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"Turning Foes into Friends: Exploiting HIV for the Gene Therapy of Inherited Diseases and Cancer"

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Turning Foes into Friends: Exploiting HIV for the Gene Therapy of Inherited Diseases and Cancer. Dr. Luigi Naldini. Milan, Italy.

Gene and cell therapies represent an emerging pillar of the molecular medicine of the third millennium, and offer unprecedented opportunities for the development of novel treatments for several deadly or debilitating genetic and acquired diseases. This promise is based on: i) the increasing power of gene transfer technologies, which allow ever more precise correction of inherited genetic defects, or instruction of novel functions to selected cell types; ii) our increasing capacity for isolating and characterizing stem cells of different types, genetically modifying them ex vivo, and successfully transplanting them back into individuals where they can engraft long-term and give rise to functional progeny bearing the corrective or therapeutic modification, thus leading to functional reconstitution of previously missing or defective cell lineages and regeneration of damaged tissues; iii) the increasing knowledge of the complex networks of immunity and our growing capacity at capturing its powerful biological weapons either to induce immune tolerance to newly inserted transgenes and transplanted cells, or instead to more vigorously fight cancer and infections. Despite these advances, we must still address substantial challenges before we can fully realize the therapeutic potential of these new therapies. Paramount among them has long been the availability gene transfer vectors that can safely and efficiently insert genes into relevant target cells, such as hematopoietic stem cells (HSC) and lymphocytes.

During a sabbatical stay in Inder Verma and Didier Trono laboratories at the Salk Institute for Biological Studies, La Jolla, from 1994 to 1996, I embarked in the development of a new gene transfer vector derived from HIV with the goal to capture the unprecedented efficiency of this fearsome and recently emerged lentivirus at infecting human cells. After 2 years of work, we could show the first proof of principle of stable gene transfer by the so-called lentiviral vectors into previously difficult to reach cells. The paper describing this work is still one of the top-cited articles in *Science*. Soon after the publication, Cell Genesys licensed the lentiviral technology from the Salk Institute and I decided to join the company to direct further development of the vector system for its eventual clinical use. In the following two years, we reported a *second* and *third "generation" self-inactivating* vector design, and developed manufacturing strategy and assays for recombinant detection, making the vector system better amenable and much safer to use.

Because we were able to substantially reduce the fraction of HIV genome used to make the vector, it became impossible to reconstitute the parental virus even from the most unlikely series of recombination occurring during production, and one could predict that any of these unlikely recombinant would have the features of a non-pathogenic vaccine strain. This work laid the foundation for the currently broad use of lentiviral vectors. What was initially received as an elegant proof-of-principle of an unlikely and fearsome technology, has now become one of the most widely used tool in biomedical research. Medline currently lists >7,300 articles under "lentiviral/lentivirus vector", but there would be many more if we could retrieve all the scientific papers that took advantage of this powerful gene transfer system to manipulate cells and living organisms and perform gene function studies, target validation, cell marking and even transgenesis.

At the end of 1998, I returned to academia as professor at the University of Torino, and in 2003, moved to the San Raffaele Telethon Institute for Gene Therapy in Milan, initially as co-director with Maria Grazia Roncarolo, and since 2008, as the director of the Institute. During those years, my laboratory engaged in further vector development, introduced several features that enhanced the efficiency of gene transfer, exploited bidirectional transcription to design vectors that allow coordinated expression of multiple transgenes, and investigated factors that limit the transduction of stem cells and developed strategies to overcome such restrictions. In a particularly rewarding development, we applied microRNA regulation to vector design and provided the prototype for making transgenes and medically used viruses stringently responsive to cell type- and differentiation-specific cues. The interest for this novel approach has since been steadily growing, not only within the gene and cell therapy community, which has broadly endorsed it, but also among investigators of microRNA biology, immunologists and scientists developing safer versions of oncolytic viruses and viral vaccines. Together with other laboratories, we showed the proficiency of lentiviral vectors at gene transfer into HSC of mice and humans. By reaching exhaustive cell marking with minimal interference with cell function, individual stem cell activity could eventually be monitored *in vivo* to unprecedented levels. An unexpected boost towards the broad use of lentiviral vectors came from our studies showing that the integration profile and the advanced design of lentiviral vectors was associated with much lower genotoxicity than conventional gamma-retroviral vectors, thus providing for a safer gene transfer platform despite the original concerns raised by the nature of the parental virus. The demonstration of high gene

