

## **Gene therapy between illusion and reality: an update for the year 001 A.G. (after-genome)**

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### **ABSTRACT**

**Molecular genetics applications have revolutionised medicine in the past 20 years. In the 80ties we started the period of 'genes as probes', in the 90ties the phase of 'genes as factories' and in the Y2K the phase of 'genes as drugs'. In the Y2K+N we shall probably witness the many post-genomic improvements of all those applications.**

**In this summary I will comment the most recent developments and concepts in the field of human gene transfer (commonly called 'gene therapy', GT). The aim is to convince the audience that gene transfer is a platform technology that can address many types of disorders: from hereditary to acquired ones. A second aim will be to compare gene-delivery problems with other drug-delivery strategies, to denote the peculiarities of the former. Another aim is to remind that in spite of the major improvements, the current vectors and methods for gene transfer cannot simultaneously satisfy all the requisites that are sometimes necessary for a 'perfect' therapy, that is: high efficiency, high specificity, good persistence/control; low toxicity. Finally, it will be demonstrated that, in spite of the many hurdles, GT has already brought an unequivocal proof-of-principle in several therapeutical areas. We shall end with a discussion on the potential of the knowledge accumulated through the human genome sequencing and on the challenge posed by cell therapy protocols.**

### *The most important disease of the Y2K in G8-countries: Ageing*

The western population has doubled life expectancy at birth since the beginning of this century (Figure 1). There is a large number of degenerative disorders that are linked with ageing. Some have a very severe outcome, such as cancer or Alzheimer's; while some others result in progressive but not lethal diminishing of the life quality, such as rheumatoid inflammation, osteoporosis, etc. That is to say, many disorders that had a minor clinical impact in the early 1900 (cancer and Alzheimer), have now become major trouble makers in the public health scenario, while others that were major killers such as tuberculosis or other infections are now listed at the bottom of the mortality list. From the 'good news' of prolonging life through better hygiene, better nutrition and better conventional medicine we reached the realm of 'preoccupying news' of the unsolved problem of 'lower life quality after the age of 50'. Provided the western world will maintain its technological standard, the solution of this problem will be one of the major challenges for our immediate future medicine, and it will require the combined effort of many medical disciplines and the discovery of novel paradigms for the treatment or the healing of late-age-onset disorders. Gene technology is one of the fields that will reveal some of these new solutions. Some of those will be found with better utilisation of conventional medications (through pharmacogenetics), others through the identification of new drug-targets (from functional genomics) and finally some through the direct use of genes as drug-providers (either biotechnologically or through direct gene transfer).

### *A crash course in 'gene-tech' dialect and some important numbers*

Materially speaking, a gene is a segment of DNA which upon two phases of expression (transcription and translation) converts a string code of nucleic acids bases into a string code of aminoacids which forms a specific protein. Though highly simplified, we can state that proteins are therefore the 'final' products of genes. For the basic functions of the cell metabolism about 5'000-10'000 genes are necessary (the so called 'house keeping' genes), whereas another set of 20'000 to N0'000 is destined to be expressed only in some specific tissues, conferring to them their differential activity. It is also important to remind that many genes can give rise to more than one single product through alternative maturation of the RNA or the protein. therefore, the final picture is extremely rich in individual functions, even if we accept the first draft of the human genome proposing something like 30'000 putative genes <sup>1</sup>. From the biological/medical point of

view we can conclude that all vital processes depend on the coherent expression of genes, and as a consequence, all pathologic processes are characterised by some incapacity of restoring this equilibrium. This means that direct interference with gene expression can help in the correction of virtually every class of pathology. When willing to interfere with cellular processes with somatic gene transfer (gene therapy) one has to bear in mind the dimensions of the problem. In a gram of tissue there are about 1000 millions cells and the entire body consists of about  $10^{14}$  cells. Thus reaching a tissue or an organ with a vector that brings new genetic information to the cells means being able to deliver the therapeutic nucleic acids to an extremely large number of cells. Since there is no natural inter-cellular transport for nucleic acids, the task is of paramount difficulty. We shall see, that in spite of this practical hurdle, gene transfer is promising to become an important therapeutical strategy in the mid term

### *The three+1 era of molecular medicine*

Before entering into the intricacies of gene transfer let's briefly recapitulate the events of the last two decades that have led us to this possibility. Gene technology, is essentially a cut-and-paste-select-grow-and-transfer ceremonial which allows the isolation, the characterisation, the experimental manipulation and the transfer of individual genes. An increasing number of genes whose malfunction is directly responsible for causing disorders or susceptibilities has been characterised. This has profoundly altered our visions of diagnostics, prevention and therapy, by including the DNA know-how into all those aspects.

Era I, 'genes as probes' The very first applications were in the diagnostic area. Over 4000 disorders are directly inherited in form of a single gene malfunction. The recognition of the genetic layout by direct biochemical analysis has been used already in the early 80ties to precisely diagnosticize a certain number of diseases such as thalassemias, haemophilia etc. The discovery of further disease-linked genes and the introduction of the polymerase-chain reaction, have further refined this diagnosis to the point in which we can make a precise analysis with very minute amounts of tissue. So, what has started in the 80ties can be considered the era of 'genes as probes'. Recently, the diagnostic capacity goes beyond monogenic disorders and can predict predispositions to various diseases including cancer, cardiovascular conditions, early-onset Alzheimer's etc. Thus, this era is not finished and is evolving into a pre-symptomatic diagnostic technology that will suscite a number of ethical-social questions about the use of such genetic information.

Era II, 'genes as factories' The genes for several pharmaceutically interesting products (insulin, interferons, erythropoietin, coagulation factors, cytokines, ...) have been isolated and engineered to be abundantly expressed in foreign organisms: bacteria, yeast, cell cultures, transgenic animals or transgenic plants. The expressed products are easily purified and are commercialised as pharmaceuticals for many indications (vaccinations, treatment of immune disorders, treatment of hormonal imbalances etc...). Thus, our pharmaceutical industry has learned how to use the power of genes to produce healing substances. Since the 90ties, the biopharmaceutical market has been growing exponentially, and it promises to maintain this trend for several years to come.

