



Use of Human Acute Myeloid Leukemia Cells to Study Graft-Versus-Leukemia Immunity in Xenogeneic Mouse Models of Graft-Versus-Host Disease

Charline Faville, Bianca E Silva, Frédéric Baron, and Grégory Ehx

Abstract

Allogeneic hematopoietic cell transplantation (allo-HCT) is the main therapeutic approach for patients with high-risk acute myeloid leukemia (AML), but the rate of relapse remains high and is associated with poor outcomes. Discovering new approaches to maximize the graft-versus-leukemia (GVL) effects while mitigating graft-versus-host disease (GVHD) should therefore be pursued. Because of the difficulties in modeling AML in mice, patient-derived xenotransplantations (PDXs) in immunodeficient NOD-scid-IL2rg^{null} (NSG) mice are preferred to study the GVL effects. In PDX, AML is typically induced through the intravenous injection of cell lines or leukemic blasts obtained from patients. GVHD and GVL effects are induced by (co)-injecting human T cells or peripheral blood mononuclear cells (PBMCs). While this approach enables the induction of systemic leukemia, notably developing in the spleen and bone marrow of the animals, it can also be associated with difficulties in monitoring the disease, notably by flow cytometry. This can be circumvented by using luciferase-expressing AML cells or transplanting the leukemic cells in Matrigel to generate solid tumors that are easier to monitor. Here, we provide detailed instructions on how to prepare human PBMCs and leukemic cells, transplant them, and monitor the disease in NSG mice.

Key words Immunodeficient mice, Xenotransplantation, AML, Bioluminescence, Hematopoietic cell transplantation, Graft-versus-leukemia effects, Graft-versus-host disease

1 Introduction

Acute myeloid leukemia (AML) is an aggressive malignancy characterized by uncontrolled proliferation of abnormal myeloid progenitor cells (blasts) of hematopoiesis. Currently, AML is the most frequent and lethal leukemia among adults, while chemotherapy results in high remission rates, ~75% of patients relapse, resulting in an average 5-year survival rate of only 40–45% in young patients

Charline Faville and Bianca E Silva contributed equally.

Ronald Sluyter (ed.), *Graft-Versus-Host Disease: Methods and Protocols*, Methods in Molecular Biology, vol. 2907, https://doi.org/10.1007/978-1-0716-4430-0_17,
© The Author(s), under exclusive license to Springer Science+Business Media, LLC, part of Springer Nature 2025

and less than 10% in the elderly [1]. Following the first cycle of chemotherapy, patients with intermediate- and high-risk AML often receive allogeneic hematopoietic cell transplantation (allo-HCT), the only curative option currently available [2, 3]. This therapy relies on two key steps: (1) administration of high doses of chemotherapy to eradicate most leukemic cells and induce medullary aplasia and (2) infusion of a combination of stem cells and mature hematopoietic cells from a genetically different healthy donor. While stem cells colonize the patient's bone marrow and restore healthy hematopoiesis, mature immune cells recognize and eradicate residual leukemic cells that survived chemotherapy, a process known as the graft-versus-leukemia (GVL) effect. Relapse prevention by the GVL effect is mostly mediated by transplanted immune cells that recognize leukemic cells as "foreign" due to the human leukocyte antigen (or HLA) mismatch between donor and recipient and/or the presentation of major histocompatibility complex (MHC) peptides derived from polymorphic genomic regions that differ between donor and recipient [4]. However, this allorecognition can also cause the immune cells of the donor to attack the healthy tissues of the recipient, resulting in an inflammatory condition known as graft-versus-host disease (GVHD) that can lead to death [5]. Therapies aimed at improving the GVL effects while mitigating GVHD are therefore under intense investigation [6].

The currently accepted AML pathogenesis mechanism describes that the malignant transformation of hematopoietic progenitors results from the effect of one or several cooperating driver mutations [7, 8]. Therefore, modeling AML in rodents relies on engineering these mutations in the germ line or retroviral transduction of bone marrow cells followed by transplantation. Apart from the obvious species-specific differences, these models are limited because engineered genetic lesions only partially mimic those found in the leukemic blasts of patients, as—even in the presence of leading cytogenetic lesions—AML blasts often contain one or several potentially cooperating mutations [9]. Therefore, using patient-derived xenotransplantations (PDXs) or cell line-derived xenotransplantations (CDX) in immunodeficient NOD-scid-IL2rg^{null} (NSG) mice is preferred when studying AML in vivo [10].

Over the last decade, our laboratory has extensively studied the pathogenesis and effects of multiple treatments on xenogeneic GVHD, either alone [11–15] or on both GVHD and GVL effects [16–18]. Our experience with these models highlighted two main technical limitations needing special attention when performing such experiments.

The first technical limitation is the capacity of primary AML cells to engraft. Indeed, this can be limited by the presence of mature T or B cells in the transplanted AML sample that mediate GVHD, thereby limiting AML engraftment and/or leading to the

death of the mice before AML engraftment [19]. Therefore, T and B cells should be depleted from primary AML cells, or cell lines should be used. Alternatively, in experiments only focused on GVL effects and when T and B cells cannot be depleted, NSG mice lacking mouse MHC molecules can be used as they develop GVL effects without GVHD [20]. Here, it is worth mentioning that an extended period is sometimes needed to reach detectable engraftment of leukemic cells when using some primary cells or cell lines. This has caused several investigators to conclude that some AML samples cannot engraft in NSG mice (6 weeks posttransplantation), while others demonstrated that by using an extended follow-up time (several months), virtually all AML cells eventually engraft [21]. Therefore, patience, in addition to T- and B-cell depletion, can be the key to engrafting AML cells. Finally, NSG mice expressing stem cell factor, granulocyte-macrophage colony-stimulating factor, and interleukin-3 (NSG-S) can be used to boost the engraftment of AML cells [22].

The second technical limitation is the capacity to detect AML cells to monitor either the engraftment or the GVL effects. We observed that T cells are the main immune cell population engrafting in NSG mice upon intravenous infusion of peripheral blood mononuclear cells (PBMCs) [12]. Consequently, the detection of AML cells could be based on flow cytometry analyses of the CD33⁺ population in the blood of mice transplanted with AML cells only or in combination with PBMCs. However, such analyses are limited by the capacity of AML cells to exit the bone marrow and be mobilized to the peripheral blood [23, 24] and by the expression of a specific marker, such as CD33, by leukemic cells. Indeed, only 75% of patients with AML express this marker and the subset of cells that regenerate leukemia in immunocompromised mice does not tend to express CD33 [25]. Consequently, using luciferase-expressing cell lines or Matrigel-based subcutaneous injections of primary cells (generating measurable tumors) may be needed to enable the monitoring of GVL effects [12].

In this chapter, we provide detailed instructions on how to prepare human PBMCs and leukemic cells, transplant them, and monitor the disease in NSG mice.

2 Materials

Ensure that all reagents intended for injection into mice remain sterile. All plasticware, including 5 mL round bottom polystyrene (FACS) tubes, are sterile. Prepare and store all reagents at room temperature (unless indicated otherwise).

2.1 Cell Line

Preparation

1. AML cell line, expressing luciferase or not (*see Note 1*).
2. 0.4% Trypan blue solution (trypan blue).
3. 1.5 mL Microfuge tube.
4. Dual-chamber counting slides for cell counter.
5. Automatic cell counter (Bio-Rad, TC-20).
6. Centrifuge.
7. Dulbecco's phosphate-buffered saline, pH 7.4 (PBS).
8. FACS tubes.
9. 70 μ m Cell strainer.
10. Ice.

2.2 Preparation of

Primary Blasts

1. Peripheral blood or bone marrow aspirate from a patient with AML.
2. 50 mL Conical tubes.
3. *Red blood cell lysis buffer (5X)*: 8.28 g of ammonium chloride, 0.036 g of EDTA, and 1 g of potassium bicarbonate in 1 L of deionized water. Adjust to pH 7.1–7.2 and sterilize by autoclaving (*see Note 2*).
4. Rocker.
5. Centrifuge.
6. Water bath set to 37 °C.
7. *Supplemented RPMI 1640 medium (RPMI)*: 10% Heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin-100 μ g/mL streptomycin in RPMI, preheat at 37 °C.
8. Hematological cell counter.
9. FACS tubes.
10. 70 μ m Cell strainer.
11. 14 mL (17 mm) Non-graduated polystyrene culture tubes.
12. *Flow cytometry staining buffer*: 3% FBS in PBS.
13. Anti-CD3-biotin antibody (clone SK7) and anti-CD19-biotin antibody (clone HIB19).
14. PBS.
15. EasySep Human Biotin Positive Selection Kit II (STEMCELL Technologies, Ref. 17,663).
16. Vortex.
17. EasySep Magnet (STEMCELL Technologies, Ref. 18,001).
18. Ice.
19. Human Fc Blocker (BioLegend, Ref. 422,302).

