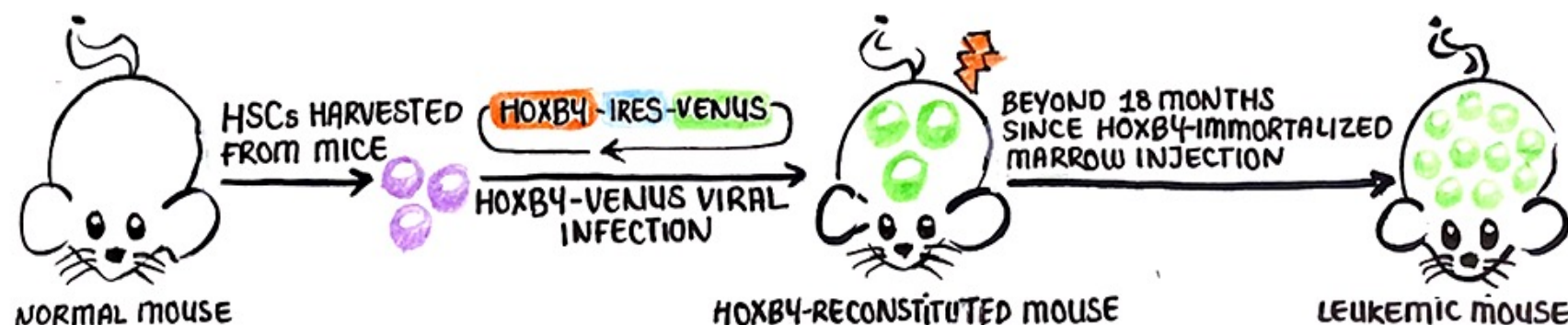


## Introduction



- The hematopoietic hierarchy has cell stages with differing self-renewing capacity and differentiation potential. At the top of this hierarchy are the hematopoietic stem cells (HSCs).
- Further down the hierarchy, cells become more differentiated, lose sustained self-renewing capacity, and lose the endogenous expression of the homeobox genes. The Iscove lab has been studying if the loss of self-renewal is related to the downregulation of homeobox genes in later stages.



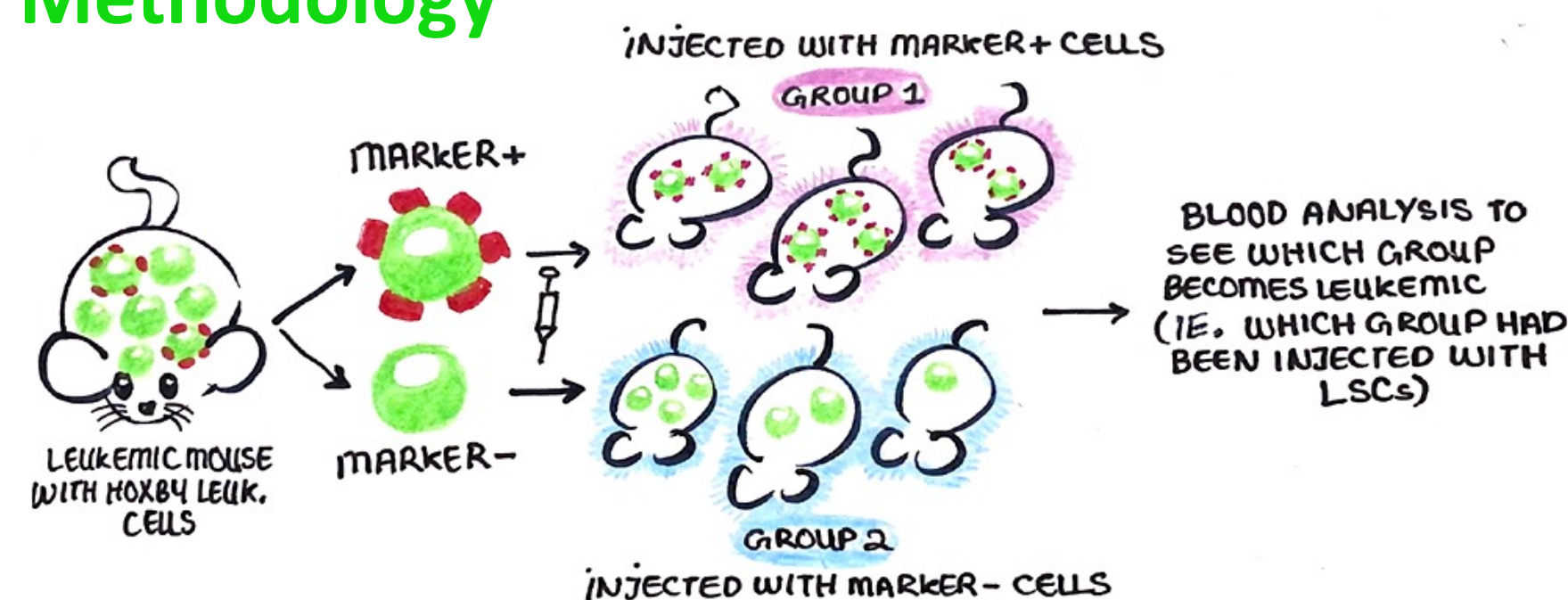
- HOXB4-infected HSCs are injected into irradiated mice. The cells these HSCs differentiate into will express HOXB4, ensuring that expression of HOXB4 is enforced in later stages where endogenous HOXB4 expression stops. The enforced expression of HOXB4 in later stages of this hierarchy endows them with self-renewal characteristic of HSCs. Immortalized by the HOXB4 transgene, these progenitors accumulate gene mutations. In the Iscove lab, a myeloid leukemia arose 18 months after HOXB4 transduction.