Era III, 'genes as drugs' However, the use of gene as templates for biotechnological protein production is not yet 'gene therapy'. This term is reserved to the situation in which genes (or fragments thereof) are directly transferred to the organism to correct the damages of a malfunction. The malfunction does not need to be inherited. For instance, there is increasing optimism for the so called DNA-based vaccinations, in which genes encoding the antigens to be immunised are temporally transfected intramuscularly instead of injecting the antigens in form of inactivated viruses, bacteria or protein extracts. It seems that the organism can mount a better immune response when those antigens are directly expressed. DNA based vaccination is thereby a bona fide 'gene therapy' and will probably be the first form of broad scale application of this concept of human gene transfer. The conceptual and prototypical basis of gene therapy was established in the nineties, but the first documented successes happened at the century turn. thus we can say that gene therapy is a technology of the Y2K.

Era IV, 'genomes and other 'omes' The sequencing of the entire genome of bacteria and most recently also of multicellular organisms including mammals <sup>2</sup> has changed the paradigms of molecular biology. Today, we are no longer searching for genes starting from a function, but viceversa, we try to understand gene function starting from their expression pattern. For many modern applications, we do not follow the expression of individual genes, but of many thousands

of genes simultaneously. So besides the structural genomics, one has seen the emergence of functional genomics (represented by transcriptomics and proteomics). New 'omics' are adding every month, such as phosphoproteomics, metabolomics, lipidomics, glycomics etc. So, for the Y2K+N the accumulated knowledge will have a profound impact in the diagnostics, in the use of genes as sources of biopharmaceuticals, and to a more targeted use of genes as drugs. This means that the post-genomic era will integrate and refine the technologies of the preceding eras, and strongly ameliorate their utilisation. It is a bit early to foresee the temporal dimensions of this impact, but we can certainly expect the first genomics-driven applications to be on the clinical implementation within the next five-ten years.

#### *Therapeutical delivery of genes, differences with conventional drug delivery*

We have defined above gene therapy as the delivery of nucleic acid sequences (DNA/RNA/oligonucleotides) into somatic cells with the aim of preventing, treating or healing different types of disorders. Thus, in this case the nucleic acids are directly used as 'drugs'. If we compare this technique to conventional pharmacology we immediately realise the many differences and peculiarities that differentiate this therapeutical approach.

In gene therapy the drug is a segment of either DNA or RNA and this imposes major constraints to the delivery. In conventional pharmacology the drugs are molecules of limited size (hundreds of Daltons) that either freely enter into cells due to their lipophilic character or are hydrophilic and destined to either act in the extracellular space or to be imported through specific biological channels. The classical pharmacological drugs are designed to act over a relatively short time and their therapeutic concentration is usually controlled by calibrating re-administration regimens. Oral delivery is possible for most of those molecules, upon appropriate formulation. A termination of the administration results in a dilution and termination of the pharmacological effects. Nucleic acids do not share many of the above properties: they have a large molecular size (1 megadalton for a segment of 1500 base pairs), are destined to work in the cell nucleus but are neither lipophilic, nor can count on a physiological import system. Once delivered into the nucleus they either integrate and persist for the rest of the cell's life or are maintained episomally for variable amounts of time. Therefore, the usual pharmacological strategies only marginally apply to the delivery of these gigantic molecules. Oral delivery is essentially inconceivable and even direct injection does not permit a significant permeation into the cells. In order to render them permeable to the cell membrane, one has to either compact them into lipid-containing particles or into viral envelopes or capsids or to accompany the delivery with some physical stress (pressure, electric shock, micro particle bombardment etc.). This means that in most cases the units of delivery are no longer single, soluble molecules but relatively large (100-500 nm) and only partially soluble aggregates. This latter aspect makes the work with nucleic acids as medicines very arduous and still poorly reproducible in the complexity of a living organism. To conclude these considerations we will mention that in the jargon of the gene therapists, the transferred gene is also usually referred to as the 'transgene'. The use of this term will hopefully simplify the reading of the further paragraphs.

#### *How to reconcile: (+)efficiency, (+)specificity (+)persistence and (-)toxicity?*

This is an old pharmacological dilemma that applies to all sort of drugs (see above). However, solving these problems when delivering nucleic acids has been a kind of nightmare, and we have not yet approached a point where we can say to have 'the' ideal vector for gene delivery. Physically, gene transfer is a very arduous challenge also due to anatomical barriers. In an average soft tissue there are something like  $10^9$  cells per gram. If we inject any type of substance directly into a tissue, it will remain local for a very long time, unless it can be picked up either by the blood or the lymphatic circulation. However, only few cells are usually reached with this kind of delivery, on average no more than three-four cell layers around the injection path. This kind of 'in vivo, topical' delivery is a good option when the therapeutical gene is destined to act locally (for instance for intra-tumoral intervention, or in specific areas such as brain sectors, joints, eyes, mucosae. Theoretically the best way to reach more cells would be via the blood vasculature, which is evolved to provide oxygen and nutrients through a finely dispersed network of capillaries. However, the intravenous injection poses other problems such as the unspecific trapping by undesired capillary systems (depending on the injection site: lungs, liver, or kidney) and also the difficulty in specifically accumulating the vector into desired organs. The recent advances in the

understanding of the zip-code of the blood-vasculature will be of great assistance in attempting to design vectors that can reach specific organs and avoid others when delivered systemically.

Finally, there is the possibility of explanting cells, treat them with a transfer vector in vitro, and subsequently re-implanting them. This type of approach has been mostly adopted with bone marrow or peripheral blood cell transplants. The great advantage of this technique is that the efficiency and specificity of transfer can be controlled outside the body. Furthermore, the excess unabsorbed vector can be removed before re-implantation, ensuring a lowering of immune or inflammatory responses that are generally cause by the excess particles. The major disadvantage of the ex-vivo approach is that it requires relatively invasive surgery, with the consequent costs and complications.

Thus, the properties of the ultimate gene therapy vector should comprise: high efficiency (few particles needed for close to 100% transfer); high specificity (e.g. could be accumulated in specific organs even after systemic administration); long persistence / good regulation (that is the transgene should preferably integrate in the host genome and remain still properly regulated); low toxicity (e.g. the transfectious or infectious particles should not contain intrinsically toxic or highly immunogenic/pro-inflammatory substances). Reconciling these divergent needs is like trying to construct an engine that combines high power with low noise and low consumption. That is, at some point one has to compromise on one or another of those issues.

