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2005: Gene therapy is growing teenage, what have we learned?

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ABSTRACT

Molecular biology of recombinant DNA exists since about thirty years. In the last twenty years this know-how has been progressively applied to medicine. We can distinguish four major periods: a) the era of 'genes as probes' (started in the 80ties) where molecular genetics has been first used for precise diagnostics of monogenic diseases; (b) the era of 'genes as factories' (started in the 90ties), where genes transferred into cell cultures have permitted the industrial production of biopharmaceuticals; (c) the era of 'genes as drugs' (coming into clinics in the 90ties) where gene transfer into human tissues and organs should permit the cure or treatment of otherwise untreatable diseases. The era of 'genes as drugs', better known as 'era of gene therapy' has indeed started to enter clinical trials in 1990. Fifteen years later we can count over 900 trials and about 4500 experimentally treated patients. In spite of that, gene therapy is still far from being widely clinically applicable, in spite of a recent commercialisation of a gene medicine in China. This report summarizes which are the basic ingredients and players in somatic gene therapy, and what have been the achievements and frustrations in this research field. The conclusions are that the potential of this approach is indeed very high, although this is no longer collectively perceived by the medical field.

Foreword

The practical utilisation of empirical genetics is as old as civilisation. However, molecular genetics is a research field that has marked only the last three decades of 1900 . In spite of its rather short history, it has produced an incredible number of effects and has transformed biology from a dusty, museum-like discipline into a billions-generating business. Among the most recent applications of gene technology we count therapeutical human gene transfer (also called 'gene therapy'). This concept, although it has not yet reached the clinical application, has already suscitated an intense debate which often was marked by inappropriate spectacularisation of positive and negative events (slide 2).

Genetics, genes, genomes

The research has led us to a number of principles that we shall briefly recapitulate in the next slides. The basic principle in genetics is based on the dogma of the information flow, by which a segment of DNA (= a gene, slide 3) can generate several copies of a specific mRNA (a transient transcript) which in turn can be translated into corresponding polypeptides. The concept of '1 gene -> 1 function' that most of us learned in the schoolbooks has become definitively obsolete. Today we know that one DNA segment can give rise to different forms of RNA and that these RNAs can be translated alternatively into different forms of proteins. Recently it has been also realised that many proteins have more than one distinct function, depending on the context of co-factors present in the surroundings. Thus, we'd better say: '1 gene encodes from one to several functions'. This multiplicity of functions may become an important player in terms of side effects when aiming to use gene transfer as a therapeutical treatment.

The structural and functional elements of a paradigmatic gene are illustrated in [slide 4](#), where we recapitulate the concepts of regulatory sequences, transcription factors and coding sequences. In this view, a gene is defined as the information circuit of a nanodevice that produces defined amounts of mRNA which in turn will generate specific amounts of a defined protein. All this process is known under the term 'gene expression'.

A complex organism is composed of organs and tissues ([slide 5](#)) whose elementary building blocks that are the cells. Each cell has been derived by sequential replication from the original fertilised zygote, and thus bears the essentially identical genome. However each cell type can 'express' a distinct panel of genes, according to its specialisation level. Although the concept is still hotly debated, it is believed that the human genome can encode at over 30'000 genes (though many must still probably be discovered). According to the previous concept '1 gene more than one function', this could imply more than 100'000 individual functions, that can be encoded by the genome. When aiming at somatic gene transfer, we need to re-insert genes in the nucleus of somatic cells. For this, it is important to remember that in one gram of tissue there are about 1 billion cells. This gives a first idea of the complexity of somatic gene transfer in terms of 'delivery'.

To conclude, the reductionist paradigm of molecular biology ([slide 6](#)) foresees that (a) a given function will be intact and correctly manifested if the corresponding gene(s) is (are) intact, (b) that a functional alteration can be caused by a genetic alteration (gain or loss of function), and (c) as a consequence, function(s) can be transferred by gene transfer.

Genetic defects, diseases, molecular medicine

Defective genes can lead to two types of disturbances: those that are immediately manifested (which can be monogenic or polygenic) or those that lead to some predisposition ([slide 7](#)). In general, monogenic diseases are very rare (from 1/10'000 to 1/1'000'000) while polygenic conditions are much more frequent (from 0.5 up to over 10%). If we take in account the genuine genetic diseases and all the predispositions, we come to the conclusion that there is statistically no 'disease free' genome.

In addition, neither the disease status nor its gravity are exclusively determined by our genome but also by a combination external factors (either behavioral or environmental). The contribution of the three aspects is different for each type of disease ([slide 8](#)). In addition there are disease situations that are, yes, caused purely by external influences (traumatic lesions, intoxications, infections), but whose severity of development and outcome still may depend of the individual genetic setup. These reflections are important to emphasize that also those types of disease can be considered for therapy by gene transfer.

Nevertheless, the major and most ravaging disease of this century is caused by the increase of longevity ([slide 9](#)). Most of the genetic predispositions become manifest and clinically important only after the age of 40. Thus diseases such as cancers or Alzheimer, were not a significant challenge for public health when the longevity was around 45 years (beginning of 1900) but have become major challengers these days. Even a young discipline such as gene therapy which still relies on few model diseases for its experimental verification will soon have to cope with this kind of age-related diseases.

Medicine has three major missions in disease identification (diagnosis) disease prevention and therapy ([slide 10](#)). The application of molecular genetics know-how (resulting in the so called molecular medicine) has had a major impact on all these three sectors. In the first era, molecular medicine has provided the tools for precise genetic diagnostics (genes as probes, [slide 11](#)). In a subsequent phase very powerful biopharmaceuticals have entered the routine clinical treatment (genes as factories). Finally in recent years, the possibility of directly using gene transfer for therapeutic purposes has started to attract the attention ('genes as drugs'). The post-genomic era, which will permit the understanding and utilization of poly-genic networks, has given and will give a strong impulse to all those techniques.

