, translational research into larger animal models, such as sheep, and hopefully in the clinic is challenging (24, 25).

In perspective as of today, one can expect the promise for a curative therapy for CFTR may not rely on gene therapy, but on “protein-decay” therapy, with the phase II clinical development of a small molecule, miglustat, by Actelion, which has been shown to slow down the mutated protein degradation and enables it to be exported to the membrane (26).

3.1.2. Duchenne's Muscular Dystrophy

DMD is an X-linked inherited disorder that leads to major systemic muscle weakness and degeneration. Muscle fiber necrosis is related to the dystrophin gene deficiency itself (27). Becker muscular dystrophy (BMD) has clinical picture similar to that of DMD but is generally milder than DMD, and the onset of symptoms usually occurs later. The clinical distinction between the two conditions is relatively easy because (1) less severe muscle weakness is observed in patients with BMD and (2) affected maternal uncles with BMD continue to be ambulatory after age 15–20 years. The cloning of the dystrophin gene opened the door for gene therapy (27–30). However, as in systemic disorders, there are major roadblocks including (1) the large amount of skeletal muscle (basically half the body weight of a healthy human being), (2) the involvement of cardiac and the peritoneal muscles in the disease, and (3) the extremely large size of the dystrophin protein, 427 kDa, encoded by a 79 exons gene (28, 31, 32).

In one study, nine DMD/BMD patients were injected with a naked dystrophin gene-carrying plasmid into the radialis muscle. Patients were divided into three cohorts, each injected with one of following three doses: 200 µg once, 600 µg once, or 600 µg twice (2 weeks apart). Biopsies were then retrieved 3 weeks postinjection, and amplicon DNA could be detected only in 6/9 patients. Patients from the first cohort and one patient from the second cohort exhibited 0.8–8% of weak, complete sarcolemma labeling (29), while 3–26% of muscle fibers showed incomplete/partial labeling. The third group showed 2–5% complete sarcolemma labeling and 6–7% showed partial labeling. There were no observed adverse effects to the treatment. The study concluded

that the expression of dystrophin was low (29), and thus, the study was not pursued. One may question why the study was initiated despite the product obviously failed to meet basic efficacy requirements to reach future clinical application and even worse was facing major industrial bioproduction pitfalls given the clinical doses that could be inferred from preclinical studies.

For several years, several preclinical studies have been initiated, and finally several concurrent clinical trials were initiated using various pseudotyped adeno-associated viruses (33) as a vehicle to deliver either truncated versions of the gene (mini or microdystrophin) or an exon-skipping RNA structure, all thought to achieve truncated albeit functional dystrophin protein expression (28, 34, 35). The AAV vector, whatever the serotype, provides superior transduction efficiency to the skeletal muscle but is also a source for potential immune response that remains to be carefully understood (36–38). No conclusive result has been drawn yet from the current clinical studies. However, the intramuscular high-dose pharmacokinetic profile in relevant preclinical models and eventually in humans is yet to be thoroughly documented prior to launching any efficacy clinical gene therapy.

However, the last 5–7 years, reviewed elsewhere (11), have seen unrivaled progress in efficient systemic delivery of synthetic and chemically modified oligonucleotides again used to enforce mutated exon splicing (39). This progress has led to several more clinical trials, which are labeled as “small molecule” trials, i.e., out of the boundaries of gene therapy. The most advanced clinical trial, led by a company called Prosensa in Holland, is completing a phase IIb and has led to finalize a collaborative agreement with GSK in October 2009, marking the return of large pharmaceutical companies in the plain field.

3.2. Gene Therapy Potential Promise to Disease Treatments

The above examples clearly illustrate how gene therapy has progressively moved from “systemic” administration routes toward more pragmatic local administration regimen or to alternative small molecule innovative therapeutics. We now review the most promising local gene therapy clinical protocols.

3.2.1. Parkinson's Disease

Parkinson's disease is primarily due to the local degeneration of nigrostriatal neurons projecting into striatum, and a subsequent shortage of dopamine in this target region. Predisposing and risk factors are numerous but disease mechanism remains unclear. More than a million patients are affected both in Europe and the USA. So far, the main treatment has been oral administration of L-DOPA, a dopamine precursor, but patients generally encounter motor complications after 5 years of treatment. Deep stimulation surgery, therefore, becomes the second phase of disease management for 0.5% of patients in France each year.

The therapeutic challenge is then to trigger continuous release of dopamine into striatum neurons. Gene therapy is a plausible approach, as far as cellular therapies could be. In addition to be continuous, dopamine release should remain local, to avoid dyskinesia effects observed in systemic administration of the precursor in the pharmacologic treatment.

