

RECOMMENDATIONS OF THE GTAC/CSM WORKING PARTY ON RETROVIRUSES

May 2005

INTRODUCTION

In September and December 2002 reports were received of a leukaemia-like illness in two patients enrolled in a French retroviral gene therapy study for the treatment of X-SCID. In March 2003 a joint working party of the Gene Therapy Advisory Committee (GTAC) and the Committee on Safety of Medicines (CSM) met to:

- Review the current state of knowledge in relation to the risks of insertional mutagenesis in retroviral gene therapy,
- Review current UK retroviral gene therapy clinical trials,
- Make recommendations in relation to retroviral gene therapy and UK retroviral gene therapy trials.

The Department of Health commissioned research into the safety of retroviral vectors to help address GTAC/CSM's recommendations 1 - 6 of April 2003¹.

In late January 2005 a report was received that a third child in the same French retroviral gene therapy study for the treatment of X-SCID had developed a T-lymphocyte proliferation. In March 2005 the joint working party of GTAC and CSM met to review the data available to date from the French X-SCID trial and the UK X-SCID trial. In addition data from alternative treatments using stem cells and cord blood was discussed. The recommendations of March 2003 were considered and revised in line with the current knowledge and information available.

The following recommendations (which relate to pre-clinical research, vector production, vector design, ongoing studies, future approvals, monitoring, consent and SAE reporting) from the GTAC/CSM Working Group were ratified by both Committees.

¹ Brief details of this DH funded research are given in the GTAC annual report <http://www.advisorybodies.doh.gov.uk/genetics/gtac/publications.htm>, Further details of this and other research can be found by searching the National Research Register <http://www.nrr.nhs.uk/search.htm>.

THE RECOMMENDATIONS

FUTURE RESEARCH

The working party determined that there is still little firm evidence that can be used in estimating the extent to which the risk described by the French leukaemia cases may be translated to other trials because;

- The gamma-c therapeutic gene itself may contribute to the pathogenesis of leukaemia due to its lymphocyte growth promoting activity and its over-expression in the context of the vector.
- The primary disease (X-SCID) may also predispose to the development of leukaemia due to the lack of a normal T-cell compartment or by another mechanism.
- The basis of selection of vector insertion sites is not fully understood although the leukaemias appear likely to be due to preferential engraftment of those cells with over-expression of particular genes such as LMO2. Integration preference now appears unlikely to play a significant role in the process.
- Differences between the French and U.K. trials including the vector envelope and packaging cell line used might make a significant difference to the risk profile.
- Other issues such as the age of child at treatment and protocol details such as vector dose, number of transductions or number of transduced cells transplanted could have a bearing on the risk of leukaemia development. It was noted that the third child to develop a T-cell proliferation was older at the time of treatment than in the previous two cases, and that cells had not successfully engrafted in one child who received a lower dose of cells.

On this basis the working party recommends to the Department of Health that it continue to support research into the safety of retroviral vectors. The areas of research which should be prioritised are covered in recommendations 1 – 9.

The Role of the Transgene

Recommendation 1: The intrinsic oncogenicity of gamma-c should be assessed in a relevant *in vivo* model. Work already funded is addressing this question through the generation of new strains of gamma-c transgenic mice. *In vitro* models involving the over-expression of LMO2 in T-cells with gamma-c should be developed to address fundamental questions about the effects of such events on the biology of the cell - e.g. effects on responses to growth regulatory cytokines.

Stem Cell Biology

Recommendation 2: Stem cell biology research is a major priority for several U.K. funding bodies at present. Some of these existing resources should be channelled to address issues pertinent to gene therapy e.g. the effects of vector/genetic modification on integrity of stem cell function.

Recommendation 3: Retrospective analysis of samples in patients who have remained healthy or developed leukaemia should be undertaken to examine the evolution of malignant clones. This can be achieved e.g. by direct PCR analysis of the LTR-LMO2 insertion site in sequential blood samples. Similar analyses of transduced cell populations in vitro might be used in future to exclude hazardous cells prior to engraftment.

VECTOR PRODUCTION

Recommendation 4: Although initial studies have not revealed integration of endogenous murine viruses in leukaemias arising in X-SCID patients, transduction of such elements has nevertheless been detected in vivo. The possible oncogenic contribution of endogenous mouse viruses present in vector producer cell lines should continue to be assessed and reported from the UK and French trials.

Recommendation 5: If endogenous retroviruses or similar elements are implicated as risk factors, future protocols should employ human producer cell lines. Greater standardisation of these key resources is required.

VECTOR DESIGN

The working party identified a number of advances in vector design that may be advantageous to pursue in the longer-term and for future generations of retroviral vectors:

Recommendation 6: Additional safety features should be considered for retroviral protocols, including the use of self-inactivating vectors and non-viral promoters to drive therapeutic genes. Ideally, new vectors should be selected on the basis of improved safety in preclinical testing models (in vitro and/or in vivo). However, the current lack of validated systems remains a constraint to application of this principle.

Recommendation 7: Vectors incorporating suicide genes are already in use in some protocols, but these might have deleterious effects where long-term survival of transduced cells is required. In addition, this strategy may not be entirely effective because it is likely that not all cells will express the suicide gene. Research should continue to explore the safety and utility of established and new suicide gene vectors in animal models.