20. *Depletion assessment mix of antibodies for flow cytometry staining*: 2 μL of anti-CD4-FITC (clone SK3), 1 μL of anti-CD33-PE (clone HIM3-4), 1 μL of anti-CD19-APC (clone HIB19), 1 μL of anti-CD3-V450 (clone UCHT1), 1 μL of anti-CD8-APC-Cy7 (clone SK1), 0.5 μL of anti-CD45-BV510 (clone HI30), 1 μL of anti-CD117-APC-R700 (clone YB5.B8), 1 μL of anti-CD56-Per-Cy7 (clone HCD56), and 1 μL of anti-CD34-PerCP-Cy5.5 (clone 8G12) antibodies per tube to label (*see Note 3*).
21. Flow cytometer equipped with violet (405 nm) and red (561 nm) lasers, and 780/60- and 450/40 nm band-pass filters.

2.3 PBMC Isolation from Healthy Volunteers

1. Human buffy coats or whole blood.
2. PBS.
3. 50 mL Conical tubes.
4. Ficoll-Paque (density 1.077 g/mL, endotoxin low).
5. Centrifuge.
6. Hematological cell counter.
7. 70 μm Cell strainer.
8. FACS tubes.
9. Ice.

2.4 Transplantation of Cells into NSG Mice

1. NSG mice.
2. *Antibiotics*: 100 $\mu\text{g}/\text{mL}$ enrofloxacin in sterile water.
3. AML cells.
4. PBS.
5. Matrigel matrix (*see Note 4*).
6. 1 mL Syringe.
7. 26-gauge \times 13 mm Syringe needle.
8. 1 mL Insulin syringe with 29-gauge \times 13 mm needle.
9. Weighing scales.

2.5 Monitoring AML Engraftment by Flow Cytometry

1. Mouse restraining device.
2. *Antiseptic solution*: 70% Ethanol in deionized water.
3. Infrared lamp.
4. 26-gauge \times 13 mm Syringe needle.
5. 75 \times 1.6 mm Heparized microhaematocrit capillaries.
6. EDTA microtainer tubes.
7. Hematological cell counter.
8. FACS tubes.

9. *Red blood cell lysis buffer (10X; Invitrogen eBioscience, Ref. 00430054)*: Dilute tenfold with deionized water to prepare red blood cell lysis buffer (1X).
10. Vortex.
11. PBS.
12. Centrifuge.
13. *Flow cytometry staining buffer*: 3% FBS in PBS.
14. Human Fc Blocker (BioLegend, Ref. 422,302).
15. *Mix of antibodies for flow cytometry staining*: 1 μL of anti-CD33-PE (clone HIM3-4) and 1 μL of anti-CD45-BV510 (clone HI30) antibodies per tube (*see Note 3*).
16. Flow cytometer with violet (405 nm) and blue (488 nm) lasers and 585/42- and 525/50 nm band-pass filters.

2.6 Monitoring AML Engraftment by Bioluminescence Imaging

1. D-Luciferin, potassium salt.
2. PBS.
3. Vortex.
4. IVIS Spectrum Imaging system (Revvity).
5. 100% Isoflurane.
6. 1 mL Insulin syringe with 29-gauge \times 13 mm needle.
7. Living image software (Revvity).

2.7 Monitoring Subcutaneous Tumor Size with Calipers

1. Digital calipers.

3 Methods

All cell preparations are carried out in Class II Biological Safety Cabinets to maintain the sterility of the samples before transplanting mice and to protect the operator from possible pathogens present in human blood samples. Diligently follow all waste disposal regulations when disposing of biological waste and materials. Wear protective gloves and a laboratory coat at all steps of the following protocols. Carefully follow the guidelines of your local animal ethics committee and ensure that all human participants have signed informed consent approved by your local ethics committee to donate blood.

3.1 Cell Line Preparation for Transplantation into NSG Mice

1. Combine 20 μL of the AML cell line suspension (either expressing luciferase or not) with 20 μL of trypan blue in a microfuge tube and mix well by gently pipetting up and down 10 times.

2. Within 5 min of mixing, pipet 10 μL of the mixture into the outer opening of either of the two chambers of the counting slide.
3. Insert the slide into the slot of the cell counter to activate cell counting.
4. Based on the cell count obtained, collect the appropriate volume of the AML cell line to inject one million cells per mouse to be transplanted. Consider preparing additional cells beyond the exact requirement to accommodate potential sample loss during the injection process. Typically, allocate one dead volume for every three mice to be transplanted.
5. Centrifuge the cell suspension at $500\times g$ for 5 min at room temperature and carefully remove the supernatant with a pipette.
6. Resuspend the cells at a concentration of ten million cells/mL with PBS.
7. Filter the suspension with a 70 μm cell strainer into a sterile FACS tube.
8. Keep tubes on ice until transplantation.

3.2 Preparation of Primary Blasts for Transplantation into NSG Mice

1. Place 10 mL of peripheral blood or bone marrow aspirate from a patient with AML into a 50 mL conical tube and add four volumes of 5X sterile red blood cell lysis buffer (*see Note 5*).
2. Homogenize the tube by inversion immediately after adding the lysis buffer.
3. Place the tube on a rocker set to 25 oscillations/min and incubate for 15 min.
4. Centrifuge the tube at $300\times g$ for 10 min at room temperature.
5. Carefully remove the supernatant with a pipette, leaving 1–2 mL of supernatant above the pellet, and resuspend the pellet with a micropipette.
6. Add 5 mL of supplemented RPMI (*see Note 6*).
7. Centrifuge the tube at $500\times g$ for 5 min at room temperature.
8. Carefully remove as much supernatant as possible with a micropipette.
9. Resuspend the pellet in 5 mL of supplemented RPMI.
10. Count the cells with the hematological cell counter and record the white blood cell (WBC) counts.
11. Transfer one million cells into a FACS tube to evaluate the depletion efficacy of T and B cells (*see step 31*).
12. Adjust the remaining cells to 100 million WBCs/mL with flow cytometry staining buffer, filter the cell suspension with a 70 μm cell strainer, and transfer the cells to a 14 mL non-graduated polystyrene culture tube.

13. Add the anti-CD3-biotin and anti-CD19-biotin antibodies at a concentration of 0.3–3 $\mu\text{g}/\text{mL}$ of sample, mix, and incubate for 20 min at room temperature.
14. Wash with 10 volumes of PBS.
15. Centrifuge at $300\times g$ for 10 min at room temperature with brake set to low.
16. Carefully remove the supernatant with a pipette and resuspend the pellet in the same volume of PBS as in **step 12**.
17. Add the “Selection Cocktail” (from the EasySep Human Biotin Positive Selection Kit II) to the sample at a concentration of 100 $\mu\text{L}/\text{mL}$ of sample (do not vortex the cocktail before adding it to the tube) and mix and incubate for 15 min at room temperature.
18. Vortex the tube of “RapidSpheres” (from the EasySep Human Biotin Positive Selection Kit II) for 30 s.
19. Add the “RapidSpheres” to the sample at a concentration of 50 $\mu\text{L}/\text{mL}$ of sample, mix, and incubate for 10 min at room temperature.
20. Top up the tube to 10 mL with PBS and resuspend gently with a pipette.
21. Place the tube (without the cap) into the EasySep magnet and incubate for 5 min at room temperature.
22. After the incubation, keep the tube inside the magnet and carefully tilt the magnet to pour off the supernatant into a new 14 mL non-graduated polystyrene culture tube.
23. Place the new tube into the magnet.
24. Repeat **steps 21–23** four times to perform a total of five incubations in the magnet to ensure complete depletion. After the last incubation, pour the supernatant into a new tube.
25. Count the WBCs with the hematological cell counter.
26. Centrifuge the cell suspension at $500\times g$ for 5 min at room temperature.
27. Carefully remove the supernatant with a pipette.
28. Resuspend the cells at a concentration of ten million cells/mL in PBS.
29. Filter the suspension with a 70 μm sterile cell strainer into a new 14 mL non-graduated polystyrene culture tube.
30. Transfer one million cells into a FACS tube and keep the remaining cells on ice until transplantation.
31. Centrifuge the one million cells from **steps 11** and **30** for 5 min at $500\times g$ at room temperature.