Several clinical trials have been undertaken (40,41). In California, Avigen, later taken over by Genzyme, initiated a trial with an AAV-vector to express the L-DOPA converting enzyme, and another biotechnology company, Ceregene, conducted a phase I open label study with 12 patients, then a phase II trial with an AAV-based vector expressing neurturin (CERE-120), a neuron survival factor (42). Very recently, Ceregene has reported additional clinical data from a double-blinded, controlled phase II trial of CERE-120 in 58 patients with advanced Parkinson's disease. The company, however, announced that the phase II trial did not meet its primary end point of improvement in the Unified Parkinson's Disease Rating Scale (UPDRS) motor off score at 12 months of follow-up, although several secondary end points suggested a modest clinical benefit. An additional, protocol-prescribed analysis reported focused on further analysis of the data from the 30 subjects who continued to be evaluated under double-blinded conditions for up to 18 months, which indicate increasing effects of CERE-120 over time. A clinically modest but statistically significant treatment effect in the primary efficacy measure (UPDRS motor off; $p=0.025$), as well as similar effects on several more secondary motor measures ($p<0.05$), was seen at the 18 months end point. Not a single measure similarly favored sham surgery at either the 12 or 18 months time points. Additionally, CERE-120 appears safe when administered to advanced Parkinson's disease patients, with no significant concerns related to the neurosurgical procedure, the gene therapy vector, or the expression of neurturin in the Parkinson's disease brain. Long-term safety was also performed in a primate model and was satisfactory (43). The company also reported the results of an analysis of neurturin gene expression in the brains from two CERE-120 treated subjects who died of causes unrelated to treatment. These analyses revealed that CERE-120 produced a clear evidence of neurturin expression in the targeted putamen but no evidence for transport of this protein to the cell bodies of the degenerating neurons, located in the substantia nigra. In addition to the known cell loss in Parkinson's disease, and in agreement with the perspectives defined elsewhere (44), these findings suggest that deficient axonal transport in degenerating nigrostriatal neurons in advanced Parkinson's disease impaired transport of CERE-120 and/or neurturin from putaminal terminals to nigral cell bodies, reducing the therapeutic effect of CERE-120.

In parallel to this study, Oxford Biomedica, in collaboration with a group in Hospital H. Mondor in France, has built an

equine lentivirus-based vector to express three genes involved in dopamine synthesis. The product (ProSavin) is administered locally to the region of the brain called the striatum, converting cells into a replacement dopamine factory within the brain, thus replacing the patient's own lost source of the neurotransmitter. A phase I/II study was initiated in December 2007 in France with patients with mid- to late-stage Parkinson's disease who are failing on current treatment with L-DOPA but have not progressed to experiencing drug-induced movement disorders called dyskinesia. After a first cohort of three patients who showed no side effect or an antibody response (42), the dose-escalation stage of the study has progressed to the second dose level. The 6-month data from the first dose level suggest ProSavin is safe and well tolerated and showed encouraging evidence of efficacy (42).

3.2.2. Severe Combined Immunologic Disorders

Another successful albeit often controversial is the case of ex vivo gene therapy. This is the case of severe combined immunologic disorders (SCID) treatment. Soon after the first US trial led by Blaese and colleagues (5), a network of European groups led by A. Fischer in France, A. Trascher in the UK, and M. Roncarolo in Italy initiated similar protocols for the treatment of SCID. The successful treatment of the first patients was greeted with a lot of enthusiasm when it was first reported in 2000 and 2002 (45–47). However, this euphoria turned to a serious alert at the end of 2002 when two of the first ten children treated in France developed SAE, described as leukemia-like conditions (48). As demonstrated later, the insertion of the therapeutic DNA into the patient cells had occurred next to one specific locus LMO2 (the proto-oncogene LIP domain only two locus) (49–51). With the news of this devastating event, most SCID-X1 gene therapy trials were placed on hold worldwide. However, in view of patient overall and lack of alternative treatment, some ADA and SCID-X1 trials were pursued, with extremely careful monitoring and better vector types designed so as to reduce the odds of such adverse effect. Work is now focusing on correcting the gene without triggering an insertional oncogenic event.

Between 1999 and 2007, gene therapy has restored the immune systems of at least 26 children with two forms [ADA-SCID (nine children) and SCID-X1 (ten children)] of the disorder, and four of the ten SCID-X1 patients had developed leukemia-related SAE (52). As of today, 20 children have been treated, four of them have developed leukemia-like adverse effects and one patient has unfortunately died from leukemia. From a clinical point of view, patients, who have been able to lead a normal life for periods up to 3 years, should be considered cured by this pioneering gene therapy treatment. Otherwise, 10 years later, none of these 20 children would be alive today without gene therapy.

Based on this clinical success, several important protocols are now entering the clinical stage. A major example is that of the

Wiskott–Aldrich syndrome (WAS), which is a complex primary immunodeficiency disorder associated with microthrombocytopenia, autoimmunity, and susceptibility to malignant lymphoma. At the molecular level, WAS is caused by mutations in the gene encoding the Wiskott–Aldrich syndrome protein (WASP). WASP is a cytosolic adaptor protein mediating the rearrangement of the actin cytoskeleton upon surface receptor signaling, which in turn is instrumental for cognate and innate immunity, cell motility, and protection against autoimmune disease (53). WASP confers selective advantage for specific hematopoietic cell populations and serves a unique role in marginal zone B-cell homeostasis and function (54).