#### *Vectors/delivery methods, in the light of the four fundamental questions*

Recombinant viruses. To solve the problem of efficiency of transfer the gene therapists have exploited the highly sophisticated properties of viral capsids, into which we have packaged recombinant viral genomes<sup>3</sup>. The initial challenge was to construct packaging systems that can produce large amounts of infectious particles. Viral vectors have been instrumental in demonstrating that functional genes can be transferred and can provide a therapeutical effect. The principle behind viral vectors is relatively simple (Figure 2). The viral genome is engineered to substitute pathological (pro-replicative) genes with the gene of interest (reporter gene or therapeutical gene). Those replication-defective genomes cannot be packaged in normal cells, and are transferred into packaging cells where the missing viral functions are anchored in the genome or provided by a helper virus. The viral capsids containing the recombinant genomes have the same infectious properties of the natural virus, but cannot produce further infectious particles. as of today, the major classes of such recombinant viruses include adenoviruses, adeno-associated viruses, retroviruses (including HIV derivatives) and herpes viruses. This panoply of systems (table 1) permits to transfer genes into a variety of tissues and with great efficiency, and some of those vectors could even be redesigned in their specificity, and the first important successes in gene therapy could indeed be achieved thank these recombinant viruses<sup>3</sup>. This may sound great, however these vectors suffer of two major drawbacks: a) they generally permit the packaging of a limited amount of DNA, thus they do not permit the incorporation of large genomic constructs; b) their capsids generally include proteins that have intrinsic toxicity or are at least highly immunogenic, thus posing some pharmacological threats and rendering problematic a re-administration.

**Table 1. Properties of current common viral vectors**

<i>Virus</i>	<i>G-type<sup>a</sup></i>	<i>G-Size<sup>b</sup></i>	<i>Pack<sup>c</sup></i>	<i>Integ<sup>d</sup></i>	<i>Qiesc<sup>e</sup></i>	<i>Titre<sup>f</sup></i>	<i>Tox<sup>g</sup></i>
Adeno	dsDNA	36 kb	8-30 kb	No	Yes	10 <sup>10</sup>	high
Ad-assoc.	ssDNA	5 kb	4.5 kb	Y/N	Y/N	10 <sup>8</sup>	low
Retro-	ssRNA	10 kb	9 kb	Yes	No	10 <sup>6</sup>	mid
Lenti-	ssRNA	10 kb	9 kb	Yes	Yes	10 <sup>6</sup>	mid
Herpes	dsDNA	150 kb	8-100	Y/N	Yes	10 <sup>8</sup>	mid

Legend: (a) ds, double stranded; ss, single stranded; (b) genome size in kilobases or kilobase-pairs; (c) packaging size for transgene; (d) ability to integrate in host genome; (e) ability to transduce non-dividing (quiescent) cells; (f) average amount of infectious particles per millilitre supernatant; (g) intrinsic toxicity/immunogenicity of the infectious particles

*Viral-free gene transfer.* Non viral gene transfer procedures have been designed to circumvent the intrinsic problems discussed above. Several protocols that exploit either physical (electric discharges, pressure, ultrasound, microprojectiles) or biochemical (cationic liposomes, cationic polymers, mixed particles) properties have been proposed (table 2, reviewed by <sup>3</sup>). Here also, the choice is great and seems to fulfil a number of requirements. However, none of these gene transfer methods comes close to viral transfer in terms of efficiency. When considering the number of molecules/cell and the final efficiency, the difference between viral and non-viral approaches is still of several logarithms. In spite of these limitations, non viral gene delivery is still widely pursued because it offers a greater versatility and simplifies the large scale production of therapeutic material. Since no viral proteins are included, one expects a much reduced toxicity/inflammatory action. Recent tests have however suggested that even naked DNA has very strong pro-inflammatory properties <sup>4</sup>, and therefore also this problem will have to be addressed in the optimisation of non-viral gene transfer techniques.

**Table 2. Synopsis of current nonviral transfer techniques**

<i>Method</i>	<i>cell cult<sup>a</sup></i>	<i>tissue<sup>b</sup></i>	<i>effic<sup>c</sup></i>	<i>others<sup>d</sup></i>
Direct naked DNA	No	muscle	10 <sup>-4</sup>	local
Cationic liposomes	Yes	all	10 <sup>-4</sup>	general
Polycations	Yes	all	10 <sup>-4</sup>	general
Electroporation	Yes	surface	10 <sup>-3</sup>	local
Biolistic	Yes	surface	10 <sup>-1</sup>	small surface
Pressure	No	limbs/expl	10 <sup>-2</sup>	local

Legend: (a) used also in cell cultures; (b) preferred/documentated target tissue; (c) efficiency of gene transfer; (d) other characteristics.

Therapeutic oligonucleotides. Finally, the use of short sequences rather than entire genes has been shown to generate specific and promising therapeutical effects. Besides the established concept of 'antisense' oligonucleotides that interfere either with the splicing or the translation of a specific mRNA, there have been other players. DNA with catalytic properties have been successfully used to block specific pathways <sup>5</sup>, double stranded oligonucleotides have been shown to compete the undesired action of pro-proliferatory genes in clinical trial for treatment of restenosis <sup>6</sup>. This plethora of possibilities (Table 3) can become even larger when the therapeutical potential of triple strand forming oligonucleotides and PNA will be documented clinically. Thus, oligonucleotides promise to be an important sector of therapeutical development.

**Table 3. Currently used therapeutic oligonucleotides**

<i>Type</i>	<i>length<sup>a</sup></i>	<i>Application<sup>b</sup></i>	<i>Perm<sup>c</sup></i>
antisense (ss)	10-30	cancer/infect/inflamm	no
anti-splice (ss)	20-30	monogenic	no
DNAzymes	25-35	various	no
Ribozymes	20-30	various	no
decoy (ds)	20-30	cancer/restenosis	no
triple-strand	10-20	cancer/inflamm	no/yes
triple str-guided repair	30-40	monogenic/point mut.	yes
chimeroplast	60-80	monogenic/point mut	yes

Legend: (a) average size of the used oligonucleotides; (b) documented/preferred clinical applications; (c) designed to exert a permanent effect.

Finally, in the oligos class, we should not omit the mentioning of sequences that can prompt specific gene repair, by creating mismatches with genomic sequences. So far, this has been achieved with triple-strand guided oligos <sup>7</sup>, with chimeroplasts <sup>8</sup> and also with simple, conventional oligonucleotides <sup>9</sup>. The key issue in these approaches is the percentage of correction. Under some circumstances, the levels attained are extremely promising, such as the reporter 20-40% in liver delivery protocols. However, the general applicability and the reproducibility of these techniques

are still matter of hot controversies, and we have to wait some years before we can get an objective opinion on the real extendibility of the gene-correction approaches. It is clear that, if proven to be efficacious, those approaches will be strongly preferred to the 'conventional 'gene addition' strategies which are intrinsically genotoxic, because the vector integrates randomly in the genome. If the efficiency can be pushed to very high levels, specific gene correction could become biologically compatible with germ line interventions, thus resuscitating the debate on this very delicate matter.