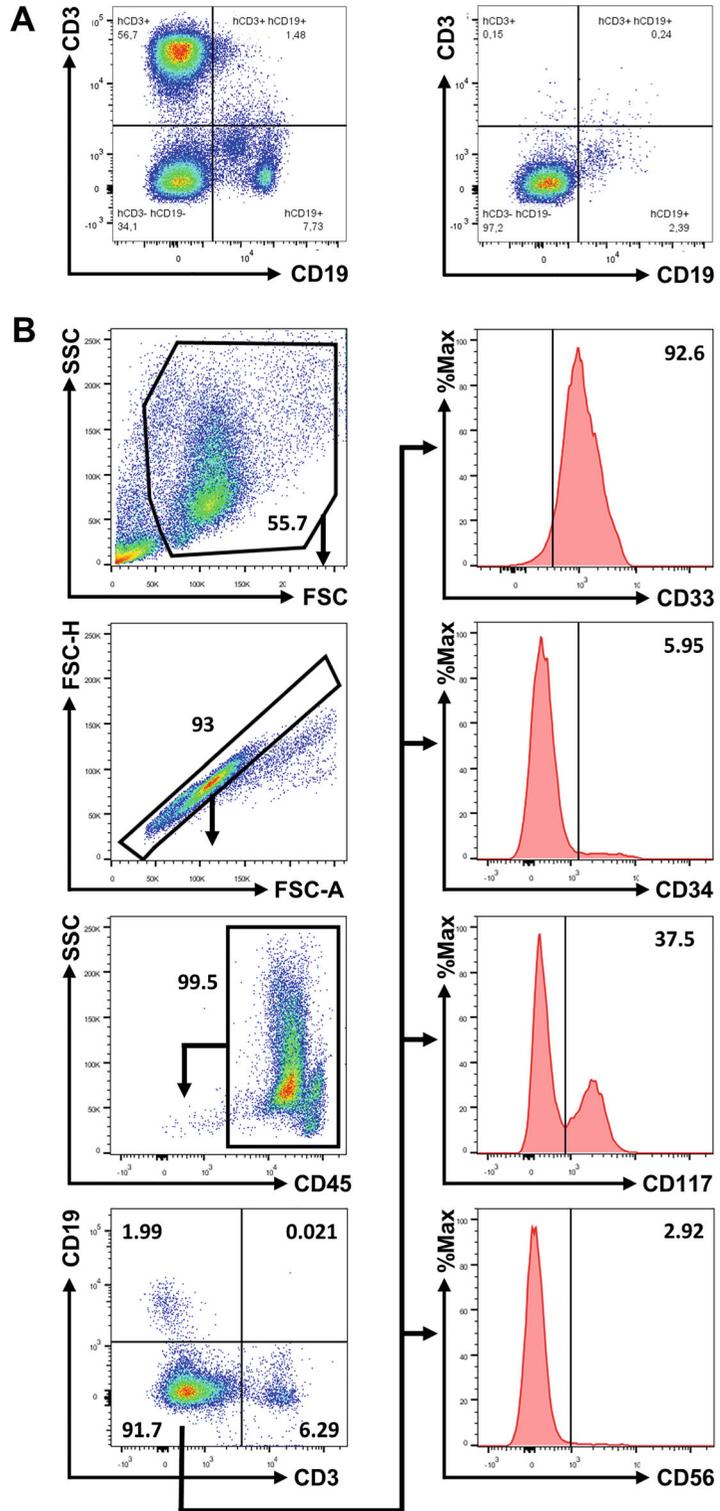


Fig. 1 (a) Depletion of T and B cells from primary AML samples with the EasySep Human Biotin Positive Selection Kit II. The plot on the left corresponds to the cells from Subheading 3.2, step 11 (before depletion), while the plot on the right

32. Discard the supernatant, resuspend the cells in 1 mL of flow cytometry staining buffer, and centrifuge for 5 min at $500\times g$ at room temperature.
33. Discard the supernatant and resuspend the cells in 50 μL of flow cytometry staining buffer.
34. Add 5 μL of Fc Blocker to each tube, vortex for 2 s, and incubate for 10 min at room temperature.
35. Add 9.5 μL of depletion assessment antibody mix to each tube, vortex for 2 s, and incubate for 20 min at 4°C (*see Note 7*).
36. Add 1 mL of PBS to each tube, vortex for 2 s, and centrifuge for 5 min at $500\times g$.
37. Resuspend the cells in 200 μL of PBS and analyze the T- and B-cell depletion efficacy, as well as the blast frequency in the sample to be transplanted into the mice (Fig. 1a, b).

3.3 PBMC Isolation from Healthy Volunteers

Here, it is assumed that PBMCs are injected simultaneously with AML cells. Alternatively, PBMCs can be transplanted upon observing that AML cells have reached the desired level of engraftment. This is especially relevant when using primary AML samples, which can take several weeks to reach detectable engraftment levels. Delaying the PBMC transplantation can prevent the development of lethal GVHD (occurring typically within 3–6 weeks posttransplantation [12]) before the engraftment of AML cells. Finally, lower numbers of PBMCs can be transplanted by sublethally irradiating the mice 24 h before transplantation (*see step 9*).

1. If working with buffy coats from healthy volunteers, transfer 17.5 mL of cell suspension into a 50 mL conical tube and add 17.5 mL of PBS, mix by inversion. Otherwise, proceed to **step 2**.
2. Transfer 15 mL of Ficoll–Paque into a 50 mL conical tube.
3. Carefully overlay 35 mL of peripheral blood or diluted buffy coat onto the Ficoll–Paque (*see Note 8*).
4. Centrifuge for 20 min at $500\times g$ at room temperature with no brake.
5. With a serological pipette, discard ~10 mL of plasma from the top layer and with a new pipette collect the PBMCs, located at the interface between the Ficoll–Paque and the plasma (thin white layer right under the plasma), into a new 50 mL conical tube.

←
Fig. 1 (continued) corresponds to the cells from Subheading 3.2, **step 30** (after the depletion). **(b)** Gating strategy for the analysis of the phenotype of AML blasts in primary samples by flow cytometry

6. Adjust the volume of PBMCs to 50 mL with PBS and centrifuge for 5 min at $500\times g$ at room temperature.
7. Discard the supernatant and resuspend the cells in 5 mL of PBS.
8. Count the cells with the hematological cell counter.
9. Adjust the WBC concentration to 200 million cells per mL. If mice have been irradiated with 2 Gy 24 h before the transplantation, PBMCs should be adjusted to 20 million per mL.
10. Filter the suspension with a 70 μm sterile cell strainer into a sterile FACS tube.
11. Keep the cells on ice until transplantation.

3.4 Transplantation of Cells into NSG Mice

NSG mice are typically transplanted between the ages of 8 and 11 weeks. As NSG mice are highly immunodeficient, supplement their drinking water with antibiotics to reduce their risk of developing infections during the experiment.

1. For intravenous transplantation, either dilute the AML cells twofold with PBS to reach a final concentration of five million cells per mL or combine the AML cells with PBMCs at a 1:1 ratio to reach a final concentration of five million AML cells per mL and 100 million PBMCs per mL.
2. For subcutaneous injections, dilute the AML cells twofold with Matrigel and mix well by pipetting up and down. Keep the cells on ice until injection.
3. For intravenous transplantations, inject 200 μL of suspension into the tail vein of each mouse (one million AML cells plus 20 million PBMCs/mouse, or one million AML cells plus two million PBMCs/mouse if mice have been irradiated) with a 26-gauge needle fitted to a syringe.
4. For subcutaneous transplantations, inject 200 μL of the AML cells–Matrigel suspension (one million AML cells per animal) into the flank of each mouse with an insulin syringe.
5. If subcutaneous AML transplantations are being performed, dilute the PBMCs twofold with PBS and inject 200 μL of suspension into the tail vein of each mouse (20 million PBMCs per mouse or two million if animals have been irradiated).
6. Once the AML cells and/or PBMCs have been transplanted, the animals might develop symptoms of AML and/or GVHD. These symptoms include abnormal weight change (increasing or decreasing), anemia (*see Note 9*), hunching, and reduced activity. Score the animals every 2–3 days for these symptoms (two grades of severity for each symptom, with a weight change of 10% = grade 1 and a weight change of 20% = grade 2).

Thoroughly follow the guidelines of your local ethics committee.

7. Sacrifice all animals reaching a score of 6 out of 8, having a tumor size of 1 cm³, or experiencing a weight change greater than 20%.

3.5 Monitoring AML Engraftment by Flow Cytometry

Starting from 7 days posttransplantation, the engraftment frequency of AML cells can be monitored in the peripheral blood of NSG mice to evaluate the GVL effects. A low engraftment (frequency of AML cells in peripheral blood) could reflect greater GVL effects. This procedure can also be used to monitor the evolution of the AML engraftment in mice that did not receive PBMCs simultaneously with AML cells.

