

Introduction

Although HSC transplantation is an established curative procedure for a variety of inherited disorders, the high incidence of adverse immunological and drug-related effects associated with engraftment of allogeneic cells remains problematic. Autologous HSCs have therefore become attractive targets for the development of alternative strategies based on gene correction or augmentation. Effective gene transfer to HSC and their progeny requires stability, which currently can only be achieved efficiently using integrating vectors, most commonly based on mammalian retroviruses. Models of haematopoietic stem cell (HSC) gene therapy in mice and large animals have reproducibly demonstrated that a significant proportion of cells participating in long term engraftment can be stably transduced by integrating vectors based on mammalian retroviruses. Though effective, this leads to difficulties associated with variegation of transgene expression (dependent on the local chromatin environment), and potential for harmful mutagenesis (Baum et al. 2004; Challita & Kohn 1994; Klug, Cheshier, & Weissman 2000; Yao et al. 2004). Thus far, almost all clinical studies have employed murine gammaretroviruses, which are dependent on active proliferation of the target cell population for effective gene transfer because the nuclear membrane must be disrupted for entry of the pre-integration complex. As HSC are mostly quiescent, extensive studies have been dedicated to the identification of optimal ex-vivo culture conditions that stimulate HSC proliferation, without inducing differentiation and loss of long-term repopulating ability. These are readily applicable to clinical studies, and have been instrumental in the recent success of several HSC gene therapy trials for human disease. Vectors based on lentiviruses and foamy viruses are under investigation as alternatives to murine gammaretroviruses as they are less dependent on cell division for effective gene transfer, and are highly efficient.

Primary immunodeficiencies as models for HSC gene therapy

Primary immunodeficiencies (PID) are a heterogeneous group of disorders in which inherited genetic defects compromise host immunity (Fischer 2004). The most severe forms of PID are known as severe combined immunodeficiency (SCID), in which T lymphocyte development is invariably compromised, and associated with diverse disorders of development and functionality of B lymphocytes, and natural killer (NK) cells (Buckley 2004). Although clinically severe, bone marrow transplantation is usually highly successful if a genotypically matched family donor or unrelated donor is available (Antoine et al. 2003); (Buckley et al. 1999). However, for the majority of individuals, this is not the case, and survival from mismatched family (usually parental donor) transplants is substantially lower and associated with predictable toxicity arising from the administration of chemotherapeutic agents to ensure adequate HSC engraftment. SCID is a particularly attractive target for gene therapy as a profound growth and survival advantage is conferred to corrected cells (though this may be variable between different molecular types). In other words, due to the huge proliferative capacity of the haematopoietic system (and particularly the lymphoid compartment), effective gene transfer to a small proportion of bone marrow precursor cells can result in substantial correction of the immunological deficit.

X-linked severe combined immunodeficiency (SCID-X1): (SCID-X1) accounts for approximately 50-60% of all SCID, and is caused by mutations in the gene encoding the common cytokine receptor gamma chain (γ_c). This is a subunit of the cytokine receptor complex for interleukins (IL) 2, 4, 7, 9, 15 and 21. In the absence of γ_c signaling, many aspects of immune cell development and function are compromised. The classical immunophenotype of SCID-X1 is absence of T and NK cells, and persistence of dysfunctional B cells (T-B+NK- SCID). If a genotypically matched donor is available, bone marrow transplantation is a highly successful procedure with a long term survival rate of over 90%. The high survival rates are partly due to the fact that the absence of T and NK cells in SCID-X1 patients allows engraftment in the absence of myelosuppressive conditioning. In contrast, extensive data from European transplant centres and from our own centre suggests that mortality associated with HLA-mismatched procedures is at least 15% (probably dependent on the age and health of the child at the time of treatment), and that the quality and durability of immune reconstitution is variable.

Many incremental advances in gene transfer technology have contributed to the successful application of gene therapy for SCID-X1 and adenosine deaminase deficiency (ADA-D) (including the optimisation of cell culture and gene transfer conditions ex vivo), which complement the intrinsic profound selective growth advantage imparted to successfully transduced cells (for SCID-X1, this is probably even more potent than that observed following restoration of ADA activity) (Aiuti et al. 2002; Cavazzana-Calvo et al. 2000; Gaspar et al. 2004), Gaspar and Thrasher, unpublished). Two studies (including 10 patients in France, and 7 patients at our

