Stem Cell Technology and Other Innovative Therapies
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HEMATOPOIETIC *STEM CELL* GENE THERAPY

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To date the best evidence for the efficacy of stem cell therapy has been provided by the success of allogeneic hematopoietic stem cell transplantation to cure a number of diseases. The potential ability of hematopoietic stem cell (HSC) to give rise, life long to all blood cell lineages [1] is a rationale for the treatment of a number of genetically determined blood cell diseases, by relevant gene transfer into HSC. There are however a number of technical limitations, the major one being related to the resting stage of HSC. Indeed, retrovectors so far used in clinical practice to induce gene transfer into host genome, can only permit gene integration into dividing cells in which the nuclear membrane is temporally dissolved. Thus clinical applications will require to develop vectors such as lentiviral based vectors enabling gene transfer into non dividing cells and/or mean to induce HSC to cycle without loosing their self renewal capacity.

Gene Therapy of Severe Combined Immunodeficiencies (SCID)

At present, however, there is a favorable setting for which gene therapy has demonstrated its efficacy [2]. It is the treatment of severe combined immunodeficiencies (SCID). SCID are rare inherited conditions characterized by a complete block in T lymphocyte differentiation. Genetic defects leading to SCID conditions have been almost all identified, opening the possibility to perform relevant gene transfer in autologous progenitors. SCID conditions are lethal within the first year of life in the absence of treatment. Allogeneic HSCT can cure these conditions although, when the donor is not fully matched, risk of failures is significant and long-term decline in immune functions makes this therapeutic approach partially unsatisfactory. Within the SCID condition, SCID-X1 characterized by a block in both T and NK lymphocyte development is the most frequent. It is

caused by mutations of the gene encoding γc, a cytokine receptor subunit expressed by all hematopoietic-derived cells. γc role in T lymphocyte development is known, it enables IL-7 to provide survival and proliferation signals to T cell precursors. IL-15 is similarly acting on NK cell precursors. It was thus thought that γc expression triggered by γc gene transfer into a few deficient lymphocyte progenitors could give rise to a tremendous proliferation of T cell precursors giving rise to long-lived mature T cells. This is the concept of selective advantage conferred by the transgene expression on which gene therapy of SCID is based. Experimental data as well as consequences of spontaneous reverse mutation correcting the γc gene mutation provided further rationale for this approach [2].

Principle of gene therapy consists in ex vivo inserting the γc gene into bone marrow precursors cells by using a retroviral vector made unable to replicate. The γc gene is placed under the transcriptional control of the viral Long Terminal Repeat (LTR). Insertion of the provirus occurs in active regions of the genome. This method has been tested in vitro showing efficacy, including long lasting expression of γc in cell lines as well as in vivo in a murine model of the γc deficiency. The immunodeficiency has been corrected, efficacy is sustained and no adverse effects have been observed. Based on these preclinical data, gathered in a 6-year period of time, a clinical trial has been initiated in 1999. Between 1999 and 2002, 10 patients with SCID were treated likewise in Paris. In 9 out of 10, a sustained correction of the immunodeficiency has been achieved [3, 4]. T cell became detectable in the blood within 10 to 12 weeks, reaching normal cell counts, now being sustained for almost 5 years for the first treated patients. The γc transgene is expressed in all T and NK cells, as well as by a minority (1‰ to 1%) of B lymphocytes and myeloid cells. All characteristics of transduced T cells indicate a full correction of the immunodeficiency, including T cell receptor diversity, ongoing production of T cells in the thymus, in vitro and in vivo reactivities to antigens.

By analyzing the transgene integration sites, which represent a 'signature' of the transduced precursors, it was estimated that a few hundred clones gave rise to the whole T cell pool, emphasizing the selective advantage conferred to these transduced precursors. Each cell contains on average one copy of the provirus. Production of immunoglobulins has been also restored to an extent, which is strong enough to avoid immunoglobulin supplementation in 7 out of 9 patients. Similar findings have since been observed in 4 additional patients with SCID-X1 similarly treated by A. Thrasher's group in UK [5]. The analysis of the provirus integrations sites

in transduced myeloid cells as well as in marrow precursors cells provided evidence that progenitors able to differentiate in both lymphoid and myeloid cells were transduced. These data together with the sustained detection of both transduced lymphocytes and myeloid cells over time strongly suggest that very immature progenitors, possibly stem cells were targeted, rising hope for very long term efficacy. Thus, this therapy is bringing a clear benefit to the patients, enabling them to live a normal life and coping with infections. It seems, that albeit the low number of treated patients and the maximum 5 years-follow up, gene therapy is more efficient that HSCT, the alternative treatment. Efficacy of gene therapy has now be demonstrated for another form of SCID (Adenosine deaminase deficiency) in 4 patients [6]. It is now logical to try to extend this strategy to the treatment of other forms of T cell immunodeficiencies for which the selective advantage concept will apply. This altogether should include about 10 diseases including SCID and the Wiskott-Aldrich syndrome. Extension to other genetic defects of hematopoiesis will require the usage of vectors enabling to transduce a much higher number of HSC possibly combined with chemotherapy to reduce the competition with non-transduced HSC.

