Gene therapy: has it delivered what it promised?

Gene therapy was hailed in the late 1970s and early 1980s as the great 'miracle cure' for a wide range of diseases. With the increasing genetic knowledge and skills available it was possible to envisage a world where every genetically defined disease would be amenable to some sort of genetic intervention and potential cure. Although we have continued to make remarkable progress in understanding the genetic basis of disease, particularly through the success of the human genome project, many feel that the therapeutic improvements that were expected have failed to materialize.

This is mostly because of unrealistic expectations: despite huge investments in time and money, it is only recently that the necessary knowledge and tools to deliver therapeutic benefit have been available. It is only in the last few years that we have been able to optimize gene delivery techniques, to scale them up for clinical use and to deliver genes to the appropriate target cell types in a clinical setting. Recently clinical trials of gene therapy for some forms of primary immunodeficiency have started to yield successful results and these will pave the way for further expansion, both in terms of numbers of patients and in the variety of diseases that will be treated in the future.

Initial clinical trials for ADA-SCID

The first clinical trials of gene therapy were attempted in the early 1990s. The initial target disorder was an inherited severe combined immunodeficiency (SCID) caused by lack of an intracellular enzyme, adenosine deaminase (ADA). All forms of SCID are usually fatal in the first year of life without effective treatment by haematopoietic stem cell (HSC) transplantation, although ADA-deficient SCID (ADA-SCID) patients can be maintained by administration of exogenous bovine ADA. Unfortunately bovine ADA treatment may only be partially effective and it is very expensive.

The treatment of choice, HSC transplantation using a matched donor, is highly effective. However, in an increasing number of cases such a donor may not be available and this necessitates the use of a mismatched donor which is associated with considerable risks of morbidity and mortality. Thus, since the ADA gene was cloned in 1984, ADA-SCID was considered as the prime candidate disorder for the development of gene therapy.

Retroviral vectors were (and continue to be) the vectors of choice for this application since they allow stable gene transfer to dividing cell types, such as HSCs, ex vivo. Once the genetic material is incorporated into the cell's genome it is replicated in cell division and passed on to daughter cells. This means that theoretically the treatment, if successful, needs only to be performed once in a patient's lifetime, but repeat treatments need not be ruled out if necessary. There is a disadvantage to this process, in that chromosomal integration can lead to inappropriate gene activation or inactivation and subsequent deleterious effects (see below).

Although it was possible to introduce the transgene into the patient's cells in these early trials, these studies were largely unsuccessful in correcting the immune defect. Perhaps the most important lessons learned from these trials was that much more needed to done to improve transduction efficiency and to understand the molecular mechanisms at play in these processes. It was also clear that, at least for ADA-SCID patients, it was going to be necessary to 'make room' in the bone marrow to allow transduced cells to engraft.

Gene therapy for SCID-X1

By 1993 the common gamma chain (γc) was identified as being the causative gene in the X-linked form of SCID (SCID-X1). Once the molecular basis of this disease became more thoroughly understood, it became apparent that SCID-X1 might be a much better choice of disease for initial clinical trials. Evidence from SCID-X1 patients with somatic reversion events suggested that it might be possible to perform gene therapy without having to ablate the recipient's own immune system first, something which was identified in the earlier trials as being a likely prerequisite for success in treating ADA-SCID. This, together with improvements in gene transfer technology and cell handling protocols over the years, has led recently to the development of highly effective clinical protocols.

The results of these trials are remarkable and represent the first definitive successful application of gene therapy in 'curing' human disease. To date a total of 19 children with SCID-X1 have been treated in two main centres by these protocols, with evidence of success in the majority (Cavazzana-Calvo et al, 2000; Gaspar et al, 2004 and unpublished data). In two older patients treated the treatment was less successful, indicating that in this disease early intervention is likely to achieve greater success (Thrasher et al, 2005).

In the study of 11 children in Paris, there were three cases where immunological reconstitution was successfully achieved, however, there was the subsequent development of a T-cell lymphoproliferative disease about 3 years later (Hacein-Bey-Abina et al, 2003a and unpublished data). Although two of these patients have responded to treatment, unfortunately one has relapsed and subsequently died. In the case of the two patients analysed to date, it appears that the enhancer sequences in the retroviral vector have activated the potent LMO-2 proto-oncogene leading to unrestricted T-cell proliferation (Hacein-Bey-Abina et al, 2003b and unpublished data).

The lack of such serious adverse events (SAEs) in other clinical studies and in many murine studies would suggest the involvement of other additional factors and it is unclear whether all patients undergoing this type of gene therapy may be at risk, or whether the risk may be restricted to SCID-X1.

In the second study in London eight children were treated and to date there have been no indications of SAEs, although only half of these patients are past the apparently critical 3-year mark.

