# A multi-organ integrated QSP model for hematopoietic stem cell differentiation to predict the immune cell reconstitution in ex-vivo gene therapy

Yuezhe Li[1], Eric Jordie[1], Tim Knab[1]

[1]*Metrum Research Group*

## Abstract

The differentiation of mammalian hematopoietic stem cells (HSCs) is complex and multi-scale, providing an opportunity for mathematical modeling and simulation to aid in mechanistic understanding, and ultimately, to inform drug development efforts. Historically, mathematical models that have been developed were focused on the development of a subset of cells, but mathematical models that encompass the overall cellular system's complexity are rarely available. Here, we develop an integrated quantitative systems pharmacology (QSP) model that characterizes multi-organ recapitulation of HSC differentiation by integrating literature models and adding novel features. The result is a more comprehensive representation of mammalian HSC development. We demonstrate our integrated model can accurately capture the reconstitution of RBCs, B cells, and T cells following HSC transplant in mice, and predict the reconstitution of granulocytes and lymphocytes in patients with adenosine deaminase-deficient severe combined immunodeficiency (ADA-SCID) who underwent ex-vivo gene therapy.

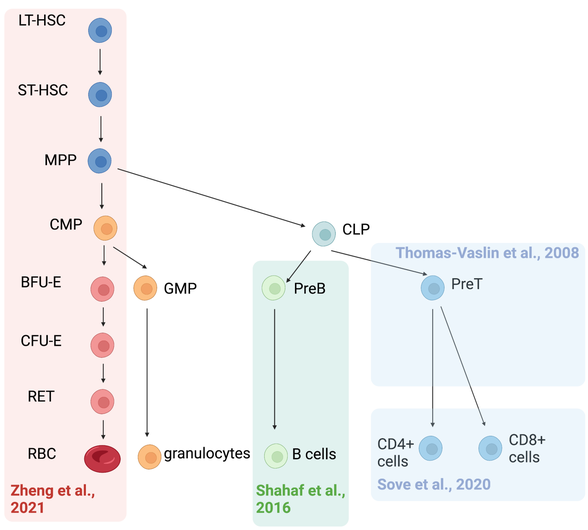
## Methods

We developed an integrated model that depicted the differentiation of hematopoietic stem cells (HSCs) into erythrocytes, lymphocytes, and granulocytes. The model was built incrementally by incorporating novel physiological-based features based on literature data while the following integrating published models:

- human HSC erythroid differentiation [1]
- mouse B cell development [2]
- mouse T cell development [3]
- human naive T cell dynamics model [4]

The model was developed in 4 steps:

- Implement an existing human HSC -> RBC differentiation model in [1]
- Scale the HSC -> RBC differentiation model from human to mouse based on mouse physiology
- Integrate T cells development model from [3], B cells development model from [2], and HSC -> granulocyte differentiation model into the mouse HSC differentiation model.
- Scale the integrated mouse HSC differentiation model to human based on human physiology




Parameter tuning were carried out to help harmonize the difference across models. The integrated models for mouse and human were validated using cell reconstitution in peripheral blood after stem cell transplant/ ex-vivo gene therapy.