Safety Issue

The occurrence of a gene therapy related serious adverse event (SAE) in 2 patients is raising an important safety issue to be thoroughly considered in the perspective of gene therapy development. A T cell clonal proliferation occurred approximately 3 years after treatment [7]. These T cells have a mature phenotype and do express the γc protein, without overexpression. Cells became blastic in appearance at a time of clinical manifestations while secondary genomic alterations became detectable, (a t 6;13 chromosomal translocation in one, a trisomy 10 and a sil-tal rearrangement in the second). Both patients were successfully treated by chemotherapy. One then required allogeneic HSCT and is still under treatment. In both cases, uncontrolled clonal proliferation was primarily caused by an insertional mutagenesis event, i.e. integration of the provirus in the LMO-2 locus. Aberrant expression of LMO-2 has previously been described in association with a rare form of T-cell acute lymphoblaste leukemia while forced expression of LMO-2 in mice is also causing leukemogenesis, albeit after a long delay (close to one year) [8]. Insertions occurred in one in the first intron of the LMO-2 locus, in reverse orientation while in the second, insertion is placed close to the hematopoietic promotor of LMO-2. In both cases, the viral LTR likely exerts

an enhancer activity on LMO-2 giving rise to aberrant continuous expression of LMO-2. That in both instances the LMO-2 locus was hit cannot be regarded as a random event. Either the LMO-2 locus is a physical hot spot for provirus integration, an hypothesis which is unlikely, given the preliminary results of the identification of the different provirus integration sites found in the patients, or among many integration sites, those occurring in the LMO-2 are selected because of their functional consequences. Since these SAE have not been observed in other clinical settings, nor in experimental conditions with a single exception [9], it is possible that functional additive or synergistic effects with the γc transgene expression plays an important role as also suggested in a murine model of leukemia [10]. This is difficult to assert, as the precise molecular function of LMO-2 is still unknown today. Another puzzling observation consists in the fact that the SAE occurred in the 2 youngest treated patients, and not in those aged 4 months or older. The likelihood that this occurs randomly is only 1.2%. It is thus very likely that age is a significant contributing factor possibly because characteristics of hematopoiesis at birth differ with a higher rate of proliferative cells and distinct pattern of gene expression [11]. These circumstances might favor integration into and selection of LMO-2-expressing clones. This hypothesis is being tested in an animal model.

These considerations are important because, if correct, they mean that the serious safety issue is limited to gene therapy of patients with SCID-X1, for the minority of patients under the age of 4 months. This would permit to continue to use this therapeutic strategy for patients above that age. In addition, in the future, vectors may be designed to increase safety, but not at the expense of efficacy. Three tracks are being tested: to use self inactivated LTR, as it is the case with designed HIV-derived lentiviral vectors, to add insulators with the hope to limit the enhancer activity to the provirus and avoid its effect on surrounding genes, and finally to add a suicide gene to be activated by a drug if a clone is escaping control.

Altogether, a careful appraisal of the benefit/risk ratio comparing the chance of alternative therapy, HSCT versus gene therapy has to be performed individually for each patient.

The Future

Further extension of stem cell gene therapy, beyond this present state, i.e. demonstration that it can work, will require further technological advances. As mentioned above, lentiviral vectors derived from HIV can pro-

vide an efficient tool to transduce HSC. Experimental results achieved in the correction of hemoglobin disorders in mice are encouraging [12, 13]. In order to reduce the risk of insertional mutagenesis, targeted integration will be a possible solution.

One strategy, which is presently tested, consists of using a bacterial integrase (Ψ C31) able to induce integration at rare positions in the mammalian genome. Some efficacy has been shown in targeting liver cells [14]. Another line of development consists of inducing homologous recombination to repair mutated genes. In order to increase the frequency of such events, a strategy based on the creation of DNA double strand break at mutation sites by a tailored integrase is being tested [15]. But a long way to go is needed to achieve high enough specific breaks without non-specific integration.

In conclusion, stem-cell gene therapy has been shown to be potentially effective. Its development requires both a careful pathophysiological analysis of diseases to be treated as well as development of technologies to improve gene transfer efficacy, and this in a safe way. HSC are very good target cells to treat a number of diseases. On the long term, newly generated stem cells following somatic nuclear cell transfer might be provide an optimal tool for disease correction [16, 17].

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