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Gene Therapy Oversold by Scientists Who Disregard Risks

Gene therapists are turning more and more to nature's worst in a desperate bid to overcome the extensive failures. The US National Institutes of Health Report by a special panel in 1995 already deplored the lack of basic research and disregard of the risks involved, and expressed concern that scientists have been overselling gene therapy. Little seems to have changed since. **Angela Ryan** reports on the continuing fiasco of attempts to genetic engineer human beings.

Last month's *New Scientist* reported the combination of two notorious killer viruses, HIV and Ebola [1], in an attempt to find an effective gene therapy vector for the treatment of cystic fibrosis. When this work was presented at a scientific meeting the audience laughed out loud.

Gene therapy is targeted at virtually every ill known to human beings, especially those inhabiting the first world, including pain relief, cosmetic hair replacement and muscle building. Massive investment has gone in but no clinical efficacy has ever been proven, despite anecdotal claims of success.

Last year in the US, gene therapy clinical trials ground to a halt amid scandalous reports of deaths and conflicts of interest [2]. The US National Institutes of Health (NIH) set up a special telephone hot line for victims that counted 652 cases of serious adverse events along with six unexplained deaths. Effects included high fevers, infections and severe changes in blood pressure, all of which went previously unreported to the NIH Recombinant DNA Advisory Committee (RAC). David Baltimore, Nobel laureate and president of Caltech, a

gene therapy based biotech company, said " I disagree we've had any benefit from gene therapy trials so far, many of us are now asking, what the hell are we doing putting these things into people?"

Sir David Weatherall, Professor at the Institute of Molecular Medicine, University of Oxford, told The UK Royal Society discussion meeting on Social Responsibility in Science [3] that "scientists have not made efforts to maintain an open and completely honest debate with the public about what they are doing. Part of the problem arose from over ambition or pressures to publish, to attract research funding".

Misinformation has generated much hype in the media about the promises of gene therapy. One main problem identified by Weatherall is that, "many scientists working in the molecular sciences are not clinically trained, even though their work impinges more and more on human molecular pathology. They know a great deal about the technicalities of their field but nothing about the complexity of human beings and their diseases". Scientists have over-exaggerated their work, for newspapers don't like 'ifs' and 'buts'.

The US Food and Drug Administration (FDA) and the NIH responded to widespread concern about risks, especially after the 1999 death of teenager Jesse Gelsinger in a phase I clinical trial. Many laboratories were shut down, public meetings were held, reviews and investigations commissioned and administrative changes have been put in place to deal with the crisis [4]. But the troubles run deep within the heartland of biomedical science, where the most important concern remains the issue of *safety*.

Gene therapy targets diseases based on the transfer of genetic material into an individual, rather than a drug. It uses genes as the therapeutic agent, and it is qualitatively very different from other forms of treatment. Despite the serious health risks involved, clinical trials have been underway since 1990. The recently released NIH 1995 report on gene therapy research documents a plethora of scientific and clinical risks associated with gene therapy [5], many of which have been highlighted independently in an ISIS report [6].

There are major technical problems with all aspects of gene therapy [7]. Furthermore, few pre-clinical data have been published and toxicological evaluations are seldom found in the literature. The potential for generating new viruses, known as replication - competent viruses (RCV) needs to be thoroughly evaluated, particularly as genetically modified viruses are used in gene therapy. The spread of viral vectors to non-target tissues throughout the host is also a major safety concern. There is no way to predict the virulence or disease potential of recombinant viral vectors, and a case-by-case approach had to be applied. It has been shown, however, that viral vectors can induce toxic shock following administration [8].

The NIH expert panel found that all gene transfer vectors are ineffective and it is not understood how they interact with the host. Basic studies of disease pathology and physiology have not been done, which are critical for designing treatment. It is not possible to extrapolate from animal experiments to human studies. In the cases of cystic fibrosis, cancer and AIDS, animal models do not have the major manifestation of the disease in humans. Gene transfer frequency is extremely low and results of gene therapy protocols rely on qualitative rather that quantitative assessments of gene transfer and expression. There are no controls, and biochemical or disease endpoints are not defined.

The panel concluded "only a minority of clinical studies, illustrated by some gene marking experiments, have been designed to yield useful basic information" [as these at least track the fate of the genetic vector]. The report states that there is "concern at the overselling of results of laboratory and clinical studies by investigators and their sponsors, either academic, federal, or industrial, leading to the widespread perception that gene therapy is further developed and more successful that it actually is".

In gene therapy, DNA is delivered, either by direct administration of viral vectors, or naked DNA, into the bloodstream or the tissues, or indirectly, through the introduction of cells that have first been genetically modified. In human studies, only somatic cells are the target of gene therapy, not germ cells (eggs and sperm), although germ line gene therapy is common practice in animals. Four main types of disease are targeted; single-gene inherited disorders, multi-factorial disorders, cancer and infectious diseases.