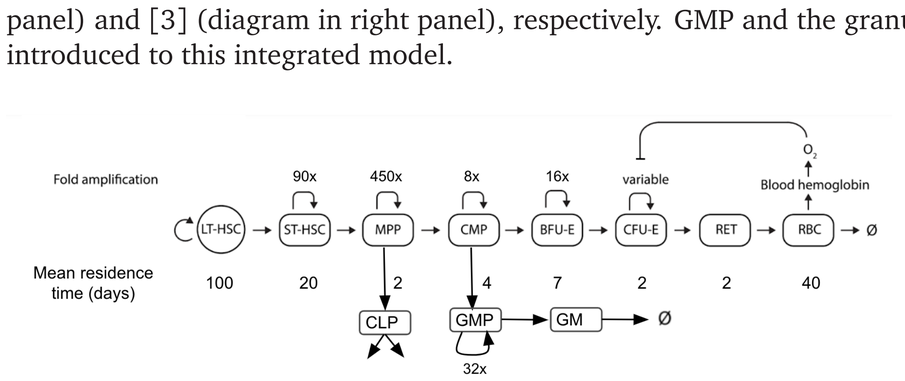
## References

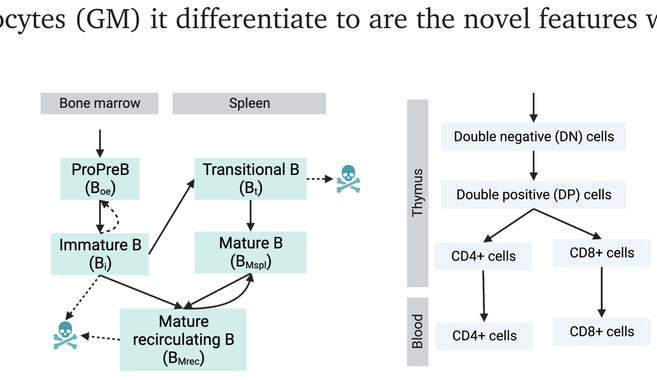
[1] Zheng, Bo, et al. "A systems pharmacology model for gene therapy in sickle cell disease." CPT: Pharmacometrics & Systems Pharmacology 10.7 (2021): 696-708. [2] Shahaf, Gitit, et al. "B cell development in the bone marrow is regulated by homeostatic feedback exerted by mature B cells." Frontiers in immunology 7 (2016): 77. [3] Thomas-Vaslin, Veronique, et al. "Comprehensive assessment and mathematical modeling of T cell population dynamics and homeostasis." The Journal of Immunology 180.4 (2008): 2240-2250. [4] Sové, Richard J., et al. "QSP-IO: a quantitative systems pharmacology toolbox for mechanistic multiscale modeling for Immuno-oncology applications." CPT: pharmacometrics & systems pharmacology 9.9 (2020): 484-497.

## Source code

## Results

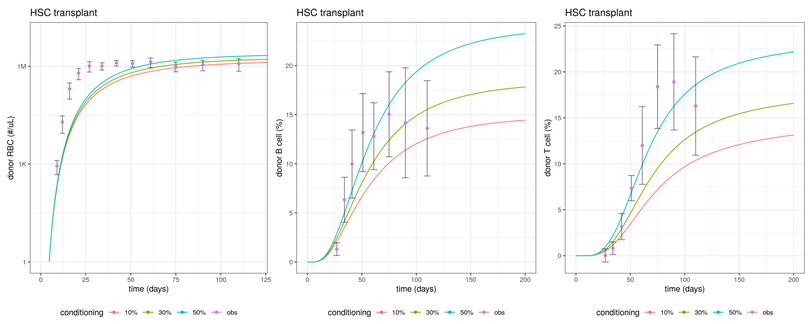
The integrated HSC differentiation model for mouse can be summarized by the figures follow. The arrows represent direction of cell differentiation, proliferation, trafficking, and death. The long-term hematopoietic stem cells (LT-HSC) is capable of self-renewal and can differentiate to short-term hematopoietic stem cells (ST-HSC). ST-HSC can differentiate to multipotent progenitor cells (MPP). MPP can differentiate to common myeloid progenitor (CMP) and common lymphoid progenitor (CLP). CMP can differentitiate to burst forming unit-erythroid (BFU-E) and granulocyte-monocyte progenitors (GMP). BFU-E can further differentiate to colony forming unit-erythroid (CFU-E), then reticulocytes (RET) and red blood cells (RBC). Furthermore, hemoglobin (Hb) can be synthesized in RETs and RBCs. The concentration of Hb in blood determines the oxygen level in blood, and subsequently imposes a negative feedback on CFU-E amplification. The erythrocytes differentiation submodel was taken from [1] (left panel) and scaled from human to mouse by reparameterizing mean residence time and amplification number using physiologically informed values. CLP is a novel feature we add to link the HSC differentiation with B cell and T cell development taken from [2] (diagram in middle panel) and [3] (diagram in right panel), respectively. GMP and the granulocytes (GM) it differentiate to are the novel features we introduced to this integrated model.



