Patient-Derived Xenografting Methods

Mouse strains:

For most xenografts we use: NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ, Stock Number: 005557

https://www.jax.org/strain/005557

For myeloid xenografts with long engraftment times we use: NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl} Tg(CMV-IL3,CSF2,KITLG)1Eav/MloySzJ, Stock No: 013062

https://www.jax.org/strain/013062

For xenografts prone to graft-versus-host disease we use: NOD.Cg-*B2m*^{tm1Unc} *Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ, Stock No: 010636

https://www.jax.org/strain/010636

Generating Patient-Derived Xenografts from Primary Tissue:

- For primary peripheral blood or bone marrow samples, extract lymphocytes using Ficoll-Paque PLUS (GE Life Sciences #17-1440-02). For primary leukapheresis samples, remove red blood cells using RBC lysis buffer (Qiagen #158904). Count viable cells using Trypan blue or equivalent method. Re-suspend cells in a volume of sterile PBS that will allow injection of 250uL per mouse intravenously in the lateral tail vein. Inject total number of cells split evenly into two mice, up to 20 x 10^6 cells per mouse for primary samples into P0 generation. Considering that not all samples will engraft we use two mice for primary samples; this minimizes waste when samples don't engraft but also allows for duplicates in case one mouse dies prematurely. The exception is when engrafting B-ALL; since B-ALL samples have a high rate of engraftment and almost always serially re-passage, you may decide to inject more mice with primary sample to bank significant numbers of P0 vials instead of expanding to P1. For expansion into P1 generation inject cells into at least 5 mice to maximize available tissue for banking. Cells should be injected into 4-8 week old mice, or if >8 weeks old, should be irradiated at 2Gy within 24 hours prior to injection.

Xenografting Cells:

- <u>For in vivo expansion</u> (with goals of maximizing likelihood of engraftment and expediting engraftment): Thaw cryovial of cells using standard methods. Count viable cells using Trypan blue or equivalent method. Resuspend cells in a volume of PBS that will permit an injection volume of 250 ul per mouse. Optimal cell dose is $1x10^6$ cells per mouse (range 10^5-10^7 cells/mouse), which would correspond to $4x10^6$ cells/mL of PBS. *Of note, while higher cell doses within this range can enhance engraftment within an individual mouse, for the purposes of expanding cells it is generally more useful to inject a moderate number of cells into a larger number of mice than to inject a very large number*

of cells into one or two mice. However, This is a general rule and will vary by specific xenograft. Mice should be 6-8 weeks old NSG strain unless otherwise specified. If mice are >8 weeks old, engraftment rates are enhanced by irradiation (200 cGy) within 24 hours prior to xenografting. Inject cells into mice via tail vein approach using a 1 mL syringe with a 27-gauge needle.

- For in vivo treatment studies (with goal of promoting temporal consistency of engraftment): Thaw cryovial of cells using standard methods. Count viable cells using Trypan blue or equivalent method. Resuspend cells in a volume of PBS that will permit an injection volume of 250 ul per mouse. Optimal cell dose is 10^6 cells per mouse (minimum 10^5 cells/mouse), which would correspond to $4x10^6$ cells/mL of PBS. Mice should be female (to permit prolonged cohabitation), 6-8 weeks old, and NSG strain unless otherwise specified. If animals are >8 weeks old, engraftment rates are enhanced by irradiation (200 cGy) within 24 hours prior to xenografting. Inject cells into mice via tail vein approach using a 1 mL syringe with a 27-gauge needle.

Specific Xenografting Methods

- Tail Vein Injection: Resuspend viably frozen and thawed cells or freshly harvested cells in a volume of PBS that will permit an injection volume of 250 ul per mouse. Optimal cell dose depends upon xenografting intent (expansion versus treatment versus other). Inject cells into mice via lateral tail vein approach using a 1 mL syringe with a 27-gauge needle.
- Subcutaneous Injection: Resuspend viably frozen and thawed cells or freshly harvested cells in 30% BD Matrigel and 70% PBS. Inject at least 100ul subcutaneously in the flank.
- Subcutaneous Tumor Fragment Implantation: Anesthetize mouse according to local IACUC-approved methods and shave the desired location. Clean with alcohol and make a small (~0.5cm) incision. Open a pocket with your scissors and insert tumor fragment. Close with 1 or 2 staples.
- Sub-Renal Capsule Implantation: *See separate file given greater level of detail involved*.