Somatic gene therapy (SGT)

After this essential recapitulation we are ready to tackle the intricacies of somatic gene therapy (SGT). In slide 12 we find the definition as 'transfer of nucleic acids in somatic cells with the intent of curing or treating a disease condition'. The targeted disease can be of inherited or acquired type and the type of treatment can be for chronic, acute or preventive purpose.

Putting back genes into cells to restore a healthy balance, sounds simple, yes, but the devil is often in the details (slide 13). There are many things in common life that are in principle simple like getting a train ticket, parking a car, or counting votes, ...but quickly become damn complicated in some particular contexts. Thus, it should not be so surprising to hear that the very same SGT clinical protocol had been considered as 'non working' for over ten years, although it was perfectly working as demonstrated recently (ADA treatment with ex vivo transfer of ADA gene by retroviruses (1))

The current view in gene therapy remains confined to the 'somatic' cells because of intrinsic geno-toxicity of the current gene transfer approaches. If these treatments would leak out into germ cells (slide 14) we would be facing potential random mutagenesis of the descendants, and this is a non tolerable risk. If SGT could manage the precise and targeted correction of genes (e.g by homologous recombination or targeted repair) then germ-line contamination with those treatments would remain only a moral issue and no longer a medical one.

In principle all diseases are amenable to be treated by gene transfer, although a certain number of current restrictions apply, as illustrated in slide 15. Most importantly we have to realise that, given the current risks associated with gene transfer, the application of SGT remains as of today, restricted to those disorders that are either lethal or cause major reduction of life quality, or for which no satisfactory alternative therapy exists. Yet, once identified a good candidate therapy, one still has to consider a large number of variables. In particular it will be important to assess at which point of the disease progression the treatment is indicated. There is little sense in treating after the point-of-no-return. For some genetic diseases like those that impair the development of the neural system or organogenesis, treatment after birth may be already too late, and this raises supplementary obstacles to SGT. In spite of these logical reasoning, recently gene transfer has suscitated the interest of the sports doping arena. This illustrates well my personal definition of the difference between intelligence and stupidity: only the former has limitations (!).

As for any conventional pharmacological treatment, four questions must be kept in mind for the therapeutical approach with gene therapy (slide 16): efficiency of delivery (transfer), specificity, persistence/regulation and toxicity. It is not so easy to make those parameters match for each individual treatment, since this depends on the vector type, the target organ, the delivery system and the type of disease.

From elementary pharmacology to gene pharmacology

In slide 17 we recapitulate the properties and features of conventional small molecular weight drugs (first panel). These drugs are designed to diffuse with precise kinetics and whenever necessary to be imported into cells by specific transport systems. Protein therapy (second panel) is more complex, specially if aimed at acting intracellularly, because there are not many ways to import proteins. Furthermore these pharmaceuticals cannot be delivered orally. For nucleic acids (third panel) the situation becomes complicated due to their size (megadaltons) and the lack of import system through the cell membrane and specially into the cell nucleus. Therefore nucleic acids need to be either naturally packaged into viral particles that satisfy many of these properties or into artificial particles that offer surrogates thereof. Also the half life of the treatment is completely different, because transformation with nucleic acid can signify permanent alteration, contrary to conventional drug treatment that is intrinsically transient.

Also the route of delivery (slide 18) must be determined for each type of disorders. Hematopoietic disorders are better treated by ex-vivo gene transfer, where bone marrow cells are explanted, selected, transformed with the vector of choice and re-infused. Other conditions (such as cancer or organ restricted diseases as in joints eyes, brain etc...9 can be treated by local vector delivery (in vivo local delivery). The simplest yet most arduous approach is the systemic delivery (intravenous or analogous). For this type of delivery the

particles must have additional properties, in particular they must be able to avoid non specific trapping and to accumulate in the vascular system in the proximity of the target organs. Also they must be able to pass several cell layers. As of today there are no vectors that satisfy all these prerequisites (commented also under slides 22 and 28).

The two schools of ‘vectorology’: viruses or not?

The intrinsic difficulties of gene transfer in somatic cells have left the scientists with a dilemma: either rely on non-viral transfer methods, which are less toxic but also less efficient, or to accept the difficulties of constructing recombinant viral vectors that possess many properties that permit a very efficient gene transfer (specially to cross the intracellular barrier of the nuclear envelope. Although it is a common belief that the cell membrane is a major hurdle (which is not exactly true because one can exploit natural processes such as endocytosis), people tend to forget that in fact the translocation of the exogenous genetic material into the nucleus cannot rely on natural transport systems. Viruses have evolved all the molecular tools to piggyback on endogenous trafficking systems and are therefore capable to deliver their DNA cargo safely and efficiently in the nucleus (slide 19). The dramatic difference between viral and nonviral delivery of a reporter gene is illustrated in slide 20 where we show that conventional transfection requires about 1 million DNA molecules per cell and an overnight exposure to the cocktail to achieve a few percentage of transiently transformed cells. On the other hand, exposure for about one hour to about 3 recombinant viral particles/cell can result in a nearly 100% gene transfer. Therefore the two categories of vectors (viral versus non viral) have specific disadvantages. If one would ignore the major disadvantage of the extremely poor efficiency in gene transfer for nonviral formulations (slide 21) one would easily imagine that nonviral methods are by far superior in terms of limited toxicity, unlimited packaging, simplicity of assembly.

However, there are many additional properties that nonviral particles ideally need to fulfil to be really suitable for gene transfer, specially if delivered systemically, such as by intravenous injection. In that case, the particles should be able to accumulate in the vasculature that serves the target organ, thus should be able to decode some of the still mysterious vascular zip-code (2), which is so nicely recognised by progenitor cells delivered intravenously. Also the particles should be stable against serum components and possess further tissue-preferential docking properties. Their DNA cargo should have tissue specific regulatory elements and be further biochemically decorated in a way that favors its protection against cytoplasmic degradative actions (such as lysosomal enzymes) and to favour nuclear translocation (see slide 20). Many of these properties are concomitant in viral particles, and this explains their current popularity in gene therapy.