- The cells reconstituting this leukemia: the leukemia-reconstituting cells (LICs) exist as 1/63 cells of the leukemia cell population in BM.

## Aim

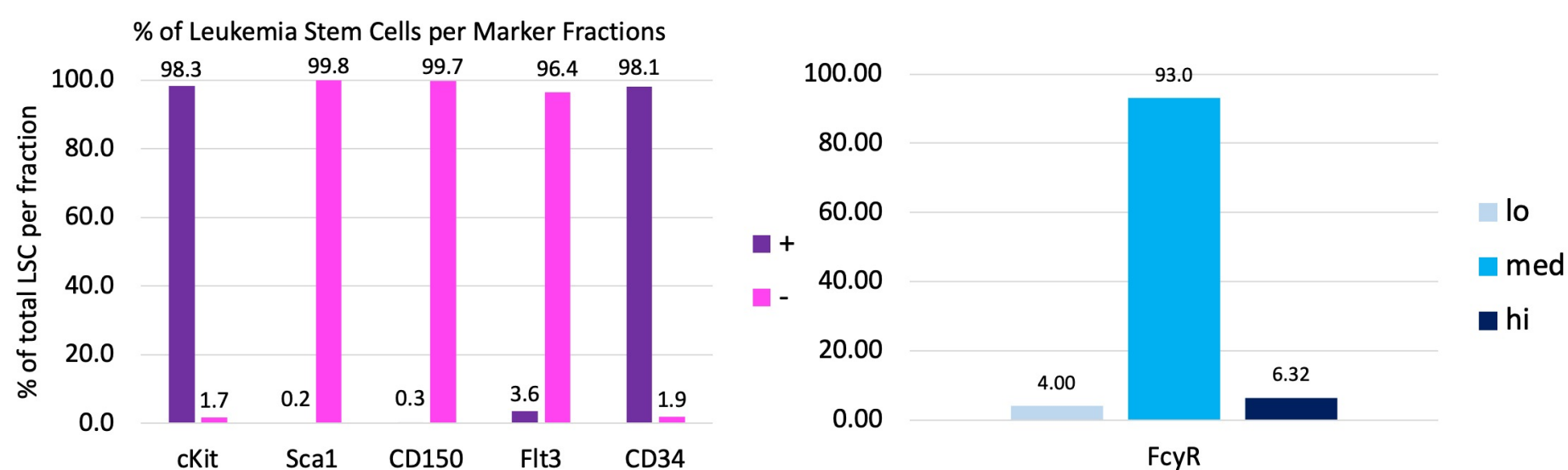
Finding the LIC antigens that distinguish them from the rest of the inert leukemic blasts in the leukemic cell population.