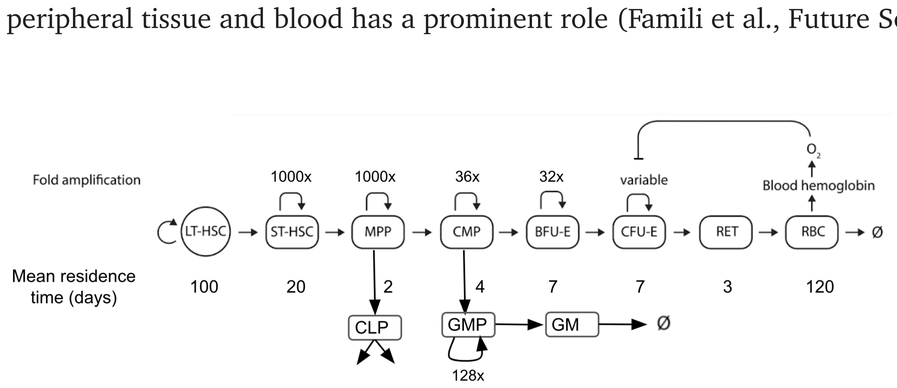



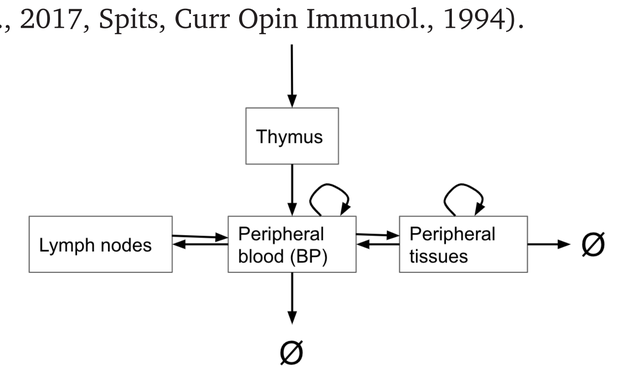

The integrated model for mouse can predict the steady state mouse cell count, as well as reconstitutions of RBC, B cells, and T cells after HSC transplant in mouse (Busch et al., Nature. 2015).

| Cell type | Simulated | Reference |
|---|---|---|
| MPP | 60k | 75k-92k |
| CMP | 2M | 755k- 3M |
| GMP | 757k | 1M |
| GM | 8.4k/uL | 1.95-12.01k/uL |
| RET | 498k/uL | 200 - 500k/uL |
| RBC | 9.9M/uL | 10.2M/uL |
| blood Hb | 13.2g/dL | 13.6 - 16.4g/dL |
| Hb in RBC | 261g/L | 270-330g/L |
| lymphocyte | 1.8k/uL | 0.12 - 24k/uL |




The integrated HSC differentiation model for human can be summarized by the figure follow. Briefly, the model was scaled from mouse to human based on physiology. Especially, the scaling of bone marrow B cell capacity was scaled based on cellularity. In addition,naive T cell dynamics in lymph nodes (LN) and peripheral tissues from [4] was also incorporated to capture the different mechanism for naive T cell maintenance: in mouse, thymic output is the main source; in human, naive T cell proliferation in peripheral tissue and blood has a prominent role (Famili et al., Future Sci OA., 2017, Spits, Curr Opin Immunol., 1994).