Small parade of principal vectors/methods

In the talk there will be a brief review (slide 22) of some relevant current vector systems (slides 23-27). The important message from those slides is that each vector has its own peculiar advantages and disadvantages that make it suitable for a specific window of applications. These are summarised also in Table 1

Table 1 vectors and properties

<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	<i>5</i>	<i>6</i>	<i>7</i>	<i>8</i>
<i>Vector type</i>	<i>pack (kb)</i>	<i>integr.</i>	<i>expr time</i>	<i>persist</i>	<i>tox</i>	<i>nondiv cells</i>	<i>clinical test</i>
adeno I-II gen	8	no	rapid	short	++++	yes	c, m, a
adeno III	30	no	rapid	med	++	yes	-
adeno-associated retroviral	4.5	yes, rnd	delayed	med-long	++	yes	m, a, n
	9	yes, rnd	delayed	long	++	no	c, m, a, n

lentiviral	9	yes, rnd	delayed	long	++	yes	-
plasmid	>20	no	rapid	short	+	no/yes	c, m, a
plasmid+ transposase	>20	yes, rnd	rapid	long	+	no/yes	-
Plasmid+ integrase	>20	yes, specif.	rapid	long	+	no/yes	-

Legend: column 1 gives the vector type, column 2 the limit of packaging into the particle, column 3 specifies if the foreign gene does integrate in the host genome (rnd means random integration), column 4 tells whether the gene transfer results in rapid or delayed expression, column 5 gives the persistence of expression of the transgene, column 6 gives the observed toxicity (includes immuno toxicity), column 6 specifies the capacity of the vector to infect/transfect nondividing cells, column 8 gives the so far tested clinical applications (c, cancer; m, monogenic diseases; a, acquired diseases; n, neurological diseases).

It is obvious that we need to await for the adenovirusesIII and the lentiviruses to enter in the clinical testing before drawing conclusions about their real efficacy. In the preclinical tests, these two categories of vectors have shown a spectacular potential of efficient and persistent gene transfer. The disadvantage of the adenovectors of third generation is their cumbersome preparation that requires a helper virus and a time consuming purification. Similarly, the disadvantage of the lentiviruses is linked to the low titre obtainable with the current safe production conditions and with the inexistence of packaging cells that permit easy amplification. By the way, also recombinant adeno-associated viruses (rAAV) are difficult to produce in large scale since there is no packaging cell that permits the amplification through infection passages. In spite of this, rAAV vectors have been accepted for clinical testing much longer before lentiviruses probably due to the lower intrinsic danger of their parental wild-type vector. The above considerations are certainly factors that have considerably slowed the clinical testing of adenovirusesIII and of lentiviruses.

Limitations and other considerations on vectorology

In slide 28 we recapitulate the major pitfalls of each vector system and we suggest that the future research seems to go in the direction of trying to artificially mimic the properties of viral particles, yet with in vitro assembled components, and trying to trade-off on their toxicity. We can dare to say that viral vectors will certainly continue to be used over the next decade, but that they will be progressively replaced by nonviral gene transfer methods. It remains that viral vectors have been indispensable to give the proof of principle, and that they will still be useful for a while.

Slide 29 (recapitulated in Table 2) gives some indications about which of the currently available vectors is most suitable for the following disease categories : chronic/metabolic, local chronic and progressive; solid tumors, trauma or infections.

Table 2 Which vector for which disease category

<i>Disease type</i>	<i>Most suitable vector</i>	<i>Remarks</i>
Chronic/Metabolic (ex OTC, Gaucher, haemophilia, hematopoietic)	AAV, Lentivir, AdenoIII, retrovir, repair oligo	persistence of alteration required, minimize readministration
Local chronic or progressive (CNS, joints, eyes)	AAV, nonviral, Lentivir	no rapid expression requested, persistence and low toxicity desirable
Solid tumors (cervical, breast, brain, skin)	AdenoI-II, Plasmid, oncolytic viruses,	rapid and mid-term transient expression of cytotoxins or immunomodulators

Trauma /Infection /poisoning

(cardiac failures, wounds, strokes, burns, acute infections, anaphyllaxis)

Adeno I-II, plasmid, modulatory oligonucleotides

rapid and short-term transient expression / modulation of gene functions

Besides the above discussed technology, there are some other procedures that cannot be considered 'gene therapy *strictu senso* (slide 30). In this we outline that some of the procedures that are inappropriately understood as 'gene therapy'. For instance, the use of antisense oligonucleotides or small interfering double stranded RNA are in essence some kind of high molecular weight pharmacology but are far from being genuine gene therapy, since the treatments have a similar pharmacological property as protein therapy with a typical half life of the active principle and an intrinsic need of re-administration for maintenance of the therapeutic doses. Also the insertion of encapsulated cells that have been engineered to produce supplementary factors (neurotrophic factors, hormones, lymphokines or cytokines (13, 14)) cannot be assimilated to gene therapy because of its absolute reversibility and because no cells of the host organism are actually genetically modified. Among the paradigms that were initiated with oligonucleotides delivery, but could be adapted to the use of persisting gene vectors, we shall mention the Small interfering RNA (SiRNA for a recent review see: Devroe, E. and Silver, P.A. (2004) *Expert Opin Biol Ther*, **4**, 319-27). This technology is analogous to the antisense technology, but it proved to be significantly more efficient. Although it is the youngest 'baby' in molecular biology, SiRNA might belong to one of the first clinically applicable principles. In fact, there are many disease forms (cancer, infective diseases, degenerative diseases) which are caused by some gain-of-function status that could be efficaciously controlled by expression of gene specific SiRNAs.