## Methodology

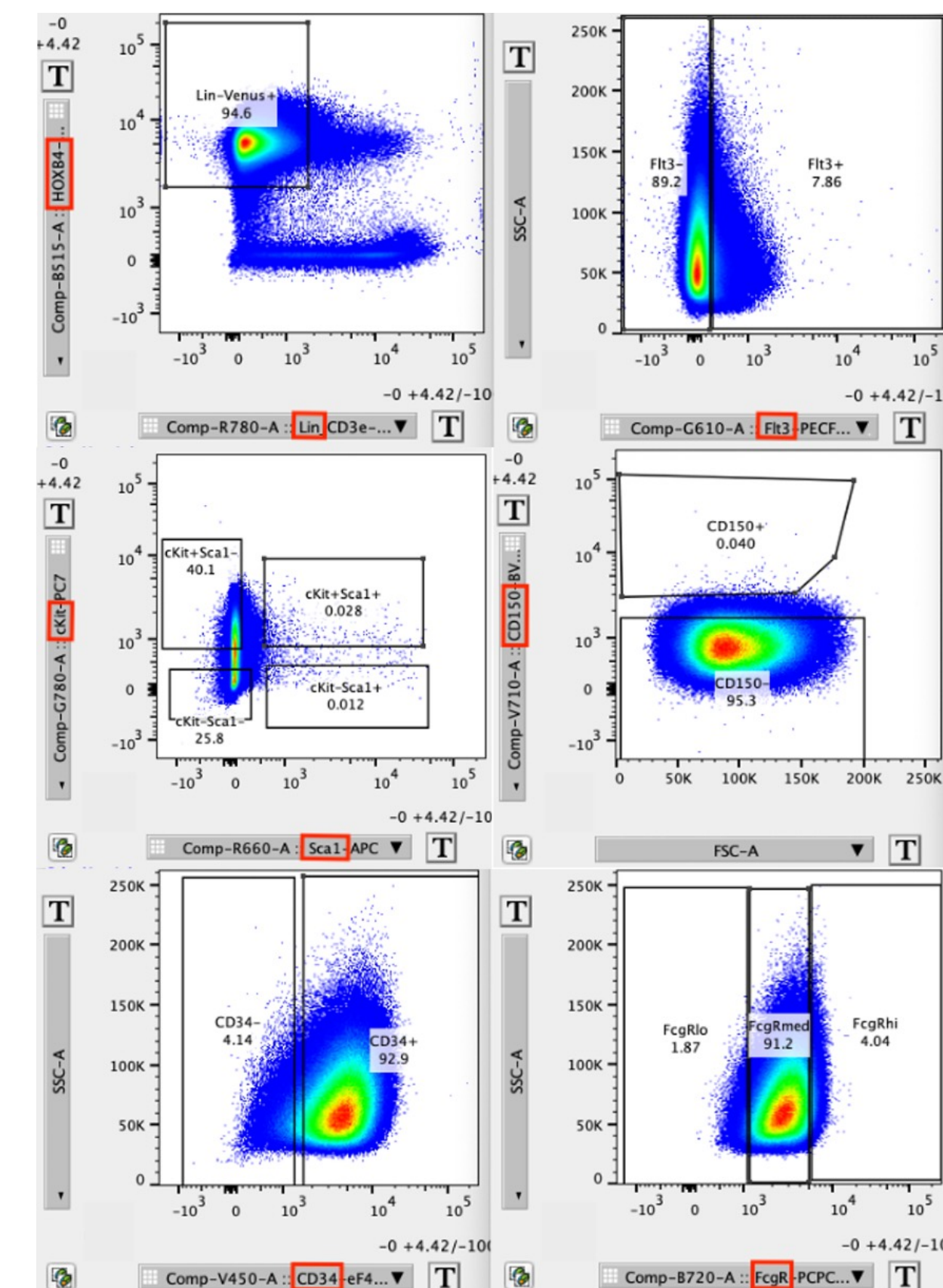


- Leukemic BM was harvested from mice with HOXB4-reconstituted marrow then sorted in a binary fashion based on the positive or negative marker expression of certain protein markers. The +/- sorted cells were then injected into two separate cohorts of recipient mice.
- The recipients were bled every 4-6 days to analyze which fraction of each marker enriches for LICs.

## Results



Using both the different rates at which the mice became leukemic and the baseline that 1 LIC makes a mouse 50%HOXB4\_VENUS+ by day 30, an estimate of how many LICs were in each fraction was calculated. The tentative distinguishing phenotype of LICs is cKit+Sca1-CD150-Flt3-CD34+FcyRmed.



24.7% of the total HOXB4-VENUS+ population remains after the LIC compound phenotype has been gated for. This percentage is a quantification of the extend to which the LICs have been purified.

## References

- Lim H et al. 2017. Committed Hemopoietic Progenitors, Not Stem Cells, are the Principal Responders to Hox Gene Transduction. bioRxiv: <https://doi.org/10.1101/174490>
- Mazzanti AD. 2019. HOXB4-mediated Enhanced Self-renewal of Committed Hemopoietic Progenitors (Doctoral dissertation, University of Toronto).
- Seita J, Weissman IL. 2010. Hematopoietic stem cell: self-renewal versus differentiation. Wiley Interdiscip Rev Syst Biol Med 2(6): 640-653.