Single-gene inherited disorders occur infrequently in populations. They are chronic conditions associated with the loss of function in a gene and relevant protein. Such single gene disorders include sickle cell anemia, hemophilia, inherited immune deficiencies, hyper-cholesterolemia and cystic fibrosis. Gene therapy aims to replace the mutant gene with its normal counterpart. The NIH panel found major problems with access to relevant cell types as well as assessing the total fraction of cells in a tissue that need to be corrected. It may not be technically possible to achieve the right level of gene expression required for correction, nor regulating the expression of the gene after it is transferred.

Multi-factorial disorders, like coronary heart disease or diabetes, involve many genes, not to mention environmental factors. The aim of gene therapy is to reverse or retard disease processes at the cellular level. The NIH panel pointed out that it is "not known how specific gene products influence cellular physiology" and therefore only purely speculative strategies have been proposed and tested.

Last year, the American Heart Association (AHA) expert panel on clinical trials of gene therapy in coronary angiogenesis found gene therapy to be unsatisfactory, especially in comparison to conventional treatments, and expressed concerns over safety [9].

Gene therapy for coronary angiogenesis involves the delivery of growth factor genes into the heart to stimulate blood vessels to grow. But the Heart Association stated "no process-specific stimuli or growth factor has ever been identified", and "re-growth of blood vessels is a complex process that involves multiple levels of stimulators, inhibitors and modulators". Therefore, for a single growth factor to work, "an entire self-propagating cascade or proliferative, migratory, chemotactic and imflammatory processes must be initiated". They leveled strong criticism to suggest that gene therapists aren’t even using the right genes.

The Heart Association is also concerned over the mode of delivery and the ‘optimal dose schedule’, which they said "is unknown". Gene therapy is very variable in the levels of the proteins produced and the duration of expression. They cite one study in which earlier-generation adenovirus vectors persisted and caused dysregulation of a number of host genes. They state that "preclinical and clinical studies *should be preceded* by tissue distribution studies to define the myocardial uptake and retention or expression of growth

factors" (author’s emphasis).

Gene therapy vectors cause immune responses, which in turn cause inflammation and transgene silencing. Attempts to make vectors safer and more efficient result in longer-term transgene expression and the American Heart Association expressed concern about deleterious effects due to prolonged growth stimulation. They are also concerned about cancer, a known risk with all gene therapy protocols due to random insertion of transgenes into the cell’s genome. The report states quite categorically "the necessary extent of cancer screening has not ever been defined".

The NIH panel pointed out that in many cancers, the cancer-causing gene is dominant and transferring a normal copy has no impact. The number of cells within a tumor is large, and the technology will only transfer genes to a subset of cells within a tumor mass. Furthermore, the mutation rate in cancer cells is very high, so the introduced gene itself may become mutated, its function inactivated, giving rise to more cancer cells. Finally, the complication of migrating cancer cells means the transfer of DNA is "not a feasible strategy".

More indirect gene therapy approaches have been considered for cancer, including the transfer of genes for cytokines or other immune modulatory factors, either outside or inside the body of the patient. This approach attempts to stimulate immune recognition not only of tumors but also cancer cells that have spread. Some of these strategies have shown promise in mouse models but none have demonstrated efficacy in humans.

A number of chronic infectious diseases have been targeted by gene therapy, HIV being the best studied. Efforts have focused in two areas; post-exposure vaccination and attempts to express genes in target cells that render HIV unable to infect or replicate. Other products have been developed and tested, including mutant proteins that inhibit virus replication, antisense RNA that blocks translation of HIV genes, ribozymes that break down HIV RNA, ‘decoy’ RNA that competes for binding of viral proteins and antibodies that prevent key HIV enzymes from functioning. All these strategies and more have been attempted, without success.

Naked DNA vaccines for HIV contain single HIV genes or combinations of HIV-1 early regulatory genes. Such HIV derived genes may recombine with other retroviral sequences, generating new strains. Viral sequences also integrate into the host genome, causing genetic damage [10].

Three main types of gene transfer vector systems are in use: DNA vectors (either naked or complexed with proteins or other molecules, RNA viruses (retroviruses), and DNA viruses (adenovirus, adenoassociated virus [AAV], herpesvirus, and poxvirus). However, none of the available vector systems are satisfactory.

The NIH report stated "the perceived advantages of each system have not been experimentally validated", and "the efficient introduction of these vectors into cells is likely to be a formidable obstacle to their use."