Monitoring Xenografted Animals for Cell Banking:

- Clinically assess xenografted mice daily for physical signs of illness, including lethargy, weight loss, hind limb paralysis, rapid/labored breathing, pallor, and/or poor grooming. Any of these physical signs should prompt euthanasia of affected animals using IACUC-approved methods. Peripheral blood can be collected by submandibular phlebotomy prior to euthanasia, or by immediate post-mortem subxiphoid cardiac puncture. Marrow can be harvested from bilateral femurs and tibias, and hematopoietic cells isolated from spleens post-mortem.
- AML PDX Lines: Of note, for AML lines, animals do not always exhibit physical signs of illness and therefore should be assessed with alternative methods, such as peripheral blood flow cytometry, at regular intervals (we recommend beginning after one month and repeating every two weeks thereafter until burden of circulating disease reaches 70%). Many AML xenograft lines can maintain high levels of circulating disease for weeks before manifesting signs of illness. The decision of when to sacrifice can therefore be more difficult in light of the desire to permit additional time for marrow and splenic disease to progress, which enhances recoverable leukemia cell yields, while also avoiding sudden mouse death prior to observed physical signs. Our practice is therefore to euthanize animals and harvest leukemic cells from peripheral blood, bone marrow, and/or spleen when an animal has maintained circulating disease at a level of at least 70% for 2-3 weeks, in lieu of physical signs of illness.
- Subcutaneous tumors: Monitor subcutaneous tumor size with calipers. Sacrifice mouse when the tumor reaches 2 cm in greatest dimension, or according to institutional guidelines. Tumor volume = (L x W x W)/2 (where length is the larger dimension, and width is shorter dimension).

Monitoring Animals for Initiation of Treatment:

- Circulating Disease: Phlebotomize animals no later than 2-3 weeks after injection and assess for the presence of circulating disease by peripheral blood flow cytometry. We use anti-hCD45 as the primary marker plus a second lineage-specific marker (i.e., anti-hCD19 for B-cell diseases, anti-hCD2 for T-cell diseases, and anti-hCD33 and/or anti-hCD34 for myeloid diseases), although more sophisticated flow cytometry strategies may be necessary in select instances. Immunophenotype data including primary flow cytometry images for individual PDX models can be found in the www.proxe.org database explorer or in the individual line report tab. Repeat peripheral blood flow cytometry at regular intervals -- typically weekly -- until mice reach a level of disease considered by the investigator to be appropriate for initiating treatment, as informed by the therapeutic agent and the objectives of the study. We typically begin treatment for acute leukemia PDX models if survival is the primary endpoint when circulating disease reaches approximately 3-5% in the majority of animals in a cohort.
- Nodal/soft tissue disease: In the case of certain lymphoma PDXs lacking a circulating component, other methods of assessing engraftment such as bioluminescent imaging may be necessary to monitor the degree and anatomic location of engraftment. PDXs already labeled in this manner can be identified in the www.proxe.org database explorer by filtering for lines with a bioluminescent marker such as luciferase. We typically begin treatment for most leukemia or lymphoma PDX models if survival is the endpoint when the average radiance (photons/second/meter^2) per animal in a cohort is between 1E6 and 1E7 and has increased over at least two consecutive weekly measurements.
- Subcutaneous tumors: Monitor subcutaneous tumor size with calipers. We generally initiate treatment when the tumor volume reaches 100-200mm3. Tumor volume = (L x W x W)/2 (where length is the larger dimension, and width is the shorter dimension).

Banking Xenografted Cells:

- Animal euthanasia: Sacrifice animal via CO2 asphyxiation, cervical dislocation, or other IACUC-approved method. For circulating diseases, phlebotomize animal prior to euthanasia via submandibular approach or perform post-mortem subxiphoid cardiac puncture. For marrow-infiltrating diseases, harvest bone marrow from bilateral femurs and tibias by flushing with PBS or media such as RPMI. For diseases involving extramedullary hematopoiesis, harvest spleen via laparotomy. For relevant lymphomas, harvest enlarged lymph nodes or soft tissue masses if observed during necropsy.
- Enriching malignant cells from peripheral blood: Eliminate erythrocytes using RBC lysis buffer (Qiagen #158902). Resuspend cells in PBS. Enrich malignant cells via fluorescence activated cell sorting (FACS) or magnetic activated cell sorting (MACS). Viably freeze cells in FBS with 10% DMSO.
- Enriching malignant cells from bone marrow: Dissect bilateral femurs and tibias from euthanized animal. Flush bone marrow from each long bone with PBS using a 3 mL syringe with a 20-gauge or smaller needle. Gently dissociate flushed marrow by passing through a micropipette tip until visible clumps disappear. Enrich malignant cells via

fluorescence activated cell sorting (FACS) or magnetic activated cell sorting (MACS). Viably freeze cells in FBS with 10% DMSO.

- Enriching malignant cells from spleen: Dissect spleen from euthanized animal. Pulverize spleen in 3 mL of RPMI or similar media. Filter through 70 micrometer mesh to remove cell clumps and connective tissue. Eliminate erythrocytes using RBC lysis buffer (Qiagen #158902). Enrich malignant cells via fluorescence activated cell sorting (FACS) or magnetic activated cell sorting (MACS). Viably freeze cells in FBS with 10% DMSO.
- Enriching malignant cells from lymph nodes or solid tumors: Excise fresh tissue from animal. Rinse and mince into submillimeter fragments. Dissociate using the Human Tumor Dissociation Kit (Miltenyi Biotec #130-095-929) +/- collagenase and a gentleMACS Tumor Dissociation Machine (Miltenyi Biotec #130-093-235). Filter the resulting single cell suspension through 70 µm mesh to remove residual cell clumps. Viably freeze cells in FBS with 10% DMSO.