1. Restrain the mouse using the restraint device with the tail protruding.
2. Disinfect the tail with an antiseptic solution.
3. Heat the tail with an infrared lamp for 2–3 min to increase blood flow in the tail and induce dilatation of the veins.
4. Puncture the tail vein with the needle of a syringe and collect the blood with a capillary (one capillary per mouse is sufficient).
5. Transfer the blood to a Microtainer tube and return the mouse to its cage.
6. Count the WBCs with the hematological counter.
7. Transfer the remaining blood (max 50 µL) into a FACS tube and add 1 mL of 1X red blood cell lysis buffer, vortex for 2 s, and incubate for 5 min at room temperature.
8. Add 1 mL of PBS to each tube, vortex for 2 s, and centrifuge for 5 min at 500× *g* at room temperature.
9. Discard the supernatant, add 1 mL of flow cytometry staining buffer, vortex for 2 s, and centrifuge for 5 min at 500× *g* at room temperature.
10. Discard the supernatant, resuspend the cells in 50 µL of staining buffer, add 5 µL of Fc Blocker to each tube, vortex for 2 s, and incubate for 10 min at room temperature.
11. Add 1 µL each of anti-CD45 and anti-CD33 antibody, vortex for 2 s, and incubate for 20 min at 4 °C (*see Note 3*).
12. Add 1 mL of PBS to each tube, vortex for 2 s, and centrifuge for 5 min at 500× *g* at room temperature.
13. Discard the supernatant and resuspend the cells in 200 µL of PBS.
14. Keep the cells at 4 °C for up to 24 h until analysis by flow cytometry (Fig. 2).

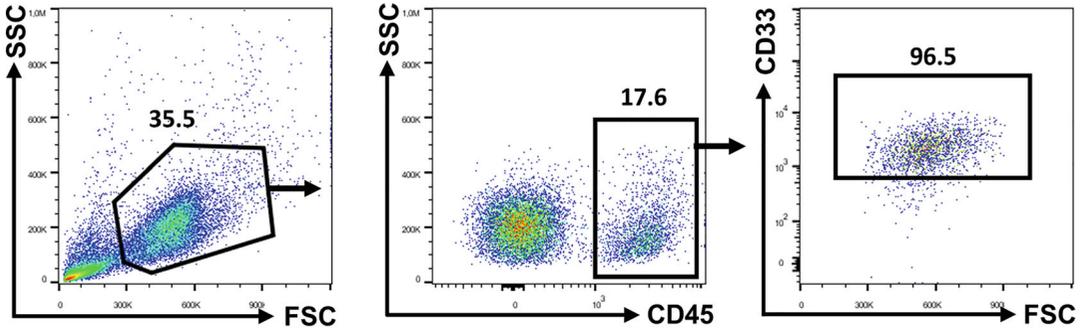


Fig. 2 Gating strategy for the analysis of AML cells (defined as human CD45⁺CD33⁺ cells) in the blood of NSG mice transplanted intravenously with AML cells

15. Based on the frequency of CD33⁺ cells among CD45⁺ cells (WBCs), and the concentration of WBCs obtained with the hematological counter, compute the concentration of CD33⁺ cells in the blood of the animals (*see Note 10*).

3.6 Monitoring AML Engraftment by Bioluminescence Imaging

If luciferase-expressing AML cells have been transplanted into mice, *in vivo* bioluminescence imaging can be used to monitor the engraftment of AML cells. The advantage of this technique is that all AML cells (not only those in circulation and/or expressing CD33) can be detected.

1. Reconstitute D-luciferin in PBS to a concentration of 15 mg/mL and homogenize by vortexing for 30 s (*see Note 11*).
2. Initialize the IVIS Spectrum Imaging system following the specific instructions of the device.
3. Saturate the inhalation chamber with isoflurane.
4. Subcutaneously inject 200 μ L of D-luciferin solution using an insulin syringe into the nape of a maximum of five mice at a time.
5. Return the mice to their cages and wait for 8 min (*see Note 12*).
6. Place the mice into the inhalation chamber until all animals are asleep. Then, place the mice into the chamber of the IVIS Spectrum Imaging system with isoflurane masks, keeping them under anesthesia.
7. Capture an initial image with the acquisition time set at 1 min, then adjust the acquisition time accordingly: Decrease if the signal is oversaturated or increase if the signal is absent.
8. Image analysis can be performed with Living Image software. The light intensity for each tumor or across the whole body of the animal (if intravenous transplantation was performed) is proportional to the engraftment of AML cells (Fig. 3).

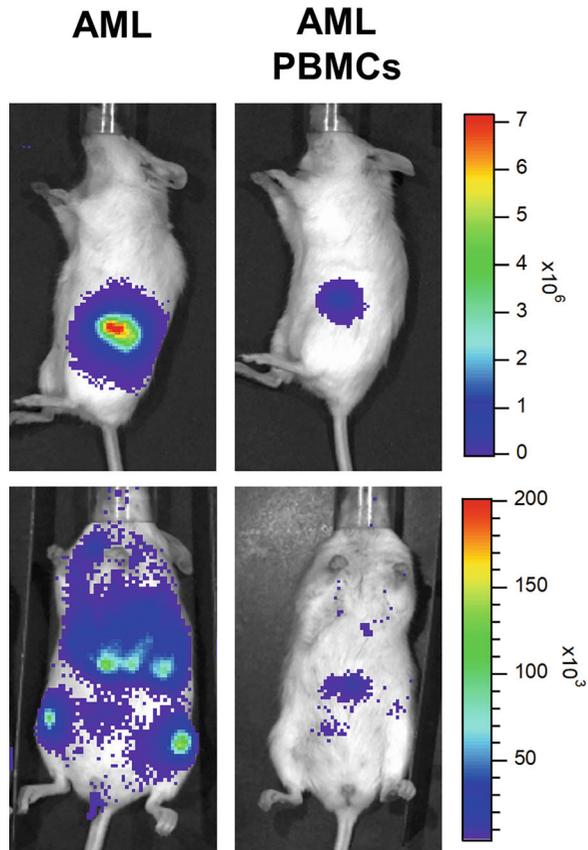


Fig. 3 NSG mice were transplanted with AML cells (THP-1-luc) either subcutaneously in Matrigel (top) or intravenously (bottom). Mice received PBMCs (right) or not (left). Bioluminescence imaging (photons/sec) shows that the presence of PBMCs reduces the growth of AML cells (GVL effects)

3.7 Monitoring Subcutaneous Tumor Size with Calipers

If AML cells (either expressing luciferase or not) are transplanted subcutaneously, tumor size can be monitored with calipers.

1. Securely hold the tail of the mouse between your outermost and middle fingers, grasp the loose skin at the nape between your forefinger and thumb, and align the animal in your hand to expose the tumor.
2. With a caliper, measure the length (L) and width (W) of the tumor.
3. Assuming that the tumor is an ellipsoid, use the following formula to compute its volume: $V_T = 0.5 \times L \times W^2$.

4 Notes

1. OCI-AML3-luc can be obtained from Ubigen (YC-C022-Luc-P). Conventional OCI-AML3 cells can be obtained from multiple biobanks but those from DSMZ (ACC 582) were shown to present less genetic drift from the original cell line compared to other biobanks [26].
2. Store the stock red blood cell lysis buffer at 4 °C and protect it from light. Pre-warm the buffer to room temperature before using it to lyse red blood cells.
3. Volumes are for commercial antibodies available from various providers. Researchers should determine the optimal volume for each respective antibody prior to using.
4. Matrigel is preserved at –20 °C but needs to be thawed at 4 °C for 24 h before transplantation. Keep thawed Matrigel on ice.
5. If necessary, and if enough blood or bone marrow aspirate is available, prepare as many 50 mL tubes as needed. A peripheral blood volume of 50 mL from an AML patient with active disease is typically sufficient to transplant 10 mice.
6. If a significant number of red blood cells remain visible after the initial lysis, a second lysis can be performed.
7. Alternatively, the cells can be incubated overnight at 4 °C to maximize the staining efficacy [27].
8. When overlaying the diluted blood sample, do not mix the Ficoll–Paque with the sample.
9. Anemia in NSG mice can be evaluated by monitoring the color of their ears. A pink color is considered normal, while a completely white color is considered a sign of severe anemia (grade 2). An intermediate color is considered light anemia (grade 1).
10. Since normal granulocytes and monocytes do not engraft in NSG mice, the frequency of CD33⁺ cells can be used as a proxy of the engraftment of AML cells.
11. The D-luciferin solution can be aliquoted and kept at –80 °C.
12. In our hands, 8 min is the time needed for D-luciferin to circulate properly in the animal and reach all AML cells transplanted.

Acknowledgments

This work was supported by the FNRS Belgium, the Leon Fredericq Foundation, and the ME-TO-YOU Foundation. CF and BES are research fellows at the FNRS. GE and FB are research associates at the FNRS.