Applications, examples, achievements and frustrations

In slide 31 we present some 'classical' models for somatic gene therapy. It is important to note that not all models are based on a monogenic loss of function and that the transferred gene is not necessarily related to the lesion that may be at the source of the disease, but rather represents a short-circuit of the symptomatic degeneration. The classical example is the transfer of genes encoding vascular endothelial growth factor (VEGF). This treatment is suitable to cure peripheral or cardiac ischemia. Analogous examples could be mentioned for the treatment of some neurologic diseases, where the transfer of neuro-protective factors can correct or slow down the progression of some neurodegenerative pathways ((13, 14)). Thus the gene which is transferred does not need to be related directly to the etiologic lesion factor.

Gene therapy trials started in 1990 with the nowadays famous treatment of adenosine deaminase deficiency (ADA deficiency) by French Anderson and colleagues (15). Since then (slide 32) many further clinical trials have followed. Actualized data about registered clinical trials can be visualized at the dedicated site of Wiley & Sons: (<http://www.wiley.com/wileychi/genmed/clinical>). According to this database, by July 2004 there have been more than 980 registered clinical trials with over 4500 treated patients. Perhaps somewhat surprisingly for some of the readers of this overview, the majority has been in the cancer treatment field (more than 70%). The trials aimed at treatment of monogenic inherited diseases represent only less than 20%. It must be emphasized that nearly 1/5 of the registered trials reported in this database are still pending or have not yet (or will never be) initiated and therefore the number of patients per valid trial may be higher at the end.

Nevertheless, the major lesson that we can distil from this summary is that thirteen years after the first trial, still less than 2% of the trials has achieved genuine phase III (1.5% if counting also the hybrid phases II-III). The reasons of this markedly slow progress are analysed in the next paragraphs. In Switzerland there have been over 300 treated patients and many clinical trials. During five years part of the research had been sponsored and coordinated by the national research program 37 (NFP37, www.unifr.ch/nfp37). The

subdivision of research categories within the nfp37 program was comparable to the world's trends, with a strong emphasis on cancer research and a relatively small impact of clinical applications.

Many successes, but not all recognized

In spite of the many hurdles and difficulties, gene therapy has managed to give us many satisfactory answers in different application fields (slide 33, gene therapy clinical milestones).

Even the very first trial initiated in 1990 by Anderson and colleagues can be counted as a success, although it has been considered for many years a failure, because it appeared that the transferred gene was not expressed over long time. This was shown to be in fact due to the concomitant enzyme therapy (PEG ADA) that did not permit the genetically altered cells to exert their selective advantage. C. Bordignon and colleagues demonstrated in 2002 (1, 16) that in absence of PEG-ADA treatment the ex-vivo transduced cells (Bordignon used essentially the same vector as in the early 90ties) did efficiently recolonise the immune system and cured the young patient.

Among the other clinical successes we shall mention the diminishing of restenosis incidence by transfer of decoy oligonucleotides that reduced the proliferative potential of smooth muscular cells of the transplanted veins in bypass surgery (17, 18); the treatment of critical limb ischemia by transient expression of VEGF via local intramuscular injections of naked plasmid DNA vector (19, 20); and the treatment of hemophilia by intramuscular transfer of recombinant AAV vectors encoding factor VIII (21). The major clinical success has been of course the series initiated in 1998 by Alain Fischer who reported in 2000 and 2002 (22, 23) the stable and long term cure of two young patients suffering from a lethal immune deficiency (X-SCID). Meanwhile Dr. Fisher has treated 10 patients. One of them was not responding because the therapy was applied too late, and two have developed a subsequent leukemia-like condition and one of those died recently (see the information assembled at our WEB site

<http://www.unifr.ch/nfp37/adverse.html>). This adverse event will be also commented later (slide 35). From congress communications, at least 6 X-SCID patients were treated in the UK by the team of Prof. Ch. Kinnon, with a very similar protocol. Although these results have not yet been published, the current understanding is none of these UK patients has manifested a similar adverse event. Besides that at least five ADA deficiency patients have been also treated analogously (ex-vivo transfer in hematopoietic precursor cells) by the team of Claudio Bordignon. Also in that case, the current communications inform us that no patient has experienced yet such a serious AE. Further wind in the sails of gene therapy has been blown by the successes in cancer treatment with oncolytic viruses (Reid, T., Warren, R. and Kirn, D. (2002) *Cancer Gene Ther*, **9**, 979-86) and more recently by the announcement of the approval of a gene medicine in China (more information about that story at our WEB site www.unifr.ch/nfp37/sibiono.html).

In spite of the excellent results achieved with some diseases, two important monogenic conditions for which many hopes had been raised early and emotionally in the public opinion (cystic fibrosis and muscular dystrophy) remain without satisfactory gene therapy solution (Slide 34). The reasons are due in part to the large size of the therapeutic gene (thus to the difficulty to engineer that into a clinically acceptable viral vector) and to other anatomical barriers. In particular, we shall mention the failure in the attempts of delivering genes via a route that seemed most straightforward, like by inhalation in the lung. The barrier posed by the mucus and the absence of viral receptors in the luminal part of lung epithelial cells has shown to be almost unbreakable. This should remind us that things that seem simple, can become unsuspectedly complicated for very trivial reasons (as mentioned under slide 13).