This makes decision making difficult, especially for families who have few alternatives. Despite this setback, however, it must be borne in mind that these trials are still in their infancy and that the risks must be offset against the not insubstantial risks that would be entailed in undergoing a mismatched allogeneic HSC transplant, for which there is a 20% first-year mortality rate and significant short- and long-term morbidities. The regulatory authorities in France and the USA have put ongoing SCID-X1 trials on hold. In the UK, however, it was decided that trials can proceed on a case by case basis. Careful monitoring and analysis of larger numbers of patients will be required to address the issues.

Trials for other related diseases

Two studies have now incorporated significant changes into ADA-SCID gene therapy protocols to achieve success. In both these trials patients received a mild non-myeloablative conditioning regimen before the infusion of gene-modified cells, allowing the engraftment of a greater number of modified cells (Aiuti et al, 2002; Gaspar et al, unpublished data, 2006). Nine children have now been treated successfully to date.

Similarly, there are now protocols which include appropriate conditioning regimens for treating patients with X-linked chronic granulomatous disease (X-CGD), a disorder of neutrophil function (Ott et al, 2006). Two adult patients with X-CGD were successfully treated, suggesting that such protocols can not only be used for diseases affecting the lymphoid compartment but also for those such as CGD which affect myeloid cells.

Further diseases which are likely to be candidate disorders for this kind of therapy in the near future include other forms of SCID, caused by mutations in the receptor tyrosine kinase JAK-3, the recombination activating genes RAG-1 and RAG-2, and ZAP-70, and another severe immunodeficiency, Wiskott–Aldrich syndrome, where mutations in the WASP gene result in immunodeficiency, thrombocytopenia and eczema.

The future

The results from these studies show that HSC gene therapy can be successfully applied. The use of appropriate pre-conditioning regimens coupled with gene therapy offers much hope for many inherited haematological and immunological disorders where initial cytoreduction may be a prerequisite for successful outcome. It is likely that there will be further refinement of these techniques and the initiation of clinical trials for additional conditions over the coming years.

Further clinical progress will come from improvements in vector design to promote safety and efficacy. In order to reduce the risks of insertional events it is important to prevent transcription of non-target genes. This can be achieved using DNA sequences which interfere with the interaction between the enhancer and promoter elements, or by using self-inactivating (SIN) vectors, where transgene expression is controlled by a non-viral promoter with limited or no enhancer activity. In the future SIN vectors based on lentiviruses may allow the preservation of larger numbers of multipotential progenitor cells, which would reduce the numbers of cells required to be returned to the patient. The ideal would be to develop homologous recombination techniques to the point where they can be used as gene repair tools.

Although it may be considered that gene therapy has not yet delivered what it promised 20 years ago, we are now poised at the brink where this technology could really start to contribute to developing successful treatments for a large number of patients with different diseases. It is possible that we will be able to deliver far many more effective cures, in terms of patient numbers and diseases, in a much shorter space of time. BJHM

KEY POINTS

- After a disappointing start, gene therapy now works well for some primary immunodeficiency diseases.
- There is still room for improvements (vector development, improved transduction protocols) in the treatment of these diseases.
- As with any new treatment there will still be problems, such as insertional mutagenesis, which will be overcome with more research.
- In the longer term it will be possible to treat many more patients with a greater range of diseases.

Christine Kinnon
Head of the Molecular Immunology Unit
Wolfson Centre for Gene Therapy of Childhood Disease
UCL Institute of Child Health
Great Ormond Street Hospital for Children NHS Trust
London WC1N 1EH

Aiuti A, Slavin S, Aker M et al (2002) Correction of ADA-SCID by stem cell gene therapy combined with nonmyeloablative conditioning. *Science* **296**(5577): 2410–13

Cavazzana-Calvo M, Hacein-Bey S, de Saint Basile G et al (2000) Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. *Science* **288**(5466): 669–72

Gaspar HB, Parsley K, Howe S et al (2004) Successful gene therapy of SCID-X1 using a pseudotyped gammaretroviral vector. *Lancet* **364**(9452): 2181–7

Hacein-Bey-Abina S, von Kalle C, Schmidt M et al (2003a) A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency. *N Engl J Med* **348**(3): 255–6

Hacein-Bey-Abina S, Von Kalle C, Schmidt M et al (2003b) LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. *Science* **302**(5644): 415–19

Ott MG, Schmidt M, Schwarzwaelder K et al (2006) Sustained reconstitution of NADPH-oxidase in X-linked chronic granulomatous disease following retroviral gene therapy and nonmyeloablative conditioning is augmented by insertional activation of MDS/EVI1, PRDM16 and SETBP1. *Nat Med* **12**(4): 401–9

Thrasher AJ, Hacein-Bey-Abina S, Gaspar HB et al (2005) Failure of SCID-X1 gene therapy in older patients. *Blood* **105**(11): 4255–7