References

1. Craddock C, Tauro S, Moss P, Grimwade D (2005) Biology and management of relapsed acute myeloid leukaemia. *Br J Haematol* 129(1):18–34. <https://doi.org/10.1111/j.1365-2141.2004.05318.x>
2. Baron F, Storb R (2004) Allogeneic hematopoietic cell transplantation as treatment for hematological malignancies: a review. *Springer Semin Immunopathol* 26(1–2):71–94
3. Döhner H, Wei AH, Appelbaum FR, Craddock C, DiNardo CD, Dombret H, Ebert BL, Fenaux P, Godley LA, Hasserjian RP, Larson RA, Levine RL, Miyazaki Y, Niederwieser D, Ossenkoppele G, Röllig C, Sierra J, Stein EM, Tallman MS, Tien HF, Wang J, Wierzbowska A, Löwenberg B (2022) Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. *Blood* 140(12):1345–1377. <https://doi.org/10.1182/blood.2022016867>
4. Fontaine P, Roy-Proulx G, Knafo L, Baron C, Roy DC, Perreault C (2001) Adoptive transfer of minor histocompatibility antigen-specific T lymphocytes eradicates leukemia cells without causing graft-versus-host disease. *Nat Med* 7(7):789–794. <https://doi.org/10.1038/89907>
5. Zeiser R, Socie G, Blazar BR (2016) Pathogenesis of acute graft-versus-host disease: from intestinal microbiota alterations to donor T cell activation. *Br J Haematol* 175(2):191–207. <https://doi.org/10.1111/bjh.14295>
6. Servais S, Beguin Y, Delens L, Ehx G, Fransolet G, Hannon M, Willems E, Humblet-Baron S, Belle L, Baron F (2016) Novel approaches for preventing acute graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. *Expert Opin Investig Drugs* 25(8):957–972. <https://doi.org/10.1080/13543784.2016.1182498>
7. Grossmann V, Schnittger S, Kohlmann A, Eder C, Roller A, Dicker F, Schmid C, Wendtner C-M, Staib P, Serve H (2012) A novel hierarchical prognostic model of AML solely based on molecular mutations. *Blood* 120(15):2963–2972
8. Rose D, Haferlach T, Schnittger S, Perglerová K, Kern W, Haferlach C (2017) Subtype-specific patterns of molecular mutations in acute myeloid leukemia. *Leukemia* 31(1):11–17. <https://doi.org/10.1038/leu.2016.163>
9. Papaemmanuil E, Döhner H, Campbell PJ (2016) Genomic classification in acute myeloid leukemia. *N Engl J Med* 375(9):900–901. <https://doi.org/10.1056/NEJMc1608739>
10. Fisher JN, Kalleda N, Stavropoulou V, Schwaller J (2019) The impact of the cellular origin in acute myeloid leukemia: learning from mouse models. *Hema* 3(1):e152. <https://doi.org/10.1097/hs9.0000000000000152>
11. Courtois J, Ritacco C, Dubois S, Canti L, Vandenhove B, Seidel L, Daulne C, Caers J, Servais S, Beguin Y, Ehx G, Baron F (2021) Itacitinib prevents xenogeneic GVHD in humanized mice. *Bone Marrow Transplant* 56(11):2672–2681. <https://doi.org/10.1038/s41409-021-01363-1>
12. Ehx G, Somja J, Warnatz HJ, Ritacco C, Hannon M, Delens L, Fransolet G, Delvenne P, Muller J, Beguin Y, Lehrach H, Belle L, Humblet-Baron S, Baron F (2018) Xenogeneic graft-versus-host disease in humanized NSG and NSG-HLA-A2/HHD mice. *Front Immunol* 9:1943. <https://doi.org/10.3389/fimmu.2018.01943>
13. Hannon M, Lechanteur C, Lucas S, Somja J, Seidel L, Belle L, Bruck F, Baudoux E, Giet O, Chantillon AM, Delvenne P, Drion P, Beguin Y, Humblet-Baron S, Baron F (2014) Infusion of clinical-grade enriched regulatory T cells delays experimental xenogeneic graft-versus-host disease. *Transfusion* 54(2):353–363. <https://doi.org/10.1111/trf.12279>
14. Grégoire C, Ritacco C, Hannon M, Seidel L, Delens L, Belle L, Dubois S, Vériter S, Lechanteur C, Briquet A, Servais S, Ehx G, Beguin Y, Baron F (2019) Comparison of mesenchymal stromal cells from different origins for the treatment of graft-vs.-host-disease in a humanized mouse model. *Front Immunol* 10:619. <https://doi.org/10.3389/fimmu.2019.00619>
15. Delens L, Ehx G, Somja J, Vrancken L, Belle L, Seidel L, Grégoire C, Fransolet G, Ritacco C, Hannon M, Dubois S, Beguin Y, Baron F, Servais S (2019) In vitro Th17-polarized human CD4(+) T cells exacerbate xenogeneic graft-versus-host disease. *Biol Blood Marrow Transplant* 25(2):204–215. <https://doi.org/10.1016/j.bbmt.2018.10.007>
16. Ehx G, Ritacco C, Hannon M, Dubois S, Delens L, Willems E, Servais S, Drion P, Beguin Y, Baron F (2021) Comprehensive analysis of the immunomodulatory effects of

- rapamycin on human T cells in graft-versus-host disease prophylaxis. *Am J Transplant* 21(8):2662–2674. <https://doi.org/10.1111/ajt.16505>
17. Ehx G, Fransolet G, de Leval L, D'Hondt S, Lucas S, Hannon M, Delens L, Dubois S, Drion P, Beguin Y, Humblet-Baron S, Baron F (2017) Azacytidine prevents experimental xenogeneic graft-versus-host disease without abrogating graft-versus-leukemia effects. *Oncotargets Ther* 6(5):e1314425. <https://doi.org/10.1080/2162402x.2017.1314425>
 18. Ritacco C, Köse MC, Courtois J, Canti L, Beguin C, Dubois S, Vandenhove B, Servais S, Caers J, Beguin Y, Ehx G, Baron F (2023) Post-transplant cyclophosphamide prevents xenogeneic graft-versus-host disease while depleting proliferating regulatory T cells. *iScience* 26(3):106085. <https://doi.org/10.1016/j.isci.2023.106085>
 19. von Bonin M, Wermke M, Cosgun KN, Thiede C, Bornhauser M, Wagemaker G, Waszkow C (2013) In vivo expansion of co-transplanted T cells impacts on tumor re-initiating activity of human acute myeloid leukemia in NSG mice. *PLoS One* 8(4):e60680. <https://doi.org/10.1371/journal.pone.0060680>
 20. Brehm MA, Kenney LL, Wiles MV, Low BE, Tisch RM, Burzenski L, Mueller C, Greiner DL, Shultz LD (2019) Lack of acute xenogeneic graft-versus-host disease, but retention of T-cell function following engraftment of human peripheral blood mononuclear cells in NSG mice deficient in MHC class I and II expression. *FASEB J* 33(3):3137–3151. <https://doi.org/10.1096/fj.201800636R>
 21. Paczulla AM, Dirnhofer S, Konantz M, Medinger M, Salih HR, Rothfelder K, Tsakiris DA, Passweg JR, Lundberg P, Lengerke C (2017) Long-term observation reveals high-frequency engraftment of human acute myeloid leukemia in immunodeficient mice. *Haematologica* 102(5):854–864. <https://doi.org/10.3324/haematol.2016.153528>
 22. Wunderlich M, Chou FS, Link KA, Mizukawa B, Perry RL, Carroll M, Mulloy JC (2010) AML xenograft efficiency is significantly improved in NOD/SCID-IL2RG mice constitutively expressing human SCF, GM-CSF and IL-3. *Leukemia* 24(10):1785–1788. <https://doi.org/10.1038/leu.2010.158>
 23. Lopez-Millan B, Diaz de la Guardia R, Roca-Ho H, Anguita E, Islam A, Romero-Moya D, Prieto C, Gutierrez-Agüera F, Bejarano-Garcia JA, Perez-Simon JA, Costales P, Rovira M, Marín P, Menendez S, Iglesias M, Fuster JL, Urbano-Ispizua A, Anjos-Afonso F, Bueno C, Menendez P (2018) IMiDs mobilize acute myeloid leukemia blasts to peripheral blood through downregulation of CXCR4 but fail to potentiate AraC/Idarubicin activity in pre-clinical models of non del5q/5q- AML. *Oncotargets Ther* 7(9):e1477460. <https://doi.org/10.1080/2162402x.2018.1477460>
 24. Diaz de la Guardia R, Velasco-Hernandez T, Gutiérrez-Agüera F, Roca-Ho H, Molina O, Nombela-Arrieta C, Bataller A, Fuster JL, Anguita E, Vives S, Zamora L, Nomdedeu J, Gómez-Casares MT, Ramírez-Orellana M, Lapillonne H, Ramos-Mejia V, Rodríguez-Manzanecque JC, Bueno C, Lopez-Millan B, Menéndez P (2021) Engraftment characterization of risk-stratified AML in NSGS mice. *Blood Adv* 5(23):4842–4854. <https://doi.org/10.1182/bloodadvances.2020003958>
 25. Vercauteren S, Zapf R, Sutherland H (2007) Primitive AML progenitors from most CD34(+) patients lack CD33 expression but progenitors from many CD34(-) AML patients express CD33. *Cytherapy* 9(2):194–204. <https://doi.org/10.1080/14653240601164042>
 26. Noronha N, Ehx G, Meunier MC, Laverdure JP, Thériault C, Perreault C (2020) Major multilevel molecular divergence between THP-1 cells from different biorepositories. *Int J Cancer* 147(7):2000–2006. <https://doi.org/10.1002/ijc.32967>
 27. Whyte CE, Tumes DJ, Liston A, Burton OT (2023) Correction: do more with less: improving high parameter cytometry through overnight staining. *Curr Protoc* 3(1):e678. <https://doi.org/10.1002/cpz1.678>