The success of such blood stem cell transplantation is related to the patient's age, the conditioning regimen precell infusion, and the extent of reconstitution postcell reinfusion. Since WASP is expressed exclusively in hematopoietic stem cells, and because WASP exerts a strong selective pressure, gene therapy is expected to cure the disease (55). Cumulative preclinical data obtained from WASP-deficient murine models and human cells indicate a marked improvement of the impaired cellular and immunological phenotypes associated with WASP deficiency. A first clinical trial is currently being conducted with a retroviral construct (55, 56) with a careful monitoring of insertional events (57). However, capitalizing on experience with SCID-ADA and establishing a solid European network, A. Galy and colleagues have engineered, validated, and GMP-produced a very potent lentiviral product (58) and a three-site clinical study is due to start in 2010 (59).

3.3. Two Clear-Cut Examples of Products Successfully Reaching Registration

As stated above, the most promising gene therapy clinical results are obtained with local delivery procedures. In addition to the above examples, two key examples of successful development of candidate drugs up to the phase III are in the field of vascular/metabolic disorders.

3.3.1. Lipoprotein Lipase

The first example is that of lipoprotein lipase gene for the treatment of familial lipoprotein lipase deficiency. The product initially cloned into adenovirus and retroviruses by us in the 1990s (60–62) is now carried onto an AAV vector (63). Very encouraging data have been obtained through a direct multiple intramuscular (IM) injection in the inner limb with corrective expression obtained for several weeks postinjection (64), and the product registration has been started by European Medical Agency (EMA) in January 2010 as a centralized procedure, which is the standard route for all advanced therapies.

3.3.2. Peripheral Vascular Disease

The second example is that of peripheral vascular disease (PVD), which is predominantly affecting the lower extremities. PVD has a relatively low mortality but results in considerable morbidity and disability.

Even though angioplasty and reconstructive surgery are somewhat effective treatment options for many patients with peripheral arterial insufficiency, these procedures are associated with considerable risks, notably restenosis after peripheral angioplasty. In addition, the severity and progressive nature of this disease often limit these treatment options, resulting in persistent, disabling symptoms or limb loss. PVD, therefore, represents an attractive target for a gene therapy approach to restoration of effective limb perfusion in selected patients (65).

Dr. Jeffrey Isner and his colleagues have taken a novel approach (66) to the problem of peripheral artery insufficiency with encouraging results. This group has been at the forefront of angiogenic gene therapy for peripheral artery insufficiency, publishing several studies over the past 15 years that have set the ground (65–69) for the clinical study by Sanofi-Aventis.

Fibroblast growth factor 1, FGF1, is a proangiogenic factor acting on various cellular subtypes, and more particularly involved in preexisting microvessels sprouting, microcapillary network genesis, and arteriolic maturation. Pharmacodynamic studies of an FGF-encoding plasmid (70, 71) in two animal models confirmed the therapeutic potential of such a vector (70, 71). Several preclinical toxicity studies were also performed to document vector lack of integration as well as lack of neither oncogenic nor retinopathic potential of the product.

Two human clinical trials (phase I–IIa) were performed and have documented good tolerance to NV1 FGF as well as local angiogenesis effects limited to the injection point, confirming product safety (72, 73). Consequently, a first phase II double-blinded clinical study was performed with 125 patients, to document product efficacy and has achieved a remarkable twofold reduction of amputation in the treated group vs. placebo (74).

As of today, a large-scale pivotal phase III trial, called TAMARIS, is ongoing (75) since November 2007 (490 patients, 130 clinical centers) to document reduction of amputation and increase of life span. The study is aimed to be completed by July 2010 (76). These results, if proven positive, will most probably result in a long-awaited milestone, i.e., the registration of the first gene therapy product for a large clinical indication.

4. Future Developments and Prospects

Several lines of observations can be drawn from these past 20 years of clinical trials.

First, yet the primordial concept was meant to tackle inheritable genetic disorders, seen as *low-hanging fruits* for a fast clinical proof of concept, most of the clinical protocols have been

addressing acquired complex disorders, e.g. cancer, cardiovascular, neurodegenerative diseases.

Second, even though the science was sort of intuitively genuine, clinical gene therapy is now understood as a “difficult” clinical development field, and there is still a trend from private investors to stay away from this area, although major clinical successes are now emerging, such as for the SCID and now peripheral artery diseases (PAD).

Third, the driving force has remained often too long in the hands of academic research, and thus, clinical development has been failing repeatedly because of translational research issues, such as good laboratory practice (GLP) preclinical, clinical development, and GMP lack of expertise.

Fourth, although viral vector are considered as best in class to achieve efficacy in men, major adverse effects have been encountered such as vector-related oncogenesis in some trials and complex immunologic responses to the virus in most of systemic and local administration protocols.

However, watching the drug pipeline from the market approval end, several investigational new drugs are by now registered or close to approval, namely, RTV-ADA treated cells from the treatment of SCID-ADA in Italy (52), the AAV-LPL product in Europe (64), and NV1FGF for the treatment of PAD (76, 77).

In the new perspective of true clinical realization and positive learning experience, the mastering and practical application of the right set of tools such as vector design and scale-up production will become true strategic advantages for future gene therapy projects.

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