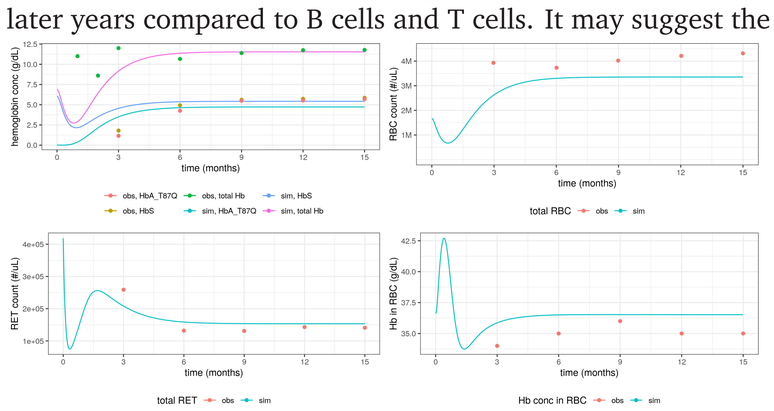



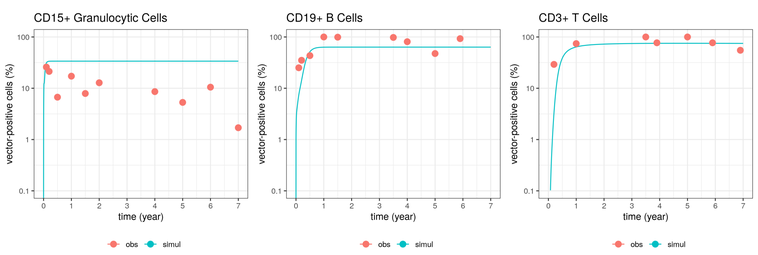

The integrated model for human can recapitulate healthy and those with adenosine deaminase deficiency severe combined immunodeficiency (ADA-SCID). The death rates for double positivr cells in thymus and splenic mature B cells were adjusted to account for ADA-SCID (Whitmore and Gasper, Front Immunol., 2016).

| Cell type | Simulated | Reference |
|---|---|---|
| RBC (/uL) | 3.9M | 4M |
| Hb (g/dL) | 13.2 | 12-15 |
| thymic output (/day) | 32M | 10M-2700M |
| granulocyte (/uL) | 1.2k | 1k-8k |
| T cell (heathy) (/ul) | 662 | 1243 |
| T cell (ADA-SCID) (/ul) | 149 | 138 |
| B cell (healthy) (/uL) | 137 | 101 |
| B cell (ADA-SCID) (/uL) | 61 | 0 |

The integrated human model can recapitulate blood cell and hemoglobin reconstitution after gene therapy in those with sickle cell disease (SCD) (left panel, data obtained from Ribiel et al., N Engl J Med., 2017). The model captured the lowering of RET count up to 6 months after the gene therapy, as well as the increase of $HbA^{T87Q}$, a Hb that only exists in transduced cells. Thee mismatch in HbA and RBC count at 3 months after the gene therapy could be due to the transfusion received by the patient.

The integrated human model can also recapitulate immune cell reconstitution after gene therapy in those with ADA-SCID (right panel, data obtained from Aiuti et al., N Engl J Med., 2009). Note the total amount of CD34+ cells infused into this patient was estimated based on the patient's age and gender (seven-month old female). Reconstitution of granulocytes matched less well in the later years compared to B cells and T cells. It may suggest the myeloid arm of this model could be further refined.




## Conclusion

Through integrating existing models and adding novel features, we developed mathematical models that present a more comprehensive representation of mammalian hematopoietic stem cell development than previous partial efforts. Our integrated models are based on physiological understanding of mouse and human, and were validated accordingly. We demonstrated our models could successfully predict cell reconstitution after ex-vivo gene therapy or HSC transplant. We believe our models provide a versatile platform for further development to inform drug development for ex-vivo gene therapy and HSC transplant.