Retroviral vectors are used extensively, as the basic biology of retroviruses is the best understood of the vector systems. But they are very expensive and complicated to prepare and validate, often having a low titer and limited insert size. Gene transfer is limited to dividing cells and expression is difficult to control and stabilize. They insert randomly in the host chromosome, which causes genetic damage and means the introduced gene does not express in the same way as it would in a normal, healthy cell. They can also lead to the creation of new viruses [11].

Adenoviral vectors have been used in about 25% of active gene therapy trials. They contain many viral genes and have been shown to be highly immunogenic. They can enter most cell types, although the factors controlling this are poorly understood. They generate RCVs by recombination and cause genetic damage by random integration into the host genome. Patients with previous infection of natural adenovirus will mount immune responses to these vectors.

Teenager Jesse Gelsinger died three days after receiving a dose of adenoviral vectors. Within the first day, tests showed he had suffered liver injury and inappropriate blood coagulation. On the third day he had trouble breathing and his vital organs began to fail. He was taken off life support on the fourth day. The autopsy revealed further abnormalities. The researchers had concentrated the vector in the liver, infusing it directly through a catheter. But significant amounts of vector were found in the spleen, lymph nodes, bone marrow and other tissues and when analyzed, duplicate sequences not engineered in the original were discovered, revealing vector recombination [12].

Since the damning NIH clinical report was published in 1995, more problems have come to light. Large-scale production of pharmaceutical-grade gene therapy vectors remains a major stumbling block to commercialization [13]. In retroviral vectors, packaging cells contain large numbers of endogenous retroviral sequences that can participate in recombination events and form new viral strains. Gene therapists try circumventing this hazard by removing as many homologous viral sequences as they can from the vector. However, it has been demonstrated that sequence homology is not necessary for viral recombination [14].

Immune-toxicity also continues to hamper progress and as vectors become more complicated in terms of construction and more chimerical in terms of origin, this problem becomes more acute. Using more than one type of vector and or having a high dose of vector particles has been ruled out as it poses a risk to health from insertion mutagenesis [15]. *Ex-vivo* gene therapy holds the greatest potential. Restoration of the common g-chain expression in X-SCID children in France was the first recorded case of a therapeutic effect, although the therapeutically efficient gene transfer and expression in human targeted cells has yet to be proven [16].

Hybrid vectors are now commonplace in gene therapy, combining elements of one viral system with another. Adenovirus-associated virus have been incorporated into herpes simplex vectors, moloney murine leukemia virus (MoMLV) has been incorporated into herpes simplex type 1 vectors and elements from adenovirus and retroviruses have been combined extensively. Retrotransposons or jumping genes are also employed in various ways, along with a whole host of other genetic fragments of diverse origin, including HIV and Ebola [17].

Viral coat proteins are also being used to help improve the uptake of viral vectors, this is known as pseudotyping. Retroviruses pseudotyped fuse with cells and do not use their normal receptors to gain entry into cells. They have a much broader host range than wildtype viruses and some are capable of infecting all organisms, showing no restriction for species infectivity [18]. Such viral particles are potentially very dangerous and should not be released from contained use conditions. They may recombine with wild viruses and relays of horizontal gene transfer events could bring about the creation of a new viral zoonosis, causing a world pandemic.

Constructing novel vectors with multiple modifications to various elements of the vector, has an additive, deleterious effect on stability: the more mosaic the vector the more unstable it is. One approach has been to use the P1 phage site-specific recombinase, cre/lox system, to remove the sequences that cause instability [19]. But this presents another risk to health, as there are many pseudo lox sites in the mammalian genome and use of the cre recombinase alone has been shown to cause large scale genomic rearrangements or scrambling in mouse studies [20].

Other groups are using different combinations of promoter/enhancer elements that exhibit cell type specific gene expression, but little has been achieved in terms of targeting the vector to specific regions of chromosomes.

The risk of inappropriate integration into the host genome that may trigger diseases such as cancer remains a central safety issue along with the creation of new types of virus.

The effect and influence of cloned hybrid genetic vectors on the function and safety of the vector, the transduced cell, the immune system and the transgenic organism as a whole is largely unknown, and requires careful long term studies.

‘Gene therapy’ has been wildly premature. All the indications suggest this so called ‘therapy’ may be worse than ‘disease’. Many scientists have pointed out that ‘complexity’ is the watchword in disease genetics [21]. Even the apparent simplicity of single-gene disorders is clouded by the specter of modifier genes that can influence disease susceptibility, severity or progression. Genetic determinism is dead [22]. Much careful work is required to tease apart the complexities of the range of factors that influence normal gene expression.

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The Institute of Science in Society
PO Box 32097, London NW1 OXR
Tel: 44 -020-7380 0908