Side effects and (apparently) slow progress: the possible reasons

As any type of treatment gene transfer can generate several undesired side effects (slide 35). Their severity goes from some mild to strong immune reaction caused either by viral capsid proteins, residual viral genes in the DNA constructs or even the therapeutic gene

itself that in some extreme cases could be recognised as foreign (see below). Another important potential side effect is caused by the random insertion of the transgene into the host genome as for instance in treatment with retroviral vectors or other randomly inserting constructs. The random insertion causes most frequently loss of functions that are compensated by heterozygous conditions. However, this loss of function could also affect tumor suppressor genes, thus rendering the affected cell more susceptible to oncogenic transformation. Most importantly, random insertion can also cause a gain-of-function by enhancing the expression of flanking genes. If this happens in vicinity of proto-oncogenes, the result may be a pro-oncogenic status that strongly increases the risk of cancer onset. The recent case manifested in the trial series of Alain Fischer in Paris could be the first concrete demonstration of this risk. In the two young patients, a kind of T-cell leukemic state appeared three years after treatment, and in the leukemic cells the provirus encoding the therapeutic gene seemed to have inserted close to the LMO2 proto-oncogene. Proof that this insertion has been the etiologic principal factor of the leukemic state is confirmed by the two independent cases. This has augmented the alert and prompted the regulatory authorities of several countries to put a moratorium on analogous trials. Dr. Fisher voluntarily suspended his own trial. Subsequent experiments showed that the risk of cancer is probably dependent on the dose and age of treatment and this has prompted Dr. Fischer to resume patients recruiting (Fischer, A. and et al. Gene therapy for SCID. Where do we stand?, (2004) *12th annual ESGT congress, Abstract Inv 28*, Tampere, 10).

To summarise, the brief clinical history of experimental gene therapy has seen four important cases of relevant adverse effects that could be directly linked to the treatment (slide 36). The first that received wide attention was the flu-like symptoms that developed in a cystic fibrosis patient that was treated experimentally by inhalation of recombinant adenoviruses (1995, (24, 25)). The second and most dramatic case, was the death of the young Jesse Gelsinger (1999 (26-29), see also the press collection at the NFP37 WEB site: www.unifr.ch/nfp37/MEDIA&NEWS/DataMedia99.html) who was inappropriately enrolled in a clinical trial aimed at correction of Ornithine Decarboxylase Deficiency by adenoviral gene transfer. A combination of the inappropriate dose and inappropriate delivery route and all this in absence of sufficient preliminary medical exams, lead to the death of the patient by multiple organ failure caused by a severe toxic shock. The third and fourth case were the ones just commented above occurring in Paris. There have been other reports and other findings of misreporting (specially in the investigation that followed the case of Jesse Gelsinger's death). However, none of them could be unmistakably linked to the gene transfer procedure as the four ones just cited.

Not only random shooting...

As commented above, gene transfer protocols where the exogenous material integrates randomly into the host genome are suitable for permanent treatment but are 'genotoxic' since random integration disturbs the resident functions and leads to insertional mutagenesis (slide 37). One of the impressions that people may have at this point is that somatic gene transfer has currently no solutions against such random shooting of artificial gene constructs in the genome. While this undesirable property applies to many of the current vectors that are suitable for permanent gene transfer such as retroviral vectors and AAV, as well as to the plasmid-based transfer with genomic integration aided by a random transposase (such as 'sleeping beauty' (3, 4)), this does not apply to all gene transfer protocols. For instance recombinant adenoviral constructs can persist for months without integrating in the genome of the host cell. These gene transfers are well suitable for transient or acute types of treatments. The same applies to RNA transfer either in form of viral vectors (example recombinant picorna viruses) or in form of in vitro generated RNA that can prompt ribozymic functions or corrective trans-splicing events ((5)). Both the transfer of genetic functions with adeno vectors or with RNA molecules are non-genotoxic since these elements do not randomly insert into the genome. Even permanent somatic genetic treatments can be performed without genotoxicity. This can be achieved with vectors that are designed to interact with homologous sequences in the genome and to correct resident genetic functions either by prompting homologous

recombination or mismatch repair. An example of such gene correction strategies are the hybrid RNA-DNA oligonucleotides also known as 'chimeroplasts' (3, 6-10). However, recent congress reports, suggested that the strong hopes in this technology might have been emphasised by some inappropriately interpreted early results (Blaese, M. Can genome editing become gene therapy?, (2004) *12th ESGT meeting, Tampere, Abstract Inv17*, 8.)

Another example of non-genotoxic procedures are those where integration of the foreign construct is achieved through a transiently coexpressed a site-specific integrase such as the pilot experimental approach proposed by Khavari and colleagues with the bacteriophage C31 integrase (11, 12) which has one single target site in the human genome. The researchers are currently trying to redesign recombinases such that they could recognise desired target sites in the genome. If those efforts would lead to the expected success, this has two advantages: a) the recombinant construct would be in a predetermined position and thus would not be subjected to random silencing by propagating heterochromatin; b) the newly inserted construct would not alter resident functions. These two properties make technically gene transfer compatible also with alteration of cells that belong to the germ line and would therefore transmit the alteration to future generation. Whether the perspective of germ line (thus inheritable) gene transfer would be acceptable from the biological-evolutionary and from the ethical aspect, that is another question.

Autoimmunity, another important source of worries

Even in conventional protein therapy (like in hemophilia) it has been demonstrated that patients can develop antibodies against the therapeutical protein. This owes to the condition of lack of function of their resident genes and to the consequent recognition of the additional factor as 'non self'. However, even ectopic expression or overexpression can become a source of immunity, and this also when the patient expresses normal forms of the same protein. This has been documented in earlier cases of treatments with some particular erythropoietin batches. In few words, it appears that a mis-folded or inappropriately processed protein can become a source of 'nonself' reaction. This is an important source of worries for those gene therapy approaches in which the foreign gene is expressed in an organ different than the natural organ that produces the same factor. With the progress of gene therapy, it was therefore unavoidable that some cases of autoimmunity ought to be documented. As shown in slide 38, this happened recently in three independent experimentations: two preclinical and a clinical one. In the preclinical experiments, it was shown that monkeys to which erythropoietin expression vectors were delivered in the lungs or in the muscle, developed anti-Epo antibodies. In two cases the monkeys developed a lethal anemia (Chenuaud et al. (2004) *Blood*, 103, 3303-4 // Gao et al. (2004) *Blood*, 103, 3300-2). An analogous observation was reported by K. High concerning patients treated with AAV vectors encoding Factor IX (K. High, ASGT meeting 2004). These observations demonstrate some important limitations of therapy via ectopic expression of factors, and is also an important signal to all those who believe in an easy sports doping (see comments under slide 15 and www.unifr.ch/nfp37/genedoping.html). This kind of doping is bound to have two advantages: it might lead to easy detectable differences of the transgenic circulating factor and it may even become extremely dangerous if it will induce autoimmunity.