*In many cases, therapeutical effect can be obtained even when the four requirements are only partially fulfilled*

The first clinical trial was registered in 1990 by FW Anderson and colleagues (1). Since then, more than 560 clinical trials dealing with some kind of gene transfer have been registered as of May 2001 ([www.wiley.com/wileychi/genmed/clinical/database.html](http://www.wiley.com/wileychi/genmed/clinical/database.html)). The majority (about 89%) of those is or was at clinical phase I (or I/II), which aims principally at assessing the degree of side effects at a single doses and are not focused on measuring the therapeutical effect. Some 10% was conducted under genuine phase II conditions and only 5 protocols are currently announced as phase III (2 thereof not yet initiated). This is the reason that makes me say that the first ten years of gene therapy were principally aimed at proving the concept rather than the efficacy of this novel class of therapy. Thus we can better understand why there has been very few reports of therapeutical success.

*Example 1: limb ischemia* (therapy in spite of low efficient gene transfer). The treatment of critical limb ischemia (CLI, see <sup>10</sup>) has shown remarkable results even in Phase I trials. Surprisingly, this comes from a protocol of deceiving simplicity: by injecting a pure DNA solution into the muscles. The success is owing to the fact that for this treatment gene transfer does not have to obey any of the rules of efficiency, specificity and persistence. Indeed the success of this gene transfer depends largely on its reduced efficiency and the short-lived persistence of its expression. For the treatment of CLI, the researchers have injected intramuscularly and in proximity of the necrotic tissue, a certain amount of DNA solution bearing a vector that can express the vascularising factor VEGF. When expression occurs, VEGF is secreted and diffuses, attracting thereby the migration of endothelial cells and the neo-vascularisation of the tissue. Direct injection of VEGF protein proved to be deleterious and to give too many side effects, while the gene transfer could be optimised in terms of 'gene-assisted drug delivery'. Thus, the gene transfer by intramuscular macroinjection produces just about the proper amount during just about the proper time necessary to restore a revascularisation of the affected limb. Analogous pictures seem to emerge for immunotherapeutic cancer treatments with lymphokines and cytokines such as IL-2, IL-4, IL-10, Interferons etc., where the gene delivery ensured local sustained distribution and less undesired effects. The paradigms that proved successful in the treatment of CLI are now being tested on cardiac ischemia, and the results are rather promising <sup>11</sup>.

*Example 2: haemophilia* (therapy in spite of unspecific and unregulated expression). The lack of blood clotting factors leads to various forms of haemophilia. In this case, regaining of a small percentage of active factor is sufficient to eliminate the majority of the clinical symptoms. Furthermore, the secreted factor VIII or factor IX do not need be synthesized by the original organs (e.g. liver). Therefore, muscle gene transfer with an AAV vector has been proven to confer therapeutical effects in large animal models and in patients <sup>12, 13</sup>.

*Example 3: treatment of Parkinson* (therapy with non-specific rescuing genes). In some cases like cancer, cardio-vascular degeneration and neural degeneration, the rescuing factor does not need to be the one which is exactly missing in the diseased cells. For instance GDNF has been proven to be capable of rescuing neurons from artificial injury in a primate model for Parkinson<sup>14</sup>.

*Example 4: treatment of restenosis with inhibitory oligos* (therapy in spite of lack of persistence). Restenosis is a major cause of failure of vein/artery grafts. If the hyperproliferation of the intima can be inhibited for few weeks, the differentiation takes another path and the restenosis is less likely. By incubating vein grafts with decoy or antisense oligonucleotides aimed to inhibit the action of the pro-proliferatory gene E2F, Victor Dzau and colleagues have shown to reduce the incidence of restenosis in a substantial fraction of grafts, both in animal models and in patients <sup>6</sup>. This treatment leads to long-term benefit even if the action of the therapeutical inhibitors lasts only for few days.

The above examples have been selected to illustrate that highly appreciable therapeutical endpoints can be achieved even with partially working gene delivery methods/vectors. The expected improvement of vectors and transfer methods will continuously expand the panel of treatable conditions.

#### *Erratic success and/or systematic failures*

Back in '94 the recent cloning of the CFTR gene and the emergence of generation I adenoviruses suscitated many hopes for the potential treatment of cystic fibrosis. Simple inhalation of the recombinant virus seemed the most straightforward approach to supplement the missing function to lung epithelial cells. Gene therapy was defined 'at reach' in the headlines of many reputed newspapers. This optimism proved fatal, since it became rapidly clear that lung epithels have natural barriers against infection and that the doses necessary to cross these barriers would cause severe side-effects. After a minor set of adverse effects a sobering report (the so-called 'Motulsky report') was issued at the NIH<sup>15</sup> and prompted researchers to intensify studies on ameliorated vectors instead of using the prototypes in the clinics. Now, after several years of efforts, we must reckon that cystic fibrosis will probably be the most difficult disease to be treated. But this was only the first of a series of judgement mistakes and most people know it because it made into the press. Other sad stories are still hidden. For instance, the success of earlier attempts of curing ADA deficiency by retroviral transfer was obscured by the continuous co-administration of the biotechnologically obtained PEG-ADA. This safety procedure, that was justifiably recommended by the ethical committees, prevented in fact the positive selection of the gene-transferred cells and masked the real success of this pioneering therapy. So, for ten years we had to hear sentences like 'in spite of having started long time ago, gene therapy has not yet proven its therapeutical value'. It was Claudio Bordignon who in 2000 unequivocally demonstrated that the original vectors/method to treat ADA deficiency was indeed efficient (C Bordignon ESGT meeting 2000).

However, the most dramatic 'coup-bas' to gene therapy came in September 1999, when the field was just few weeks away to witness the spectacular successes of Alain Fischer (the rumours of whose results were already circulating among the specialists circuits). By September 18 a young fellow, Jesse Gelsinger died three days after having received a high doses of recombinant adenoviruses that were originally designed to transfer the OTC gene which was defective in his liver. This casualty was caused by several gaps in the good clinical practice protocol<sup>16, 17</sup>. The death of the young fellow raised an incredible turmoil and revealed on subsequent investigations in the same and other clinics a preoccupying number of misconduct and misreporting<sup>18</sup>. Part of these mis-happenings were caused by potential conflicts of interests, because the principal investigators were shown to have strong financial involvement in the companies that were co-sponsoring the research. To avoid further degenerations, the ASGT has issued a memorandum requesting that a PI should not be accepted as responsible for a clinical trial if he/she has stock options or other financial bonds with the sponsoring company<sup>19</sup>. We must hope that this measure will have the desired effects and help restoring the credibility of the entire field, since the glorious reports of Alain Fischer<sup>20</sup> did not fully succeed in this job.