transfer efficiency coupled with improved safety provided by these studies has been crucial for eventually moving lentiviral vectors to the clinic.

Indeed, our efforts towards improving gene transfer have always been pursued with the clear goal in mind of therapeutic translation. Since the early days of vector development, we selected lysosomal storage disorders, and in particular metachromatic leukodystrophy (MLD), an invariably lethal neurodegenerative disease currently without effective treatment, as paradigmatic for testing the new therapeutic potential offered by lentiviral vectors. Having shown that the post-transplant recruitment of hematopoietic progenitors to the resident microglia pool can be exploited to deliver gene therapy to the central and peripheral nervous system, we traveled all the steps from the proof-of-principle studies in the mouse model, the pre-clinical validation in mice and in patient cells, to clinical testing. Two first-in-human clinical trials of HSC gene therapy were eventually launched in our Institute in 2010, one for MLD, and the other for Wiskott Aldrich Syndrome (WAS), a severe combined immunodeficiency (SCID) and platelet deficiency, for which the only treatment option is allogenic HSC transplant (HSCT) if a matched donor is available. The first clinical testing of lentiviral vectors in HSC gene therapy had just been reported in 2009 by a French team led by Patrick Aubourg, using the vector design developed by our previous work, for the treatment of Adrenoleukodystrophy, a disease related to MLD. The current results of our two trials show in an increasingly larger cohorts of treated patients very high levels of stable gene marking of the reconstituted hematopoiesis associated with long-term substantial therapeutic benefits. At the latest follow-up, which reaches up to 6 years in the earliest treated children, all MLD patients treated in pre-symptomatic stage, show marked benefit demonstrated as prevention of disease onset and/or halted progression. Treatment resulted in protection from central nervous system demyelination and amelioration of peripheral nervous system abnormalities, with signs of re-myelination at both sites. These patients showed continuous motor and cognitive development, in sharp contrast with the natural course of the disease. Similarly, all treated WAS patients clearly benefited from HSC gene therapy as if they had received a successful HSCT from a matched donor, which was not available for them.

The high levels of genetic engineering of hematopoiesis seen in our patients have been achieved without any clinical or molecular signs of adverse effects of vector insertion to the latest follow up. In-depth longitudinal analysis of the genomic landscape of vector insertion, used as readout of clonal composition and activity, provides a comprehensive picture of highly

polyclonal multi-lineage reconstitution of hematopoiesis without emergence of expanding or dominant clones, consistent with efficient engraftment of transduced HSC. These findings are consistent with the earlier predictions of our preclinical models supporting an improved safety of lentiviral vectors as compared to earlier generation vectors. Intriguingly, this analysis, if indeed achieved by neutral cell marking, provides the first comprehensive assessment of stem and progenitor activity at the clonal level in post-transplant hematopoiesis of humans and may help validating current models of hematopoiesis based on experimental systems.

Autologous HSC gene therapy, if confirmed to be safe and effective in more patients and upon longer follow-up, may thus eventually become a first treatment option at least for those patients who are candidate to allogenic HSCT but lack a fully matched normal donor. Autologous HSC gene therapy would not only be available to virtually any patient but may also substantially reduce the morbidity of the treatment as compared to allogenic HSCT, because there is no risk of graft-vs-host disease and therapeutic benefit may often be achieved by partial chimerism with transduced HSC, thus relieving the need for fully myeloablative and immunosuppressive preparatory conditioning. In some diseases, such as MLD, genetic engineering of HSC may even surpass the benefit of conventional HSCT, because it may engage novel therapeutic mechanisms, such as increased dosage and biodistribution of the replaced gene product over what can be achieved by transplanting normal HSC. Based on the encouraging early results of these trials, our Institute signed a strategic alliance with GlaxoSmithKline (GSK) - a first-of-its-kind agreement between a major pharmaceutical company and an academic centre engaged in gene therapy - to further develop HSC gene therapy for these genetic diseases into a commercially available clinical reality.

