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From stem cells to functional lymphocytes: cell differentiation and gene therapy implementation for RAG-SCID

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Chapter 9

SUMMARY (ENGLISH)

The immune system is a complex, layered mechanism of defence to prevent or limit infections and maintain homeostasis. It consists of an interactive network of lymphoid organs, humoral factors and several types of specialized cells, including B and T lymphocytes that constitute the hallmark of the adaptive immune system. These immune cells develop from hematopoietic stem cells (HSC) that undergo differentiation through a highly regulated succession of developmental steps. Each cell type of the immune system performs a unique specialized role, and their development is strictly regulated by the action of many transcription factors and their target genes.

In **Chapter 2** of this thesis, we gained additional knowledge on normal immune development, with detailed focus on the transcriptional network orchestrating T-cell development. Tcf1, the first T cell-specific protein induced in the thymus regulates the expression of two major target genes, Gata3 and Bcl11b. Tcf1 deficiency results in partial arrests in T cell development, high apoptosis, and altered development of alternative (i.e., non-T) lineages. Phenotypically, seemingly fully T cell-committed thymocytes with Tcf1 deficiency have promiscuous gene expression, an altered epigenetic profile and can dedifferentiate into more immature thymocytes and non-T cells. Restoring Bcl11b expression in Tcf1-deficient cells rescues T cell development but does not strongly suppress the development of non-T cells. In contrast, expressing Gata3 suppresses the development of non-T cells, but does not rescue T cell development. Thus, a minimal transcription factor network ensuring a properly regulated T-cell gene expression program was described: Notch signaling induces Tcf1 expression which subsequently has two target genes, *Gata3* and *Bcl11b*, that accomplish a division of labor with Gata3 suppressing non-T cell lineages and Bcl11b inducing the expression of T-cell specific genes.

The disruption of normal lymphoid development can lead to severe illnesses known as immunodeficiencies. Severe Combined Immunodeficiency (SCID) is a devastating immune disorder affecting infants lacking a functional immune system, in particular T cells. Infants with SCID will die within the first year of life unless effective treatment is administered. Therapeutic treatments are limited and confined to allogeneic HSC transplantation and emerging autologous stem-cell gene therapy.

In this thesis we focused on developing an efficient and safe lentiviral-based gene therapy approach to correct both Recombinase Activating Gene 1 and 2 (RAG1 and RAG2) immune defects. In **Chapter 3**, I discuss the pre-clinical development, safety and regulatory hurdles across the process and steps to successfully develop gene therapy for immunodeficiencies from the bench to the clinic. Autologous lentiviral-based HSC gene therapy was described in **Chapter 4** and **Chapter 5** as an efficient and safe therapy to correct RAG-SCID deficiency. Full functional immune reconstitution with our MND-c.o.RAG1 lentiviral vector and our PGK-c.o.RAG2 lentiviral vector in murine HSC was observed in murine models (Rag1-/- mice and Rag2-/- mice). B- and T- cell development in BM and Thymus were observed after gene therapy together with a successful functional restoration including immunoglobulin production, diverse T-cell receptor rearrangements

and an effective immune response. Additionally, patient HSCs transduced with our clinical RAG1 vector and transplanted into NSG mice led to improved human B- and T-cell development**.** Successful pre-clinical development together with a favorable safety described in **Chapter 4** substantiates the first phase I/II clinical trial worldwide for RAG1-SCID gene therapy. In parallel, functional immune reconstitution was achieved in the murine Rag2-/- mouse model with a safe clinical PGK-c.o.RAG2 lentiviral vector in **Chapter 5**, although RAG2 gene therapy seems to be more challenging due to the crucial role of optimal RAG2 expression levels.

Additionally, we optimized related protocols by developing novel tools and conditioning regimens for a successful allogeneic and autologous gene therapy transplantation outcome. A novel method for single-cell characterization of the transduction efficiency and transgene expression in the gene therapy product is introduced in **Chapter 6**. The branched DNA technique used showed high specificity, sensitivity, reproducibility and versatility. This method allows to study the heterogeneity within gene therapy products and to reconsider the actual proportion of potential therapeutic cells and the underestimated transgene vector copy number.

Lastly, alternative reduced-chemotherapy conditioning regimens were tested to achieve suitable cell engraftment and immune recovery while reducing short and long-term side effects. In **Chapter 7**, I describe our efforts to develop a reduced chemotherapy (busulfan) regimen by combining it with clinically approved mobilizing agents (G-CSF and Plerixafor) used to mobilize HSCs from the bone marrow to the peripheral blood. While an interesting reduction of the HSC compartment was observed in bone marrow with the novel combinations including G-CSF or Plerixafor, no significant differences were achieved in the transplantation outcome using NSG mouse model. Recent and promising developments of antibody-based conditioning are extremely attractive to the field, as they ideally will provide the ability to achieve donor chimerism without the use of toxic chemotherapeutic agents.

Altogether, the work described in this thesis moves towards a regular enforcement of gene therapy treatment for immunodeficiencies for which potential milestones during the overall procedure, e.g., newborn screening, cell isolation and transduction, pre-conditioning, standardized protocols and patient monitoring, have been optimized.