centre) have demonstrated highly effective somatic gene therapy for patients with SCID-X1 (Cavazzana-Calvo, Hacein-Bey, de Saint, Gross, Yvon, Nusbaum, Selz, Hue, Certain, Casanova, Bouso, Deist, & Fischer 2000; Gaspar, Parsley, Howe, King, Gilmour, Sinclair, Brouns, Schmidt, von Kalle, Barington, Jakobsen, Christensen, Al Ghonaium, White, Smith, Levinsky, Ali, Kinnon, & Thrasher 2004; Hacein-Bey-Abina et al. 2002). In both, a gammaretroviral vector encoding a γ c cDNA (regulated by Moloney murine leukaemia virus long terminal repeat sequences), was used to transduce autologous CD34⁺ cells *ex vivo* which were re-infused into the patients in the absence of pre-conditioning. In nearly all patients NK cells appeared between 2 and 4 weeks after infusion of cells, followed by new thymic T lymphocyte emigrants at 10-12 weeks. With some variation, the number and distribution of these T cells normalised rapidly (more rapidly than observed following haploidentical transplantation). They also appeared to function normally in terms of proliferative response to mitogens, T-cell receptor (TCR), and specific antigen stimulation, and to have a complex phenotypic and molecular diversity of TCR. Functionality of the humoral system was also restored, maybe not quite as effectively, but to a sufficient degree that discontinuation of immunoglobulin therapy has been possible in most patients. The contribution to the initial burst of thymopoiesis from relatively late T cell precursors in the original transduced CD34⁺ cell population, versus that from cells earlier in the haematopoietic differentiation hierarchy (or true HSCs) that have engrafted in the bone marrow, has not yet been determined. However, persistent long term marking in myeloid cells, albeit at low level, suggests that long-lived stem or progenitor cells have also been successfully transduced and engrafted. This may have important implications for the durability of immunological reconstitution, and for sustained production of new T cells. Ultimately the longevity of functional reconstitution can only be determined by clinical monitoring, but it may also be feasible to repeat gene therapy on multiple occasions. Definition of the effective window within which gene therapy will be effective is vitally important, as true for other more conventional therapeutic modalities. This has been clearly demonstrated by the failure of immunological reconstitution in two older patients following effective gene transfer to bone marrow CD34⁺ cells (Thrasher et al. 2005). At least for SCID, it is likely that there are temporal host-related restrictions to efficacy, for example due to the inability to reinitiate an exhausted or failed programme of thymopoiesis.

Insertional mutagenesis and risks of HSC gene therapy

The dependence of retroviruses on chromosomal integration for stability of transduction, brings with it the risk of insertional mutagenesis. On the basis of numerous animal studies and over 300 clinical trials in which patients have received gammaretroviral vectors, the risk of clinically manifesting insertional mutagenesis has been judged to be low. However, reproducible leukaemogenesis and oncogenesis has now clearly been demonstrated in pre-clinical models, and may be directly associated with vector dose or cell copy number. Co-operating effects from expression of the transgene (Li et al. 2002) or from other elements within the vector backbone may also be important, and are likely to be context dependent (Baum, von Kalle, Staal, Li, Fehse, Schmidt, Weerkamp, Karlsson, Wagemaker, & Williams 2004). In human clinical trials, 3 patients with SCID-X1 developed T cell lymphoproliferative disease approximately 3 years after the gene therapy procedure, having initially achieved successful immunological reconstitution (Hacein-Bey-Abina et al. 2003). In at least two of these patients, retroviral vector insertion into or near the *LMO-2* proto-oncogene resulted in high level expression of LMO-2 in the clones, as a result of retroviral enhancer-mediated activation of transcription.

Strategies to improve safety of HSC gene therapy

Following the reports of adverse events associated with a SCID-X1 trial, many regulatory authorities suspended all HSC gene therapy. In the UK, trials have continued on a case-by case review, and recruitment of patients remains open on this basis. According to updated recommendations from the UK Gene Therapy Advisory Committee (GTAC) and Committee for safety of Medicines (CSM), a number of advances in vector design should be pursued that may be advantageous in the longer term and for future generations of retroviral vectors.

Recommendation 6 states: '*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 pre-clinical testing models (in vitro and/or in vivo). However the current lack of validated systems remains a constraint to application of this principle.*'

(<http://www.advisorybodies.doh.gov.uk/genetics/gtac/>).

Several strategies have been suggested to improve efficiency and safety of current protocols. Patterns of integration into host chromosomes are to some degree vector dependent and could thereby contribute to the likelihood of inadvertent gene activation. However, both gammaretroviral vectors and lentiviral vectors have been shown to integrate preferentially within genes, and both are likely therefore to be susceptible to induction of mutagenic side effects. The overall design of vectors used for gene delivery is probably of most importance and modifications may be possible that will limit the risks of mutagenesis, for example by the use of self-inactivating (SIN) configurations in which the powerful duplicated viral LTR enhancer sequences are deleted. In theory, incorporation of more physiological promoter sequences (such as the human elongation factor 1 α promoter which works well in the context of gammaretroviral SIN vectors, (Schambach et al. 2005), or those less reliant on classical enhancer elements may well further decrease the potential for insertional gene activation.

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