As demonstrated by the graph in Figure 3, a substantial effort in gene transfer has been devoted to attempts of controlling/curing various cancer forms. The strategies include: the simple protection of normal tissues via transfer of drug-resistance genes; the specific boosting of anti-tumoral immune reactions; the invention of many different pro-drug activation systems, and the establishment of replication-competent viruses that preferentially replicate in tumor cells<sup>21, 22</sup>. Why so much effort in cancer treatment? I believe this is due to two coincidences: (i) cancer is the second highest mortality reason in our society (marketing considerations); (ii) in patients with incurable tumors, the risk-benefit balance of a potentially toxic intervention such as prototypical gene therapy is much more favourable. The strong percentage of cancer-related therapies is the best argument to demonstrate that gene transfer therapy can go much beyond the treatment of hereditary, monogenic disorders.

*Quo vadis GT after Gelsinger's debacle?*

Gene therapy has seen several ups and downs in its short history (Figure 4). It is one of the few medical fields in which the public discussion and public awareness largely precedes the clinical applications. Other drugs or treatments were made available without such preliminary discussion. Was this good or bad? Can't properly answer. However, it becomes evident that when new technological advances hit the society, there are many conditions for abuse or misuse (suffices to think about the side negative effects of the broad-scale availability of technological gadgets such as internet, cellular phones, erythropoietin). Thus, I genuinely believe that the discussion around gene transfer may help sorting out some problems before they are really concrete. Perhaps, the emphasis on the Jesse Gelsinger case was a very constructive element in this debate. It is a pity that this costed the life of somebody, but we must hope that this was not for nothing.

*And how much has ol'good Switzerland to say in all this?*

Switzerland has a very high reputation and a solid tradition in the life-sciences research field and it is therefore less surprising to note that our country counts a substantial percentage of gene therapy-enrolled patients. About 10% of the world-wide patients have been treated in Swiss Hospitals. Since 1996, the National Research Program number 37 (NFP37) 'Somatic Gene Therapy' has entered its active phase. The program has invested 15 million Sfr over the past five years (it expires by the end of 2001). All in all, the NFP37 has financed about thirty research teams whose activity classes follow very much the world trend ([www.unifr.ch/nfp37](http://www.unifr.ch/nfp37)). Several other clinical trials and much more basic-research investigations on gene transfer technology were initiated or conducted independently to the NFP37. Thus, in our country this particular application seems to enjoy a good popularity among the investigators. It remains to be established how much the Swiss efforts will be competitive in the final rush. Perhaps it will be important to create a stable network of gene-transfer scientists, to promote the quality of training and research this important medical investigation area.

*Now we understood what's all about, and yet, there are still people who confuse issues when it comes to GT*

Besides the common belief that GT is mainly dedicated to inherited disorders (see above) there are several other 'myths' that may deserve a comment. Many critics predict that gene therapy will be invariably expensive. The example of DNA based vaccination should be sufficient to illustrate that gene-based interventions can become much less expensive than conventional therapies, if properly designed. Another current belief is that gene therapy is withdrawing substantial public funds from conventional research. A simple calculation shows that less than 1% of the public research funds are currently put into this field in our country, and less than 2% in the US. The reason is simple: most of the financial effort in the field of gene therapy is done by private investment. Another common opinion is that GT has not yet proven its effectiveness. The examples cited above, together with the unequivocal curing of young children affected by a lethal immunodeficiency<sup>23</sup> are the best arguments to contrast this assertion.

Finally, there are two 'technical' myths that can be easily disputed: a) all the recombinant viruses are of the non-autonomously replicating type; and b) current GT protocols primarily work with 'random' gene insertion. The point (a) has been discussed already when we mentioned the use of selectively replicating viruses for the killing of tumor cells (see above). For the point (b), we shall mention again that there are emerging protocols that utilise oligonucleotide-mediated gene repair (see chimeroplasts commented above) and raised the possibility of curing disorders caused by point mutations by specifically correcting the resident defective genes.

*Illusion or reality: which accelerations/challenges are we expecting from the HGP and from cell therapy protocols?*

Recently, another myth has been added to the list. In the euphoria of the human genome sequencing, some media (probably prompted by some unscrupulous scientists) have suggested that the sequencing of the genome will accelerate the progress in gene therapy. To me, this is true but it will not be so immediate as implied in some statements. The knowledge about the genome structure will assist us in setting new hypothesis about gene regulation, in finding new gene targets etc., but those advances will only become tangible in several years to come.