Recommendation 8: In addition to the inclusion of safety features in future retroviral vectors, alternatives to retroviral gene therapy, such as non-integrating vectors, homologous recombination and gene conversion, should be investigated for the treatment of X-SCID and other diseases.

Recommendation 9: The effect of the envelope protein of the vector particle in targeting cells at different stages of the cell cycle and at different stages of

development should be investigated together with effects on the cell due to receptor-mediated signalling by the envelope.

ONGOING STUDIES OF *EX VIVO* RETROVIRAL GENE THERAPY

Recommendation 10: Data currently available on long-term outcomes of haploidentical transplants in SCID patients are limited and some patients remain on life-long immunoglobulin therapy, but success rates appear to have improved in recent years. Nevertheless, despite the potential risks highlighted by the French trial, there may still be clinical situations in which retroviral gene therapy may be more appropriate than haploidentical transplantation. Regular updates are needed with regard to the risk assessment of different techniques, namely mortality, morbidity and the risk of leukaemia.

Recommendation 11: The current provisions for case-by-case assessments of applications to treat individual X-SCID patients with retroviral gene therapy should continue for current protocols. The case-by-case assessment should be based on the severity of the disease phenotype, clinical condition of the patient, and the availability and likely outcomes of alternative treatment options for that patient.

Recommendation 12: In addition to examining expression profiles in patient samples, researchers treating patients in the X-SCID retroviral gene therapy study should specifically monitor vector insertion sites, clonality of T-cells and levels of gamma-c expression.

Recommendation 13: The current provisions for case-by-case assessment of individual patients should not be extended to UK retroviral gene therapy studies for other diseases, such as cancers or disorders associated with cancers.

FUTURE APPROVALS

Recommendation 14: Case-by-case assessment for the enrolment of individual patients should be extended to other gene therapy studies involving the modification of haematopoietic stem cells with retroviral vectors in the future.

Recommendation 15: For the foreseeable future, applications of *ex-vivo* gene therapy involving the modification and transplantation of haematopoietic stem cells should be limited to serious diseases, and those diseases associated with serious morbidities for which current therapy is inadequate or imposes an unacceptable quality of life on the individual.

Recommendation 16: Integration copy-number may be critical for optimal therapeutic advantage, but is likely to be positively correlated with risk. Where possible pre-clinical studies should attempt to define the optimal copy number of the treatment gene in relation to therapeutic effect and assess the likely integration copy

number, using different protocols, in an appropriate cell line relevant to the *in vivo* target tissue (for example in hepatocytes where the target organ is the liver).

MONITORING OF PATIENTS ENROLLED IN EX-VIVO RETROVIRAL GENE THERAPY STUDIES

Recommendation 17: Resources need to be identified to allow potential problems to be handled as and when they arise. The work that needs to be undertaken to protect patient interests in the light of the adverse events in the French retroviral gene therapy trial is of importance and may carry significant research costs.

Recommendation 18: It is fundamentally important to collect as much molecular and cellular data as possible from those patients already treated, including documenting the sites of insertion of retroviral vectors in trials world-wide. We recommend this to researchers in the wider international community.

Recommendation 19: Monitoring and follow-up research for **all** patients, not only those who have gone on to develop T-cell proliferations or other complications, are essential. Detailed analysis of patients for whom treatment has been successful over the long-term may yield vital information to direct future research towards safe and effective treatment. Resources have been supplied to provide for the monitoring of those patients already treated with *ex-vivo* gene therapy in the UK. Further funding should be identified and supplied prospectively to enable monitoring of future patients.

Recommendation 20: Patient samples should be prospectively monitored for the appearance of oligoclonal populations of T-cells as these may precede monoclonal populations. The continued application of rapid and accurate technologies to look for sites of retroviral insertion and emerging clonality is desirable.

Recommendation 21: Where treated patients are currently healthy, further intensive monitoring or additional invasive procedures, over and above those outlined above, are not recommended. However, life-long surveillance, (as is currently the case) is recommended.

Recommendation 22: Materials used in retrovirus gene therapy treatments (including serum and cell samples) should be archived as a resource for future research (before, during and after therapy). Protocols for sampling, collection, processing and storage (pre and post therapy) should be standardised. Proposals for secure storage, and access by the wider scientific community to stored materials should be included in the trial protocol.

Recommendation 23: The effects of immunisation and infection on T-cell clonal proliferation and antibody production should be monitored in recipients of haematopoietic stem cell retroviral gene therapy over an extended time period.

CONSENT

Recommendation 24: Where gene therapy is determined to be an acceptable option for a given patient, the final decision in relation to whether to choose gene therapy or alternative forms of therapy should remain with the patient / the patient's parent(s) or guardian. Appropriate information on both gene therapy and alternative therapies should be provided. Independent, non-directive counselling should be made available.

Recommendation 25: Valid consent for the retention, archiving and future use of patient materials must be sought in accordance with the Human Tissue Act 2004.

Recommendation 26: Where appropriate, UK retroviral gene therapy clinical trials should be required to refer directly to relevant data, such as the French gene therapy related T-cell proliferations, as part of their patient information leaflets, and in the process of obtaining informed consent.

SERIOUS ADVERSE EVENT REPORTING

Recommendation 27: The Working Party commended the openness and transparency with which information related to the serious adverse events in the French X-SCID trial had been shared with the international community. In the longer term we recommend that the Department of Health/Licensing Authority continue to ensure that there is adequate international collaboration in the sharing of safety information from gene therapy trials.