INDEX

A

Abatacept 9
 Acute myeloid leukemia (AML)..... 359–373
 Adenosine 5'-triphosphate (ATP).....74, 80
 Adhesion molecules75, 80
 Alemtuzumab 8, 94, 96
 Algorithm probability 79
 Alloantigens 129, 142, 287, 288, 300, 316
 Allogeneic haematopoietic stem cell transplantation (allo-HCT, allo-HSCT, allo-SCT, allogeneic HCT, allogeneic HSCT, alloHSCT or alloSCT)..... 1, 5, 14–18, 57, 58, 66, 71–73, 76, 77, 79, 85–87, 91–122, 128, 129, 141, 143, 161–181, 237–239, 259–261, 315–318, 322, 333, 334, 360
 Alloreactivity..... 6
 Ammonium, chloride, potassium lysis buffer (ACK lysis buffer) 155–156, 318, 323, 338, 351
 Anakinra..... 6
 Anemia.....59, 65, 66, 95, 98, 108, 122, 369, 373
 Anesthesia 136, 137, 211–213, 220–222, 227, 231–233, 241, 246, 264, 271, 318, 323, 331, 336–337, 345–348, 354, 356, 371
 Angiopoietin-2 (ANG-2) 75, 80, 86, 87
 Animal models.....57–67, 162
 Animal welfare174, 176, 246, 271
 Annexin V241, 242, 245, 249, 250
 Antibiotics 8, 133, 134, 157, 213, 225, 226, 235, 241, 246, 264, 271, 363, 369
 Antibody 17, 58, 142, 166, 187, 212, 241, 261, 289, 301, 320, 338, 362
 Antibody panels..... 173, 174, 180, 245, 248, 249, 251, 254, 255, 263, 265, 266
 Anticoagulant216, 222, 308, 321, 327
 Antigen presentation..... 2
 Antigen presenting cells (APCs)2, 6, 7, 58, 63, 142, 166, 188, 189, 238, 242, 243, 248, 249, 288, 300, 311, 316, 339
 Anti-metabolite 6
 Antiseptic213, 227, 363, 370
 Anti-thymocyte antibody..... 6
 Anti-thymocyte globulin (ATG) 6, 94–96, 114, 115, 213

Apheresis..... 16, 59, 62, 211, 220–222, 230, 233
 Area under the curve (AUC)73, 74, 76–79
 ARRIVE guidelines 201, 353

B

Barcode 261, 266, 273, 274, 276–278, 283
 Barcoding 261–263, 265–266, 273–274, 280
 Basiliximab..... 8
 B cells 2, 6, 9, 19, 58, 59, 61, 63–66, 76, 77, 80, 87, 162, 184, 196, 198, 233, 254, 277, 279, 334, 360, 361, 365, 367, 368
 Bedding 163, 172, 176, 178–180, 185, 189, 201, 335
 Belumosudil.....5, 9, 17, 19
 Bioluminescence.....321, 329–330, 337
 Bioluminescence imaging 321, 329, 330, 348, 350, 364, 371, 372
 Biomarkers..... 17, 58, 72–75, 77–80, 87, 129
 Biopsy 4, 75, 110, 210, 227, 229, 233
 Blood 15, 57, 128, 142, 161, 184, 208, 237, 259, 288, 299, 319, 334, 361
 Body temperature211, 232, 234, 354
 Bone128, 132, 134, 137, 148, 150, 169, 170, 179, 220, 244, 269
 Bone marrow (BM) 4, 16, 59, 64, 91, 95, 105, 128–130, 132–137, 142, 143, 146–148, 150–158, 162, 167, 169–171, 173, 178–180, 210, 212, 216, 220–225, 229, 232, 233, 244, 245, 253, 255, 269, 270, 316, 322, 331, 335, 360–362, 365, 373
 Bortezomib 9
 Bovine 145, 156, 166, 240, 248, 264, 281, 289, 301, 310, 318, 319, 338, 362
 Brain.....57, 87
 Brentuximab 6
 Bronchiolitis obliterans syndrome (BOS) 6, 9, 19

C

Cages 134, 153, 163, 165, 166,
 168, 170, 172, 174, 176, 178–180, 185, 189,
 191–193, 201, 203, 212, 224, 232, 246, 271,
 318, 320, 330, 335, 342–348, 355, 356, 370, 371

Calcineurin inhibitor (CNI) 100, 102,
 105, 111, 112, 114, 115

Calipers 364, 372

Canine 6, 142, 207–235

Carboxyfluorescein diacetate succinimidyl ester
 (CFSE) 289, 293, 294, 296, 297,
 302, 306–307, 309–312

Cardiac puncture 194, 203, 337, 349, 356

CD33 339, 361, 371, 373

CD34 60, 63, 64, 212,
 220, 222, 232, 233

Cell line-derived xenotransplantations (CDX) 360

Cell lines

- K562-*luc* cells 321, 326–330,
 334–336, 341–344, 348, 350, 352
- OCI-AML3 cells 373
- OCI-AML3-*luc* cells 373
- P815 GFP⁺ cells 318–319
- THP-1 cells 62
- THP-1-*luc* cells 372

Cell stimulation 240–241, 247,
 253, 254, 265, 272

Chemotherapy 17, 72, 95, 96, 101,
 106, 114, 115, 118, 162, 164, 166–168, 260,
 315, 333, 359, 360

Chimerism 18, 94, 96, 129,
 131, 173–175, 180, 215, 216, 220, 223,
 225–227, 229, 247–249, 254, 255, 281

Chronic myeloid leukemia (CML) 128, 316, 321,
 333–357

Ciprofloxacin 138, 320, 331

Cisplatin 108, 119, 265,
 272–273, 276, 283

Clinical scores 138, 154, 157–159,
 173, 179, 192, 203, 323, 345

Clustering analysis 276–278, 280–281

Compensation 166, 174, 181,
 246, 250, 338, 357

Complement 8, 146, 151, 156, 157

Complications

- cardiac and vascular 100, 115
- dermatologic 113
- endocrine 106
- endothelial 85–87
- gastrointestinal complications 103, 104
- late 92–94, 96, 98, 102,
 104–106, 110, 112–114, 116, 122
- muscle and connective tissue 110
- neurological and cognitive 114

- ocular 97
- oral and dental 98
- renal and urinary 105
- respiratory 99
- skeletal 111, 112
- transplant 92

Conditioning

- myeloablative conditioning (MAC) 17, 108,
 112, 129, 131, 162, 176, 180
- reduced intensity conditioning (RIC) 17,
 119, 142, 161–181

Corticosteroids 5, 72, 107,
 110–112, 115–117

Cow 65, 66

Creatinine 75, 80, 86, 105

Cyclophosphamide 17, 105, 107,
 162, 164, 167, 168, 172, 176, 179, 238, 260

Cytokeratin (CK) 78

Cytokine receptor 76–77

Cytokines 6–8, 58, 66, 74,
 76–77, 80, 142, 184, 187, 202, 205, 238, 253,
 254, 260, 262, 289, 295, 300, 304, 308, 312, 334

D

Damage-associated molecular patterns
 (DAMPs) 3, 8, 13, 73, 80

Daclizumab 8

Dendritic cells (DCs)

- conventional (cDC) 300, 311
- lymphoid 299
- monocyte-derived 287–297
- myeloid 2, 196
- plasmacytoid 299–312