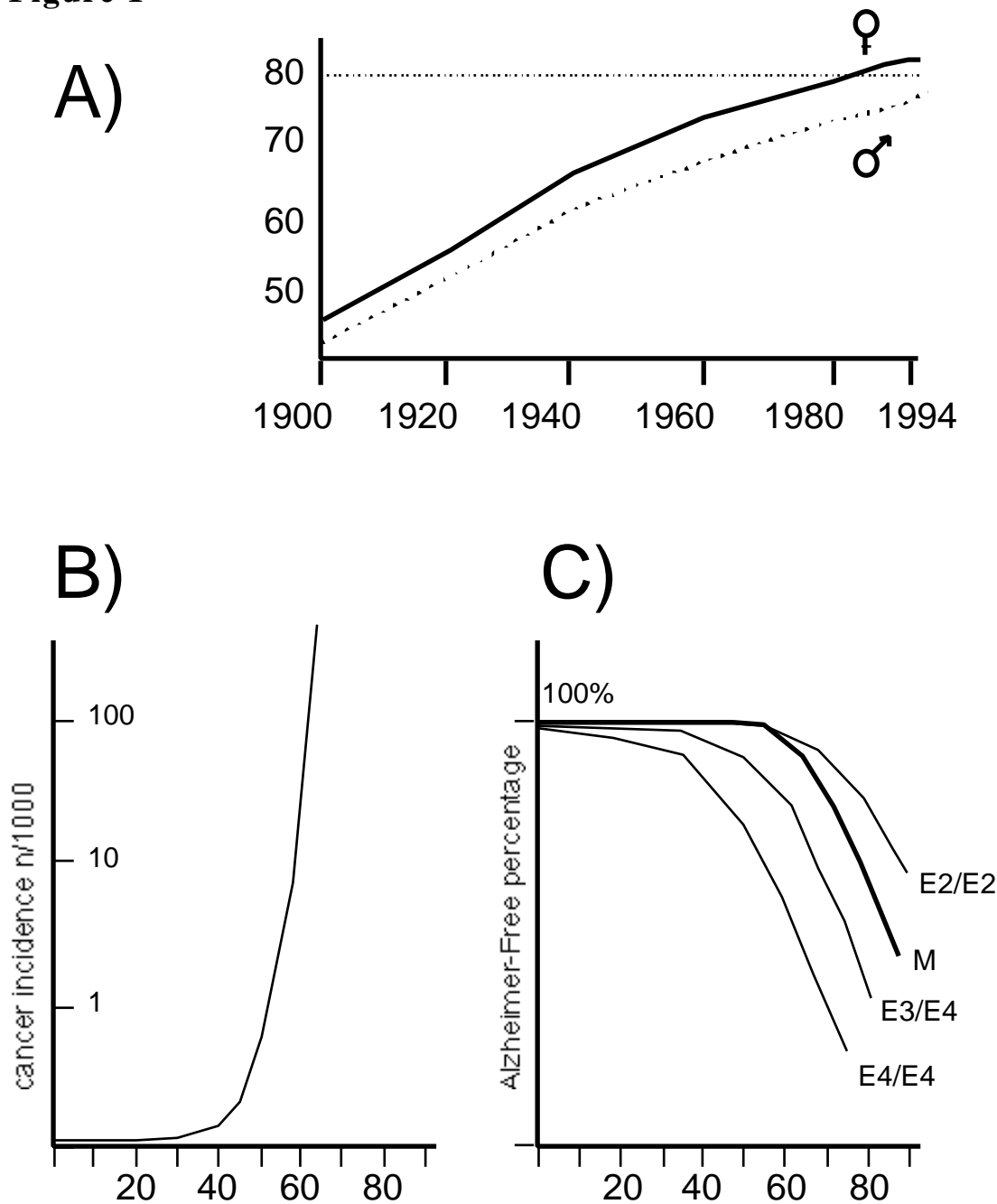
Gene therapy has started making headlines long before having concretised its most elementary requirements. But when will gene therapy become a clinical reality? The fact that we still have so few phase III clinical trials witnesses that broad-based clinical applications are not for tomorrow. On average only one out of three-five new therapeutical approaches that pass phase III is finally successfully registered. Thus, we have to wait several years before seeing gene transfer procedures translated into the daily clinical praxis. Meanwhile, the development of conventional drugs or protein drugs will also continue in parallel. This development may render many gene therapy approaches obsolete before they are finally tested. Think for instance how much less urgent the issue of AIDS has become after the introduction of tri-therapy. Also in the cancer field new and powerful small or mid-molecular weight drugs are coming into the market (think of STI571 or Herceptin, just for two examples). However, for chronic disorders, the biggest challenger seems to be cell therapy. The chasing news about progresses in the isolation and manipulation of stem cells from many different tissues make us believe that many disorders could one day be treated by cellular transfer rather than by in vivo gene transfer. It is possible that the cell transfer could be accompanied by gene transfer in vitro, but for this kind of intervention the today's gene transfer methods are amply sufficient. Will cell therapy sentence the death of gene therapy? Will they coexist peacefully with their own niches? Will they merge into a global replacement therapy? As usual, only the future will tell...

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**FIGURES AND LEGENDS**

**Figure 1**

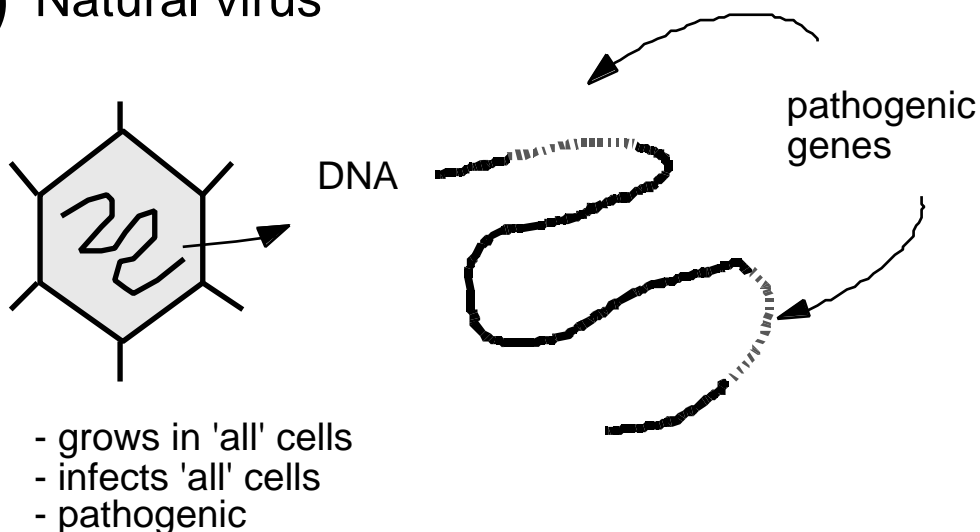
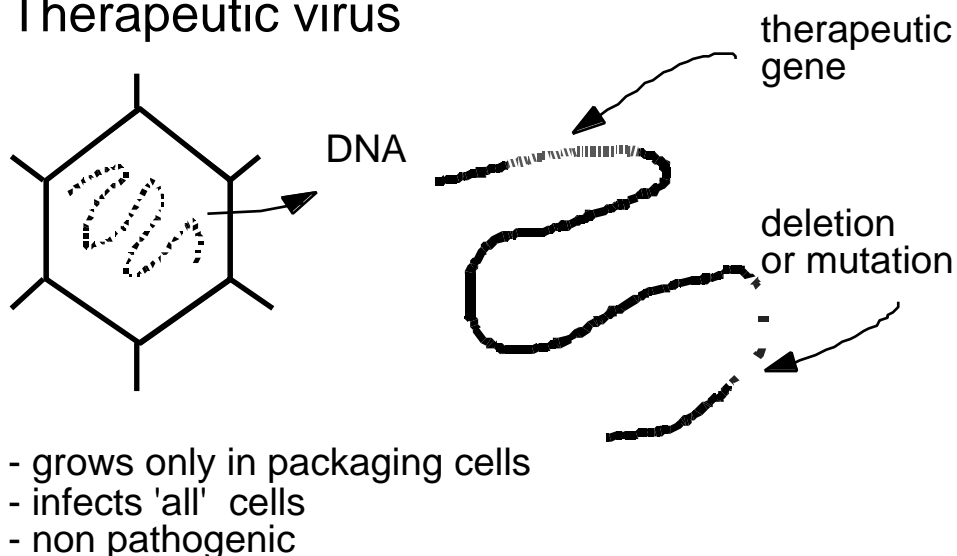


**Figure 1, Ageing of the population and disease frequency**

A) Plot representing life expectancy at birth (y axis) during this century (x axis), source Swiss office of statistics

B) Plot of the incidence of cancer (y axis) as function of age (x axis), source NIH.

