# Characterizing Leukemic Stem Cells in a HOXB4 Transgene Dependent Leukemia Model

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**Introduction, Research Question, Impact, and Outcomes/Goals**

In the hematopoietic system, there is a hierarchy of stages which vary in proliferative lifespan and degree of differentiation. At the origin of the hierarchy are the hematopoietic stem cells (HSCs). HSCs have two defining characteristics: indefinite self-renewing capacity –which allows them to produce progeny with identical proliferative capacity– and multipotency, which allows them to differentiate into different cell types. Self-renewal is the essential property which supports the permanent, sustained function of the system (Wiseman et al. 1999). Commitment of precursor cells into specific hemopoietic lineages is accompanied by a diminished self-renewing capacity and a shortened reconstituting life span (Seita and Weissman 1999).

Homeobox genes, including HOXB4, are believed to play a role in self-renewal of hemopoietic precursor cells. First, they are expressed preferentially in HSC and other primitive cell stages in the system, but are silenced as precursors mature, lose self-renewal capacity, and restrict toward a single lineage (Argiropoulos and Humphries 2007; Lim et al. 2017). Preferential expression of HOX paralogs has similarly been found in human AML leukemia stem cells relative to leukemia blast cells. Further, when HOXB4 expression is enforced by transduction of an exogenous copy into hemopoietic precursor cells, the cells exhibit an indefinite self-renewing capacity and in vivo reconstituting potential characteristic of HSCs (Kustikova 2005; Lim et al. 2017). This evidence supports the view that HOXB4 has an important role in self-renewal of primitive hemopoietic precursor cells.

The Iscove lab has studied extensively the effects of enforced expression of exogenous HOXB4 in a mouse model. Cells at various stages in the hemopoietic hierarchy were purified and then transduced with a single copy of the HOXB4 transgene. Cells with normally restricted lineage potential and only limited proliferative lifespans acquired a new capacity for indefinite self-renewal and in vivo reconstituting ability normally characteristic only of HSCs (Kustikova 2005; Lim et al. 2017). Despite the change in self-renewal properties, these cells continued to produce normally differentiated blood cells in normal numbers in vivo.

Immortalized hemopoietic clones can accumulate gene mutations which eventually lead to leukemia (Argiropoulos and Humphries 2007). Although HOXB4 is recognized as not primarily leukemogenic, leukemic transformation has been observed in the Iscove lab model after in vivo passage of HOXB4-immortalized marrow cells for periods beyond 18 months. The line of murine acute myeloid leukemia (mAML1) was derived in this way. One key property of this leukemia is its continuing dependence on the HOXB4 transgene. After excision of HOXB4 using a cre-loxP strategy, the leukemia regresses and disappears in host mice. Another key property is the evidence for proliferative hierarchy typical of clinical AML (Lapidot et al. 1994; Jordan 2007; Wiseman et al. 2014; Jackson et al. 2016). Limiting dilution analysis involving transfers of small numbers of mAML1 cells to irradiated mouse recipients was performed. The measurement established that the cells capable of reconstituting the leukemia –the “leukemia stem cells,” or LSCs– exist as only a small fraction –1 in 63 cells– of the leukemia cell population in marrow.

Elucidating the pathways supporting immortalization by HOXB4, and discovering means of disrupting such pathways in leukemia, are long-term goals of the Iscove lab. My project aims to characterize the surface antigen phenotype of the LSC in the mAML1 model in a way that would allow these cells to be distinguished from the majority of inert leukemia blasts, and further allow identification of the LSC's counterpart in the normal hemopoietic hierarchy. The knowledge gained will support future efforts to uncover the pathways essential for leukemia propagation.

My research goals are purifying LSCs and characterizing them to answer my research question: what are the distinguishing antigens on purified LSCs in a HOXB4 transgene dependent leukemia model? I expect that the outcome of this research will answer this question.

The impact of this project's outcome lies in the research opportunities that the work will open up. These results will allow comparison between the phenotype of the LSCs and the phenotypes of the different stages across the precursor hierarchy of the normal hematopoietic system. Identifying a precursor stage in the normal hematopoietic hierarchy with which the phenotype of the LSC aligns opens the opportunity to compare the transcriptome and epigenetic profiles of both the LSCs and the corresponding normal precursor cell. We can then ask: what's altered in the HOXB4 gene of the LSC relative to the precursor's gene? What's different between the transcriptomes of the immortal LSCs and the regular precursors? These questions which imply a deeper comparison at the level of genetic expression require that we first identify a normal precursor stage to which the LSC is phenotypically similar enough; this requires the characterization of the LSC.

My specific goal for this research is to identify 4-6 antigens on LSCs that distinguish them from other non-stem cells in the leukemia population. Identification of these markers will form the foundation for future purification of the rare LSC, enabling elucidation of the molecular basis which underlies their immortality.

**Brief methodology description**

The leukemia cell population is maintained in the laboratory as frozen samples and in transplantation for one host mouse to another. The integrated HOXB4 provirus also expresses fluorescent VENUS protein, allowing ready purification of leukemic cells and LSCs from the mice. Bone marrow cells from leukemia-bearing mice are incubated with antibodies conjugated with appropriate fluorescent dyes. The labelled cells are passed through the flow cytometer where cells are sorted; VENUS+ cells that are positive for the presence of a single antigen are separated in a different test tube from cells that are negative for that antigen. The cells in each test tube are injected into two separate groups of irradiated mice; the group which develops leukemia will have been injected with the LSCs. This flow cytometric isolation based on the presence or absence of certain antigens and the subsequent injection into irradiated mice will be repeated for one antigen at a time for 6 antigens (CD150, cKit, Sca1, CD34, FcγR, Flt3). With each cell sorting step based on positive or negative marker expression for a particular antigen, I'll be injecting the two sorted fractions into two cohorts of normal recipients.

**Interdisciplinary and/or international dimension**

This project is interdisciplinary in terms of its convergence of both stem cell research and immuno-oncology, both of which are of international interest. In addition, this research experiment is interdisciplinary in its use of international cell sorting technologies like FACS and flow cytometry, its dependence on the engineering of the retroviral sequence which inserts the HOXB4 transgene into host cell DNA, and its use of immunophenotyping and CD nomenclature. Also, leukemia is an international disease, so understanding leukemogenesis is an international goal. Considering this experiment aims to characterize and define the multiparametric phenotype of the LSCs –the cells propagating the leukemia– this project takes a step towards understanding the origin of leukemogenesis.

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