Density gradient centrifugation 202, 310,
 353, 354

4',6-Diamidino-2-phenylindole (DAPI) 166,
 174, 319, 325, 331

Diarrhea 59, 60, 65, 78,
 172, 174, 179, 180, 228, 234, 238, 250, 283, 323

Diphenhydramine 212, 224

DLA-88 209, 215–217, 219, 226

D-Luciferin 321, 329–331,
 336, 337, 347, 350, 364, 371, 373

DNA 103, 104, 120, 121, 209,
 210, 213, 214, 216–219, 225, 226, 230

Dog 15, 64–66, 207, 212,
 215, 220–227, 229, 231–233, 235

DRB1 210, 215, 217, 219, 220, 226

E

Ear 66, 119, 147, 163, 166, 177,
 184, 194, 227, 229, 349

Elafin 78–80

- Endothelial activation and stress index (EASIX)..... 75, 80, 85–87
- Endothelial dysfunction..... 75, 80, 86
- Engraftment 4, 6, 16, 19, 58, 60–66, 128, 157, 161, 162, 166, 173–175, 178, 188, 196, 204, 220, 221, 223, 225, 238, 320, 327–329, 331, 334, 339, 352, 357, 360, 361, 363, 364, 366, 370, 371, 373
- Enrofloxacin 157, 224, 363
- Entospletinib 6
- Enzyme-linked immunosorbent assay (ELISA)..... 233, 342
- Etanercept..... 6
- Ethics 134, 137, 138, 147, 153, 176, 192, 200–203, 321, 329, 347, 353, 354, 364, 370
- European Society for Blood and Marrow Transplantation 6, 14
- Euthanasia 132–134, 144, 159, 176, 179, 180, 186, 192, 201, 203, 215, 234, 337, 347–349, 353, 354
- Everolimus 7
- Extracorporeal phototherapy..... 7
- Eye 57, 60, 97, 174, 179, 227, 229, 336, 347, 354
- F**
- Femur..... 128, 134, 135, 148, 156, 169, 170, 244, 269, 322
- Fertility 92, 106, 108
- Fibrinogen 74, 80
- Fibrosis..... 2, 58–60, 62, 142, 229, 230
- Ficoll-Hypaque 302
- Ficoll-Paque 319, 325
- Fingolimod 6
- Fixation 232, 247, 252, 254, 255, 261, 262, 266, 273–275, 284
- Flow cytometer 144, 163, 166, 174, 187–189, 196, 200, 202, 204, 212, 243, 246, 261, 289, 301, 319–321, 324, 338, 339, 352, 357, 363, 364
- Flow cytometry 146, 149, 150, 152, 187, 188, 192, 196, 197, 199, 200, 202, 214, 222, 227, 232–234, 237–248, 261, 263, 276, 281, 283, 291–294, 296, 297, 300, 303, 305–308, 311, 312, 318–321, 323–324, 327–330, 338, 342, 351, 361–365, 368, 370
- FlowJo 166, 175, 187, 204, 241, 250, 263, 268, 276–278, 330, 331, 357
- FlowSOM 263, 281, 282
- Fludarabine 17, 162, 164, 167, 168, 172, 176, 179
- Fluticasone propionate 240, 260, 264
- Food and Drug Administration (FDA) 5, 9, 19
- Formalin 186, 194, 203, 214, 215, 227, 229, 232, 337, 349, 356
- Fostamatinib 6
- Foxp3 238, 241, 242, 245, 248, 249, 251, 254, 260, 262, 268, 279–284
- Fur 59, 60, 65, 137, 147, 154, 155, 158, 172, 174, 179, 180, 184, 192, 193, 203, 221, 250, 283, 345–347
- G**
- Gastrointestinal tract 57, 62, 63, 128, 142, 155, 183
- Glucocorticoids 100, 111, 239, 260, 270
- Graft acceptance 134
- Graft conditioning 237–248, 250–252, 259–284
- Graft rejection 9
- Graft-versus-host disease (GVHD)
- acute (aGVHD)..... 2–5, 16, 17, 19, 58, 61, 64, 67, 72, 74–78, 85–87, 129, 131, 143, 207, 208, 223, 225, 238, 239, 246, 250, 271
- allogeneic 318–319
- bone marrow 4, 180
- chronic (cGVHD)..... 2–5, 16–19, 77, 92, 93, 97–99, 103, 110, 113, 119, 122
- cutaneous 4, 64, 143, 158
- gastrointestinal 57, 62
- gut 3, 184
- human 2, 15, 17, 20, 61, 142
- hyperacute 4
- liver 59, 65, 78, 214, 223, 227
- lung 59, 60, 74
- neurological 4
- pulmonary 4
- renal 4
- skin 59, 63, 64, 78–80, 121, 214, 227
- steroid refractory (SR-GVHD) 5, 6, 17, 85, 86
- thymic 4
- xenogeneic 57–67, 319–321, 325–330, 333–357, 359–373
- Graft-versus-leukemia (GVL) 57, 58, 62, 63, 237–239, 316–319, 321–325, 329–330, 333, 334, 342, 359–373
- Graft-versus-tumour (GVT)..... 1, 5–9, 14, 15, 17, 161, 162, 237, 238, 260
- Granulocyte colony stimulating factor (G-CSF)..... 212, 220, 222, 224, 230, 233
- Granulocyte-macrophage colony stimulating factor (GM-CSF) 288, 289, 291, 292, 295, 296
- Green fluorescent protein (GFP) 243, 245, 248, 249, 251, 281, 283, 318–319, 322–325
- Gut 3, 4, 176, 184, 207, 234, 238, 260

H

Harvesting184, 233, 262, 263
 Hazard ratio (HR) 73, 75–78
 Heart87, 101, 102, 193,
 194, 229, 231, 246, 250, 349
 Heat shock proteins (HSPs)74, 80
 Hematologic malignancies 141
 Hemocytometer 132, 135, 137,
 144, 149, 165, 171, 176, 185–187, 190, 195,
 196, 232, 240, 254, 264, 265, 272, 318, 320,
 322, 324, 326, 335, 336, 338, 341, 342, 351
 Hematopoiesis 17, 95, 127, 220, 359, 360
 Hematopoietic stem cells (HSCs)57, 58, 60,
 63, 91–122, 127, 161–181, 211, 220, 221, 238
 Hematoxylin and eosin203, 214,
 227, 229, 356
 Hemolytic uremic syndrome 86
 Hepatocyte growth factor (HGF)78–80
 High mobility group box 1 (HMGB1)74, 80
 Histology 157, 194, 213,
 214, 226, 227, 342, 349
 Histopaque 265, 271, 283
 H-2Kb 267, 277–279, 282, 284
 H-2Kd 267, 277–279, 284
 Horse 58, 65–67
 Housing 153, 157, 174, 185, 335, 342
 Human leukocyte antigen (HLA) 2, 15, 72,
 96, 99, 102, 105, 110, 113, 161, 316, 360
 Human studies 6, 17, 19
 Husbandry185, 189, 335, 342
 Hypogammaglobulinemia 96

I

Ibrutinib5, 9, 17, 19
 Immunophenotyping 157, 239, 248,
 259–284, 338
 Immunosuppression 14, 114, 119,
 121, 128, 129, 142, 208, 223, 316
 Immunosuppressive therapy72, 99
 Infections2, 6, 7, 9, 78, 93, 95, 96,
 98, 99, 103, 105, 115, 128, 134, 141, 142, 155,
 157, 223, 235, 246, 271, 296, 299, 300, 353, 369
 Inflammation6, 8, 58, 63, 75, 142, 260
 Infliximab 6
 Infusions 14, 76, 113, 129, 130,
 153, 155, 157, 210, 212, 220–225, 233, 239,
 317, 318, 320–323, 326–329, 360, 361
 Injections 58, 61–64, 66,
 67, 137, 153, 157, 167, 168, 170, 172, 177–179,
 184, 185, 189, 191, 192, 201, 203, 210, 241,
 246, 264, 270, 271, 295, 321, 322, 327–329,
 334, 336, 341–344, 347, 348, 353–356, 361,
 365, 369

Integrin 8
 Interferon (IFN)268
 Interleukin (IL) 61, 74, 87, 142,
 239, 267, 288, 300, 320, 361
 Interleukin-2 (IL-2) 6–8, 61–63, 79, 142, 268
 Interleukin-3 (IL-3)302, 304, 309, 361
 Interleukin-4 (IL-4)242, 248, 254,
 268, 288, 289, 291, 292, 295, 296
 Interleukin-17 (IL-17) 254
 Intracellular staining 247, 254, 255,
 262, 266, 268, 274, 275, 284
 In vivo imaging system (IVIS) 334, 364, 371
 Irradiation7, 61–63, 66,
 129–131, 133, 134, 137, 147, 155, 162, 168,
 176, 178, 179, 212, 224, 241, 246, 261,
 264–265, 270, 320, 331
 Irradiator 134, 144, 165, 168,
 176, 179, 246, 270
 Isoflurane 211, 231, 241, 246,
 264, 271, 321, 331, 336, 347, 354, 364, 371
 Isolation of
 blood cells 184–185, 290, 335
 bone marrow 152, 155
 cells 302–306
 leukocytes 338
 liver cells 338
 lung cells 338
 spleen cells 134, 244, 245,
 255, 262, 269, 270, 272
 splenocytes 129, 150
 IVIS Spectrum CT 334, 337,
 342, 345, 347, 350, 354, 356

J

Janus kinase (JAK) 17

K

Kaplan-Meier method 72

L

Lactate dehydrogenase (LDH) 75, 80, 86, 87
 Leukapheresis 222, 233
 Leukemia128, 333–357
 Leukocytes 7, 57, 58, 62,
 63, 65, 66, 75, 96, 99, 102, 105, 110, 113, 156,
 157, 184, 186, 187, 193, 195, 196, 198–200,
 204, 207, 208, 215, 216, 227, 233, 287–297,
 299–312, 318, 322, 329, 334, 338, 339, 350,
 352, 357
 Lineage distribution analysis277–280, 284
 Live/dead cell stain 196
 Liver2, 4, 57, 59, 60, 62, 64–66,
 72, 78, 80, 87, 93, 94, 103, 104, 142, 183, 184,