Further factors that have negatively influenced gene therapy

Taken at face value, we could actually state that gene therapy has produced surprisingly few adverse events, specially when compared to other experimental therapies. Nonetheless, in the 'imaginaire collectif' of the scientific community and the public opinion, gene therapy is still regarded as a very risky procedure. This has much more to do with the emotional perception of gene therapy than with pure rational thinking. In the eyes of the public opinion there is a generalised negative perception of procedures that deal with any sort of genetic manipulation. This aversion is often worsened by the wide spread confusions around the fields of genetics and reproductive technology. For instance, I would not be surprised if the image of gene therapy would suffer substantially

from the hot debate suscitated by the human reproductive cloning claims, be them true or hoaxes 'peu importe'. Certainly we must admit that part of the skepticism towards gene therapy must be accounted to the obvious deception that follows the many spectacular promises (see also below).

In slide 39, we list some of the possible causes of this vicious circle of extreme promises-extreme deception. I think that the major player has not been the ego of some investigators (which certainly was the source of several reckless statements) but rather the greed generated by the stock market bubble in the mid nineties. This was realised also by the American society for gene therapy (ASGT, www.asgt.org) that issued a very clear memorandum for the conduction of gene therapy trials in absence of conflicts of interests (30). The concomitance of gene therapy progress with this period has led to many perversions, because the huge amount of funds had attracted a large number of poorly competent people in the field, and made many people react in totally unethical manner. Just like in sports or arts, a certain amount of funding is necessary, but when it becomes excessive it leads to grotesque cheatings.

This cocktail of good news and bad news has been very detrimental to the genuine progress of the field. In slide 40 I have attempted to represent the changes in 'mood level' of the investigators (obviously in arbitrary units) along the last one and half. By looking at these ups and downs you will certainly appreciate how difficult it was to maintain a clear view of what was and is feasible and realistic and how to overcome the periodical phases of deep discouragement.

Examined under this optic, the life of gene therapy appears very much like the life of a child that has been prematurely born and has suffered too many 'Kinderkrankheiten', such that it cannot enter the teen-age with sufficient maturity. However this picture is misleading, because gene therapy is an undoubtedly logical development of our progress in gene technology and still has an absolutely extraordinary potential. Its problem is that it was born indeed under the wrong star and suffered from sub-optimal management. Thus its muscles and bones might look still weak but the hormonal boost will soon come and will substantially reinforce that body.

Besides those technical and structural pitfalls, somatic gene therapy is also regarded (as many of the technologies related to genetic manipulation) as a direct ground for doubtful or even evil applications. Some people fear that gene therapy would quickly abandon its medical mission to relieve serious diseases, to concentrate on the more lucrative field of serving the hedonistic needs of the humans, thus slipping into a bunch of techniques aimed at amelioration and cosmetics rather than genuine therapy. Not surprisingly, the option of gene transfer has attracted the attention of the sports field (see comments under slide 15 and www.unifr.ch/nfp37/genedoping.html) Other people see a bad cocktail of economical burden and the complexity and tend to consider this technology as yet another expensive but rather useless and even deforming tool. Finally, other people fear that the knowledge gained through gene transfer studies could be directly exploited for the concoction of bioweapons. All those worries, even if they may sound a bit far fetched have some solid basis on the historical proofs of perverse use of technological advancement, and it is certainly the duty of our democratic society to make sure that we avoid entering slippery slopes.

Besides these public opinion challengers, gene therapy is facing also challenges from the scientific-medical world. The emerging news in the field of stem cells seem to promote very much cell therapy as the next best candidate for spreading unsubstantiated illusions. Obviously cell therapy based on stem cells has an incredible potential and could substitute gene therapy or be combined with gene transfer for the treatment many disorders. But also from the small molecular weight drug design we have seen some new players (example Glivec) that could take many of the niches that gene therapy has been claiming in the past years, because of their increased specificity and lower toxicity. Finally, also the biomechanical world has made some spectacular progress since there are organs that can be partially or entirely engineered such as the heart, limbs and even the eyes. Thus, gene therapy is certainly not the sole high-tech alternative for treating our future afflictions.

Some Conclusions and perspectives

In the preceding paragraphs we have analysed the features of somatic gene transfer. We have described the basic problems the essential techniques and we have seen some elements of 'vectorology'. Preclinically there has been a very significant advancement, to the point that a common semi-humorous statement circulates since several years such as: 'gene therapy has cured lots of mice but no human being'. The vectorology field has been the most investigated sector and has yielded a panel of very versatile tools that include recombinant lentiviruses (which have not yet been tested clinically). From the clinical point of view (slide 41) we have seen that although there has been a substantial effort accompanied by some genuine successes (think of the SCID cure by Alain Fischer, The ADA cure by Claudio Bordignon), still very few protocols have reached the Phase III. In fact most of the few Phase III in the database have just been registered but not yet started. We probably should have expected a larger fraction thereof if there would have been less political-emotional hurdles caused by the few adverse events. Perhaps the Chinese bravery in granting commercialisation of the first gene medicine based on relatively soft (as far as we know so far) clinical results, is not after all such a bad attitude.

Now you're teenage, what will you do when you are grown up?

It is always difficult to predict the future, but there are some signs that I would like to share with the readers (slide 42). At the level of fundamental studies we can expect that the knowledge offered by functional and structural genomics can help to better understand which gene under which conditions and under which regulatory elements could have the best desired and the smallest undesired effects. Also the study of molecular genetics never ceases to produce surprising paradigms such as example the small interfering RNA (SiRNA, called also RNAi) which appears as an incredibly powerful tool to suppress specific genetic functions (see some reviews by: (31-37)). Besides the promising lentiviral vectors that still await their clinical 'consecration' ((38-43)), there are the adenovector of third generation (44, 45) that remain to be soon clinically validated and there is a whole new generation of hybrid vectors (46, 47) and systems that permit specific integration into the genome or even gene correction via homologous recombination/repair. These vectors may solve some of the original toxicity/risk factors linked to the first wave of gene therapy vehicles, thus opening the therapy to less severe diseases than currently acceptable.