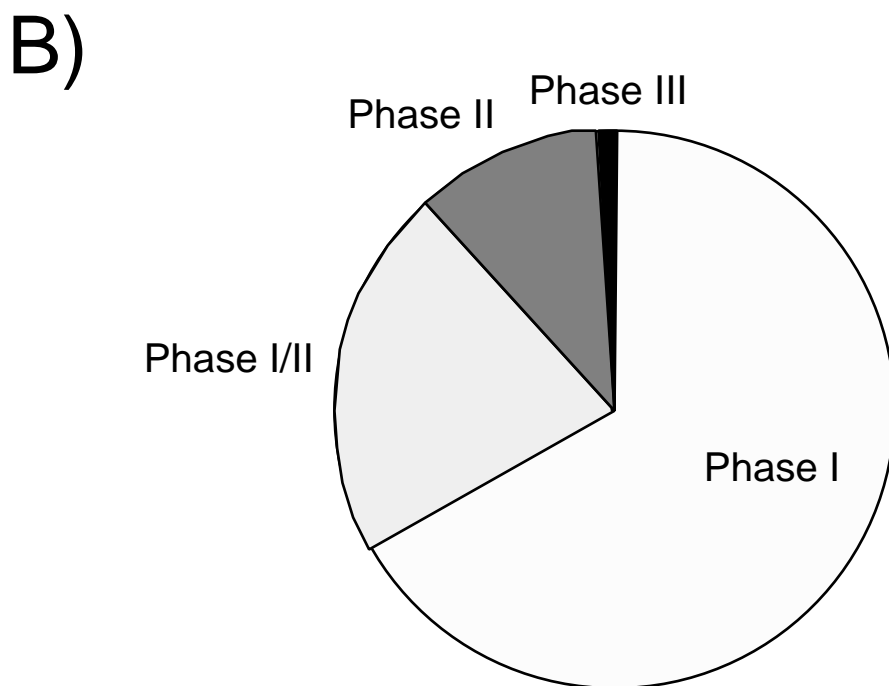
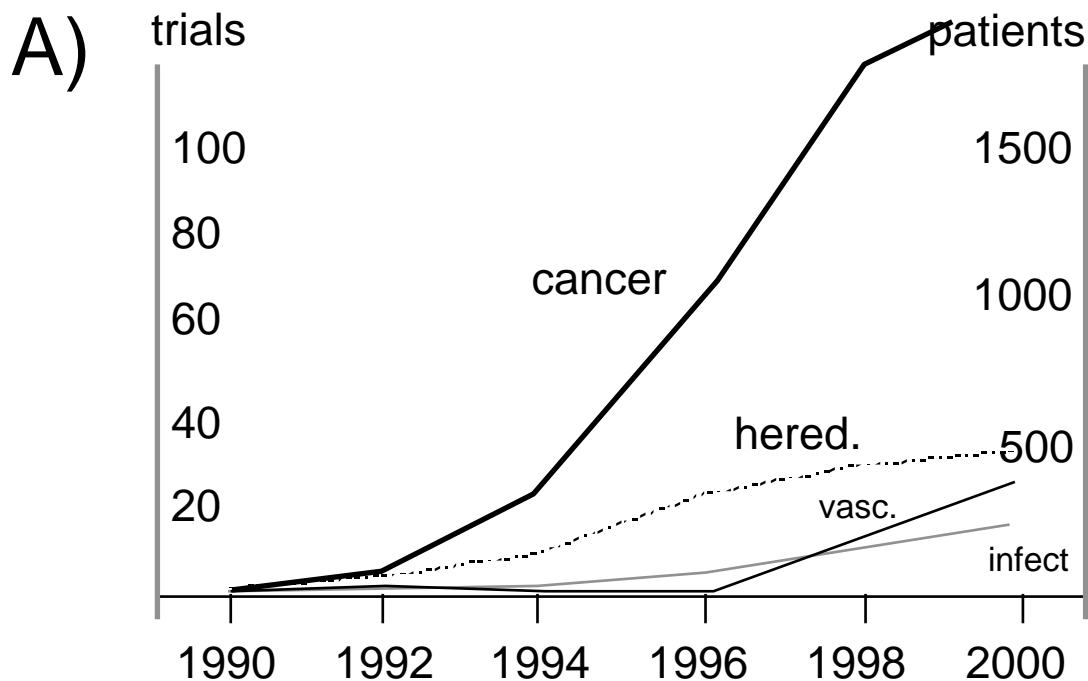
C) Plot representing Alzheimer's-free fraction as function of age. Thick line marked M, average of population; thin lines marked E(n)/E(n), curves for cohorts with distinct combinations of alleles of the apolipoprotein E gene.

**Figure 2****A) Natural virus****B) Therapeutic virus****Figure 2, Therapeutic viruses**

Natural viruses (2A) are composed of one or more protective protein (envelope or capsid proteins) that protect the nucleic acids that compose the viral genome (RNA or DNA). Natural viruses contain in their genome all the genes that are necessary for producing the coat and envelope, and for ensuring viral genome replication (dashed portions) after infection. The genes responsible for viral replication are changing the cell's genetic program and are therefore the basis of the pathogenic potential of viruses.