The promising results of HSC gene therapy for some genetic diseases have also prompted us to investigate application of this strategy to more common diseases, such as cancer. By studying the hematopoietic cell contribution to tumors, we had established a novel paradigm in which myelo-monocytic cells are recruited from the bone marrow to the tumor to provide an essential paracrine support to angiogenesis. These studies were extended to define a new lineage of proangiogenic monocytes, which selectively engage in tissue remodeling and regeneration and can be distinguished from conventional inflammatory monocytes in the blood and organs of mice and humans by their gene expression analysis, surface marker profile and functional properties. We recently started to exploit these findings in a new gene

therapy strategy in which the progeny of transplanted hematopoietic progenitors is engineered to selectively target gene therapy to tumors, thus enhancing therapeutic efficacy and avoiding systemic toxicity. We are now investigating this approach for the targeted delivery of interferon alpha to tumors by gene-modified tumor infiltrating macrophages. The local release of interferon at the tumor site reprograms the tumor microenvironment towards a permissive state for the generation and deployment of immune effector responses against tumor associated antigens, leading to tumor clearance and resistance to re-challenge. We hope to start clinical testing of this new strategy in hematopoietic malignancies in the next couple of years.

Whereas the findings summarized above support an increasing application of HSC gene therapy, current gene replacement strategies remain potentially limited by the residual risk of insertional mutagenesis and an imperfect control of therapeutic gene expression. New strategies based on targeted genome editing are emerging that may eventually increase the precision and safety of HSC gene therapy. We pioneered the use of engineered Zinc-finger nucleases to target vector integration and edit the human genome. These studies opened the way to correct, rather than replace genes, a potentially revolutionary approach that may substantially expand the scope and power of genetic manipulation. Together with Chiara Bonini's group, we demonstrated the proof-of-principle of T-cell receptor editing as a novel means of T-cell therapy, in which a new biological function is instructed to an immune effector cell by genetically re-writing its endogenous antigen specificity. In parallel, we embarked on applying targeted genome editing to HSC gene therapy. The long-term follow-up of patients treated by lentiviral HSC gene therapy mentioned above proves the feasibility to manipulate HSC ex vivo without hampering their long term repopulation potential and open the way to design improved gene therapy strategies. Whereas targeted genome editing may bring increased precision and, possibly, safety to genetic engineering, its application to HSC, however, remains constrained by a low efficiency of gene editing in these primitive cells. Recently, we could overcome at least in part these barriers by culture conditions that induce HSC proliferation while preserving their long-term engraftment capacity and provide evidence of correction of SCID-X1 causing mutations in the *IL2RG* gene. An advantage of testing gene editing in SCID-X1 is the selective expansion of the corrected T-cell progenitors, which should make the treatment effective even if only a small fraction of gene corrected cells are administered to the patients. We are now investigating this approach in an *ad hoc* humanized SCID-X1 mouse model to support the scientific rationale

and safety of the proposed treatment, and identify the conditioning regimen and degree of chimerism with edited cells required to correct the disease.

As the first ex vivo gene therapies have now progressed to the market, constant engagement of the drug companies and regulatory agencies becomes essential to define appropriate quality standards for manufacturing and release and to build suitable pipelines for supplying these personalized and expensive therapies. Whereas this is only the first step of a seemingly long journey, it is gratifying to contemplate that scientific progress and the efforts of many scientists and clinicians have been able to turn a deadly virus into a therapeutic capable to relieve human suffering and treat disease.