At the preclinical level there has been recently a number of experiments in animal models that better represent the human dimensions (such as dogs and monkeys) and the field of transgenesis /KO is constantly producing improved models for human diseases. Probably from the field of reproductive animal cloning we may be able to obtain specific models in those animals for which the ES cell transgenesis is as 'easy' as in mice.

Clinically we are all eagerly awaiting the confirmation of the spectacular preclinical data with the very promising vectors of the 'second generation' and we should be able to see the first real gene therapy protocols of the first generation being successfully registered within 3-4 years. In spite of these promising cards, the concept of somatic gene therapy could become partly obsolete (at least for many chronic diseases) if the progress in cell therapy maintains its promises. However, the bitter experience with the illusions raised around gene therapy renders us rather skeptical about concrete rapid progresses in cell therapy.

If you want to share my frank opinion on the potential progress in gene therapy, then you need to be as optimistic as I am. I tend to compare this to the progress in aviation. That means: lots of early visionary characters; lots of brave pioneers; several dramatic and spectacular accidents (slide 43); but finally resulting into a solid technology that completely changed our way of life. Just give it the necessary time and we or the future generation will be able to look back to our times, very much as we look back with a mix of pride, amusement and nostalgia to the blurred black-and-white pictures of the Wright Brothers who, just one hundred years ago, dared to launch a sputtering wood-and-paper

precursor of our monstrous jumbo jets and space crafts. We then shall ask ourselves: was it really «too early» to start at that time?

S. Rusconi, January 2005

PS the slides-show (devoid of movie clips) can be downloaded from:

<http://www.unifr.ch/nfp37/slides/ecpm2005rusconi.ppt>

References

1. Aiuti, A., *et al.* (2002). Correction of ADA-SCID by stem cell gene therapy combined with nonmyeloablative conditioning *Science* 296, 2410-3.
2. Trepel, M., Arap, W., and Pasqualini, R. (2002). In vivo phage display and vascular heterogeneity: implications for targeted medicine *Curr Opin Chem Biol* 6, 399-404.
3. Richardson, P., Thoma, C., Kren, B. T., and Steer, C. J. (2002). Strategies for hepatic gene correction *J Drug Target* 10, 133-41.
4. Yant, S. R., Ehrhardt, A., Mikkelsen, J. G., Meuse, L., Pham, T., and Kay, M. A. (2002). Transposition from a gutless adeno-transposon vector stabilizes transgene expression in vivo *Nat Biotechnol* 20, 999-1005.
5. Watanabe, T. and Sullenger, B. A. (2000). RNA repair: a novel approach to gene therapy *Adv Drug Deliv Rev* 44, 109-18.
6. Richardson, P. D., Augustin, L. B., Kren, B. T., and Steer, C. J. (2002). Gene repair and transposon-mediated gene therapy *Stem Cells* 20, 105-18.
7. Richardson, P. D., Kren, B. T., and Steer, C. J. (2002). Gene repair in the new age of gene therapy *Hepatology* 35, 512-8.
8. Kren, B. T., Chowdhury, N. R., Chowdhury, J. R., and Steer, C. J. (2002). Gene therapy as an alternative to liver transplantation *Liver Transpl* 8, 1089-108.
9. Kren, B. T. and Steer, C. J. (2002). The application of DNA repair vectors to gene therapy *Curr Opin Biotechnol* 13, 473-81.
10. Richardson, P. D., Kren, B. T., and Steer, C. J. (2001). Targeted gene correction strategies *Curr Opin Mol Ther* 3, 327-37.
11. Khavari, P. A. (2000). Genetic correction of inherited epidermal disorders *Hum Gene Ther* 11, 2277-82.
12. Ortiz-Urda, S., Thyagarajan, B., Keene, D. R., Lin, Q., Fang, M., Calos, M. P., and Khavari, P. A. (2002). Stable nonviral genetic correction of inherited human skin disease *Nat Med* 8, 1166-70.
13. Tseng, J. L. and Aebischer, P. (2000). Encapsulated neural transplants *Prog Brain Res* 127, 189-202.
14. Zurn, A. D., Widmer, H. R., and Aebischer, P. (2001). Sustained delivery of GDNF: towards a treatment for Parkinson's disease *Brain Res Brain Res Rev* 36, 222-9.
15. Blaese, R. M., *et al.* (1993). Treatment of severe combined immunodeficiency disease (SCID) due to adenosine deaminase deficiency with CD34+ selected autologous peripheral blood cells transduced with a human ADA gene. Amendment to clinical research project, Project 90-C-195, January 10, 1992 *Hum Gene Ther* 4, 521-7.
16. Bordignon, C. and Roncarolo, M. G. (2002). Therapeutic applications for hematopoietic stem cell gene transfer *Nat Immunol* 3, 318-21.
17. Kaneda, Y., Morishita, R., and Dzau, V. J. (1997). Prevention of restenosis by gene therapy *Ann N Y Acad Sci* 811, 299-308; discussion 308-10.
18. Rutanen, J., Markkanen, J., and Yla-Herttuala, S. (2002). Gene therapy for restenosis: current status *Drugs* 62, 1575-85.
19. Baumgartner, I., Pieczek, A., Manor, O., Blair, R., Kearney, M., Walsh, K., and Isner, J. M. (1998). Constitutive expression of phVEGF165 after intramuscular gene transfer promotes collateral vessel development in patients with critical limb ischemia *Circulation* 97, 1114-23.
20. Baumgartner, I. and Isner, J. M. (2001). Somatic gene therapy in the cardiovascular system *Annu Rev Physiol* 63, 427-50.
21. Kay, M. A., *et al.* (2000). Evidence for gene transfer and expression of factor IX in haemophilia B patients treated with an AAV vector *Nat Genet* 24, 257-61.
22. Hacein-Bey-Abina, S., *et al.* (2002). Sustained correction of X-linked severe combined immunodeficiency by ex vivo gene therapy *N Engl J Med* 346, 1185-93.
23. Cavazzana-Calvo, M., *et al.* (2000). Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease *Science* 288, 669-72.

