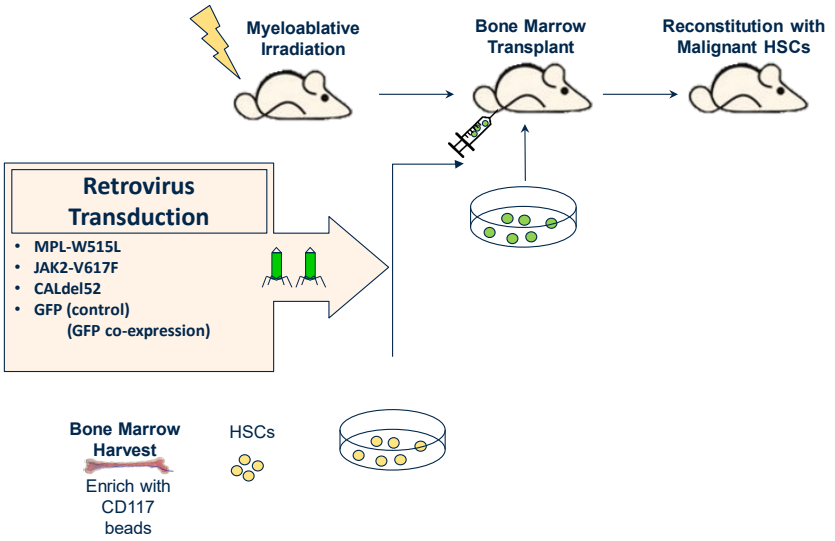
Preclinical Myelofibrosis Model in Mouse Tibia

I. Animal Model Specifications

1. Species: mouse
2. Strain: C57BL/6.
3. Sex: Female
4. Age at start of data acquisition: ~12 weeks
5. Disease induction:
    i. The JAK2 V617F ($JAK2^{+/VF}$) animal model of myelofibrosis (MF) was generated using resultant 8-10 week old female donor offspring from cross between $JAK2^{+/VF}$ mice (B6N.129S6(SJL)-*Jak2*$^{tm1.2Ble}$/AmlyJ; Charles River Stock No. 031658) and Mx-Cre mice (B6.Cg-Tg(Mx1-cre)1Cgn/J; Charles River Stock No. 003556), similar to previously described methods [12, 13].
    ii. Whole bone marrow cells were isolated from $JAK2^{+/VF}$ donor mice and mixed 1:1 with whole bone marrow cells isolated from wild-type mice.
    iii. A total of 1×10^7 bone marrow cells mixed 1:1 were injected retro-orbitally into lethally irradiated 6-8 week old female C57BL/6 recipient mice.
    iv. Polyinosinic-polycytidylic acid (10 mg/kg) was administered intra-peritoneally 10 days post-bone marrow transplant (post-BMT).
    v. Development of MF was evaluated by spleen volumes using MRI beginning 4 weeks post-BMT, continuing every 2 weeks thereafter.
6. Therapeutic intervention – Not Applicable
7. Animal prep and during imaging: 1.5% Isofluorane/air inhalation
8. Animal monitoring/support during imaging:
    i. Thermoregulated heating bed during imaging
    ii. Respiratiory montoring (SAI monitor)
9. Animal recovery: isolated cage until full recovery, then back to communal cage

MPLW515L MF mouse model

Generation of myelofibrosis mouse models can be accomplished using retrovirus transduction of gene mutations for MPL-W515L, JAK2-V617F or CALdel52 depending upon experimental needs. As an example, the MPLW515L mutant MF mouse model can be established using the following procedures.



Briefly, female donor BALB/c mice are treated with 5-fluorouracil at 150 mg kg^{-1} intraperitoneal for 5 d before harvesting bone marrow. Hematopoietic stem and progenitor cells are enriched with CD117 magnetic beads (Miltenyi Biotec), transduced with retrovirus containing MSCV-hMPL W515L-IRES-GFP, then 50,000 transduced hematopoietic stem cells (HSPCs) along with 300,000 total non-stem cells are injected intravenously into lethally irradiated (2 x 4.5 Gy separated by 24 hours) female BALB/c recipients. Development of MF can be evaluated by measurement of spleen volumes using a small animal MRI beginning ~14 d following bone marrow transplantation (BMT), continuing weekly until end of study. Mice are typically be separated into equal groups based on spleen size ~14–21 d post-BMT, then treated with vehicle or experimental drug for 14–28 d or until mice are euthanized using guidelines for end stage illness and humane endpoints.