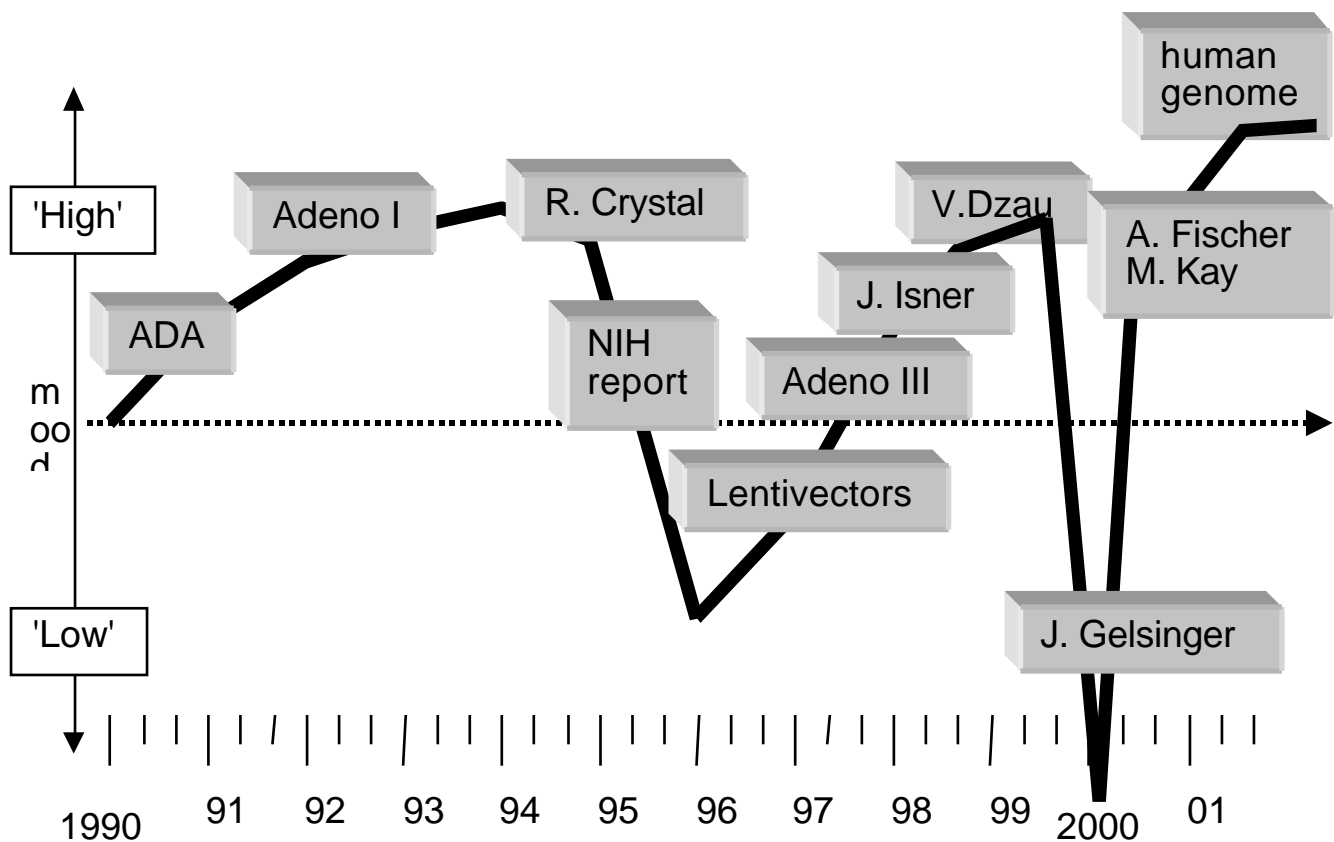
In recombinant viruses (2B) parts of the genome are substituted by the genes of interest (therapeutic or marker gene). Such recombinant viruses are incapable to grow autonomously. They can be grown in the so called 'packaging cell lines' which contain in their genome the complementing functions. One of the disadvantages of recombinant viruses is in their limited size capacity for foreign genes (see text).

**Figure 3**



**Figure 3. Trends in gene therapy**

A) Gene therapy world-wide. The graph shows the evolution of approved clinical trials (y axis left) or the number of patients (y axis right) as function of time (x axis, from 1990 to 1998). Solid line, clinical trials aimed at cancer treatment; dotted line, clinical trials aimed at treatment of hereditary disorders. Other abbreviations: vasc, vascular disorders; infect, infectious disorders.  
 B) Percentage of clinical trials by phase, situation as of May 2001 (from the Wiley.com database).

**Figure 4****Figure 4. Ups and downs in gene therapy**

The x-axis shows the time from 1990 to 2001. The y-axis has arbitrary units of my appreciation of the general mood in the field of gene therapy. Points below zero-line, discouraging or destructively-critical; above the zero-line, encouraging and constructively critical. The boxed texts indicate the emergence of crucial events: ADA adenosine deaminase; Adeno I; first generation adeno vectors; R Crystal, first clinical trial R Crystal on cystic fibrosis; NIH report, critical report on the limitations of gene therapy<sup>15</sup>; Lentivectors, first reports on HIV based recombinant vectors; Adeno III, first reports on gutless adeno vectors; J Isner, treatment of CLI; V Dzau, treatment of restenosis; J Gelsinger, death of a patient as direct consequence of the adeno transfer; A Fischer/M Kay, treatment of SCID and Haemophilia; human genome, publication of the HG sequence.

### **Short CV S. Rusconi**

S. Rusconi (1952) has obtained the degree of primary school teacher in 1972 in 1974 he enrolled in the molecular biology program at the Zürich's University. He obtained the diploma in 1979 and the doctoral title in 1982 with a Thesis on transgenic vertebrates. He continued working on transgenics in 82-84 and then he moved to UCSF for his postdoctoral stage in the field of steroid hormone receptors. He brought back this knowledge in 1986 as a group leader at the institute for Molecular Biology at the UNIZH. He became 'Privatdozent' in 1991 and was nominated full professor of biochemistry at the University of Fribourg in 1994.

His field of interest rotates around fundamental gene regulation with emphasis on steroid hormones genomic responses, including the effects of endocrine disrupters. Further interests include DNA stability in cellular ageing, engineering of recombinant viruses, in vitro evolution of bioactive nucleic acids, functional genomics, and gene transfer in animals and in humans. Between 1988 and 1998 he was treasurer and then president of the Swiss Society for Molecular Biology. The USGEB committee (Union of Swiss Societies for Experimental Biology) has selected SR as the designated president of the USGEB for the years 2003-2005. Since 1996 he is director of the Swiss National Research Program Nr 37 entitled 'somatic gene therapy'. Web sites: [www-chem.unifr.ch/bc](http://www-chem.unifr.ch/bc); [www.unifr.ch/nfp37](http://www.unifr.ch/nfp37). Mail: [sandro.rusconi@unifr.ch](mailto:sandro.rusconi@unifr.ch)