24. Crystal, R. G., *et al.* (1995). A phase 1 study, in cystic fibrosis patients, of the safety, toxicity, and biological efficacy of a single administration of a replication deficient, recombinant adenovirus carrying the cDNA of the normal cystic fibrosis transmembrane conductance regulator gene in the lung *Hum Gene Ther* 6, 643-66.
25. Crystal, R. G., *et al.* (1995). Evaluation of repeat administration of a replication deficient, recombinant adenovirus containing the normal cystic fibrosis transmembrane conductance regulator cDNA to the airways of individuals with cystic fibrosis *Hum Gene Ther* 6, 667-703.
26. Stolberg, S. G. (1999). The biotech death of Jesse Gelsinger *N Y Times Mag* 136-140, 149-150.
27. Somia, N. and Verma, I. M. (2000). Gene therapy: trials and tribulations *Nat Rev Genet* 1, 91-9.
28. Smith, L. and Byers, J. F. (2002). Gene therapy in the post-gelsinger era *JONAS Healthc Law Ethics Regul* 4, 104-10.
29. Gelsinger, P. L. (2000). Jesse Gelsinger *FDA Consum* 34, 2-3.
30. Woo, S. L. (2000). Policy of the American Society of Gene Therapy on financial conflict of interest in clinical research *Mol Ther* 1, 383-4.
31. Bass, B. L. (2000). Double-stranded RNA as a template for gene silencing *Cell* 101, 235-8.
32. Hutvagner, G. and Zamore, P. D. (2002). RNAi: nature abhors a double-strand *Curr Opin Genet Dev* 12, 225-32.
33. Hannon, G. J. (2002). RNA interference *Nature* 418, 244-51.
34. Hunter, C. P. (2000). Gene silencing: shrinking the black box of RNAi *Curr Biol* 10, R137-40.
35. Ullu, E., Djikeng, A., Shi, H., and Tschudi, C. (2002). RNA interference: advances and questions *Philos Trans R Soc Lond B Biol Sci* 357, 65-70.
36. Zamore, P. D. (2001). RNA interference: listening to the sound of silence *Nat Struct Biol* 8, 746-50.
37. Fjose, A., Ellingsen, S., Wargelius, A., and Seo, H. C. (2001). RNA interference: mechanisms and applications *Biotechnol Annu Rev* 7, 31-57.
38. Naldini, L. and Verma, I. M. (2000). Lentiviral vectors *Adv Virus Res* 55, 599-609.
39. Pfeifer, A. and Verma, I. M. (2001). Gene therapy: promises and problems *Annu Rev Genomics Hum Genet* 2, 177-211.
40. Trono, D. (2000). Lentiviral vectors: turning a deadly foe into a therapeutic agent *Gene Ther* 7, 20-3.
41. Ailles, L. E. and Naldini, L. (2002). HIV-1-derived lentiviral vectors *Curr Top Microbiol Immunol* 261, 31-52.
42. Deglon, N. and Aebischer, P. (2002). Lentiviruses as vectors for CNS diseases *Curr Top Microbiol Immunol* 261, 191-209.
43. Galimi, F. and Verma, I. M. (2002). Opportunities for the use of lentiviral vectors in human gene therapy *Curr Top Microbiol Immunol* 261, 245-54.
44. Amalfitano, A. and Parks, R. J. (2002). Separating fact from fiction: assessing the potential of modified adenovirus vectors for use in human gene therapy *Curr Gene Ther* 2, 111-33.
45. Morsy, M. A. and Caskey, C. T. (1999). Expanded-capacity adenoviral vectors--the helper-dependent vectors *Mol Med Today* 5, 18-24.
46. Lam, P. Y. and Breakefield, X. O. (2000). Hybrid vector designs to control the delivery, fate and expression of transgenes *J Gene Med* 2, 395-408.
47. Mitani, K. and Kubo, S. (2002). Adenovirus as an integrating vector *Curr Gene Ther* 2, 135-44.

some additional reviews in gene therapy:

48. Ferrari, S., Geddes, D. M., and Alton, E. W. (2002). Barriers to and new approaches for gene therapy and gene delivery in cystic fibrosis *Adv Drug Deliv Rev* 54, 1373-93.
49. Fischer, A. (2001). Gene therapy: some results, many problems to solve *Cell Mol Biol (Noisy-le-grand)* 47, 1269-75.
50. Haviv, Y. S. and Curiel, D. T. (2001). Conditional gene targeting for cancer gene therapy *Adv Drug Deliv Rev* 53, 135-54.
51. High, K. A. (2001). Gene therapy: a 2001 perspective *Haemophilia* 7 Suppl 1, 23-7.
52. Woo, S. L. (2002). Gene transfer in tissue regeneration and reparative medicine *Ann N Y Acad Sci* 961, 61-2.
53. Vile, R. G., Russell, S. J., and Lemoine, N. R. (2000). Cancer gene therapy: hard lessons and new courses *Gene Ther* 7, 2-8.
54. Reynolds, P. N., Feng, M., and Curiel, D. T. (1999). Chimeric viral vectors--the best of both worlds? *Mol Med Today* 5, 25-31.
55. Chamberlain, J. S. (2002). Gene therapy of muscular dystrophy *Hum Mol Genet* 11, 2355-62.
56. Anderson, W. F. (1998). Human gene therapy *Nature* 392, 25-30.

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