- 186–189, 194, 195, 207, 223, 225, 227, 229,
234, 238, 260, 338, 349, 350, 352, 357
- Log-rank test73, 79
- Long-term effect 354
- Long-term follow-up (LTFU) 93, 162
- Luciferase334, 362, 364, 372
- Lungs 57, 59–61, 65,
72, 74, 87, 92, 99, 183, 184, 186–188, 193–195,
203, 207, 229, 338, 349, 350, 357
- Lymph nodes..... 16, 118, 155,
210, 213, 216, 223, 225–227, 233, 318, 323,
324, 331
- Lymphocytes 7, 14, 64, 71,
113, 207, 208, 211, 220, 221, 223, 225, 251,
294, 317
- Lymphoma 63, 238
- M**
- Macrophages58, 59, 61, 64, 74,
196, 200, 279, 280, 292
- Magnet..... 144, 150, 151,
301, 304, 311, 362, 366
- Magnetic beads 150–151, 156, 289
- Magnetic bead separation 156
- Magnetic cell sorting (MACS)..... 144, 150,
156, 289–293, 295, 301, 303–306
- Major histocompatibility complex (MHC) 15,
61–64, 128, 162, 184, 189, 208, 215–217, 225,
259–284, 288, 300, 316, 360, 361
- Mannan.....74, 80
- Maraviroc..... 6
- Mass cytometer 261
- Mass cytometry 259–284
- Mesenchymal stromal cells (MSCs) 9
- Microbiome 3, 8, 153
- Microbiota..... 8
- MicroRNA (miRNA)..... 76
- Microscope132, 136, 185–187,
190, 195, 196, 214, 227, 240, 243, 264, 265,
269, 289, 291, 292, 296, 297, 301, 318–320,
324, 325, 335, 338, 341, 342, 351
- Microscopy 87, 292, 303,
308, 312, 319, 323–325
- Microtome 214, 227
- Minor histocompatibility antigen (MiHA)..... 15,
130, 131, 141–159, 162, 317
- Mixed leukocyte reaction (MLR)..... 287–297,
299–312
- Mobilisation agent 16
- Mobilising agent 212, 220
- Mocravimod 6, 9
- Monoclonal antibodies 114, 166,
187–189, 197, 199, 202, 204, 212, 222, 227,
229, 232, 233, 320, 321, 327, 329, 338, 339, 357
- Monocytes64, 74, 204,
288–292, 294–296, 373
- Mount Sinai Acute GVHD International Consortium
(MAGIC) 14, 79, 80
- Mouse
- B6.C-H2^{bm1} 131
- B6D2F1 130, 162
- Balb.b..... 131, 162, 163, 175–177
- Balb/c 131, 133, 137, 239, 246,
253, 255, 262, 270, 271
- BLT 64
- BRG 59, 61, 62
- C3H.SW 143, 145, 153, 156
- C57BL/6 143, 153, 154,
156, 158, 162, 163, 175, 177
- NCG60, 63
- NOD61, 63
- NOD-*scid* 60–64, 183–205,
319–321, 334, 335
- NOG 60, 62, 63, 67
- NPG60, 63
- NRG.....60, 63
- NSG 59, 61–67, 183–205,
327–329, 331, 334–336, 342, 346–348, 350,
352–356, 361, 363–370, 373
- scid* beige.....64–66
- Mouse models 1–20, 58–66,
127–138, 161–181, 183–205, 315–331, 333–357
- Mouse monitoring344–345, 353
- Multiplexed flow cytometric cytokine assay 187–188,
198–200, 202
- Multivariate analysis 72
- Muscles 110, 132, 134, 135,
148, 169, 170, 224, 227, 244, 269, 354
- Myelodysplastic syndrome 128
- Myeloid-derived suppressor cells (MDSCs) 2,
196, 200
- N**
- Natural killer cells (NK cells).....6, 7, 61, 279, 327
- Necropsy..... 186, 192, 210,
214, 229, 337, 347
- Negative cell isolation289
- Negative selection 304, 310
- Neoplasms 14, 17, 118, 122, 128
- Non-relapse mortality (NRM)1, 5–9,
72–80, 85, 86
- O**
- ODN 2216 302, 304, 311
- Overall response rate (ORR) 5, 6, 19
- Overall survival (OS)6–9, 72–78, 80,
162, 239, 260

P

Pain 117, 119, 174, 211, 231, 234, 355
 Palifermin 6
 Paraffin 214, 227, 229
 Pathogen-associated molecular patterns (PAMPs) 3, 73, 80
 Pathologist 227–229
 Pathophysiology 2–3, 19, 129, 142, 155
 Patient-derived xenotransplantations (PDXs) 360
 Pentostatin 7
 Peripheral blood 128, 129, 142, 157, 211–214, 220–222, 232, 299, 300, 302, 310, 311, 334, 361, 362, 365, 366, 370, 373
 Peripheral blood (PB) 16, 57, 62
 Peripheral blood mononuclear cells (PBMCs) 7, 58, 66, 189, 190, 202, 203, 226, 233, 291, 293, 302–303, 310, 326–328, 331, 340–341, 363, 366
 Permeabilization 247, 248, 252, 254, 255, 261, 262, 266, 273, 274, 284, 324
 Plasma 190, 290, 301–303, 306, 309, 310, 326, 340, 366
 Plasma cells 2
 Plasmacytoma 318
 Platelets 75, 80, 86, 87, 234, 261, 303, 310
 Plerixafor 16, 212, 220, 222, 230
 Polymerase chain reaction (PCR) 209, 213, 214, 216, 217, 220, 226, 230, 342
 Ponesimod 6
 Positive cell isolation 276, 277, 280
 Positive selection 150, 156, 303, 304, 310, 311, 362, 366, 367
 Post-transplant cyclophosphamide (PTCy) 6, 15, 17, 19, 118, 142, 238, 260
 Pregnancy 106, 108, 109, 287
 Progression-free survival (PFS) 73, 74
 Propidium iodide (PI) 241–243, 302, 306, 307
 Propofol 211, 214, 222, 231
 Prostaglandin E₂ (PGE₂) 289, 292, 296
 Psychosocial health 116

Q

Quality of life (QoL) 9, 92, 116, 179, 183

R

R848 302, 304, 309, 311
 Radiation 17, 61, 131, 184, 212, 224, 241, 246
 Radiotherapy 155, 238, 260, 315, 333
 Randomised control trials (RCTs) 6, 7, 9, 19
 Rat 58, 64, 67, 132, 134, 166, 177, 339
 Red blood cell (RBC) 149, 156, 179, 204, 216, 226, 232, 233, 240, 326, 373

Red blood cell (RBC) lysis 149, 165, 170, 179, 232, 240, 243, 244, 247, 249, 264, 269, 281, 364, 365, 370, 373
 Red cell lysis 155, 187, 195
 Red cells 65, 66, 155, 186, 187, 195
 Regenerating islet-derived protein 3-alpha (REG3α) 17
 Relapse incidence 73
 Replacement, reduction, and refinement (3Rs) 201, 353
 Reporter mouse 245, 248, 249, 281
 Respiratory rate 231
 Rituximab 9, 96, 119
 RNA 186, 194, 201, 261, 337, 349, 354
 Rodents 336, 337, 343–345, 353, 354, 356, 360
 RRGs rat 64, 65
 Ruxolitinib 5, 7, 17, 19

S

Scales 133, 137, 138, 144, 163–166, 172, 173, 177, 178, 185, 191, 192, 201, 203, 212, 244, 252, 270, 335, 344, 345, 353, 363
 Sclerodermatous 4, 7, 9, 19, 228, 229
 Sepsis 86, 87, 96, 225
 Sequencing 210, 216, 217, 219, 220, 230, 261
 Serum 85–87, 145, 156, 165, 166, 184, 186, 187, 193, 194, 198, 204, 205, 212, 223, 225, 226, 232–234, 240, 248, 250, 264, 281, 283, 289, 290, 301, 310, 318, 319, 336, 337, 349, 350, 362
 7-Aminoactinomycin D (7AAD) 146, 204, 301, 304, 305
 Severe combined immune deficiency (SCID) 58
 Sexual health 106, 108
 Signal transducers and activators of transcription (STAT) 17
 Sirolimus 7, 100
 Skin 4, 6, 7, 57, 61, 63, 64, 66, 72, 75, 76, 78–80, 134, 135, 137, 142, 147, 148, 154, 155, 158, 159, 169, 170, 172, 174, 178, 179, 183, 184, 191, 193, 194, 203, 207, 210, 214, 221, 227, 229, 231, 234, 238, 250, 260, 283, 344, 346, 347, 349, 372
 Skin lesions 59, 65, 227
 Software 144, 166, 175, 187, 188, 196, 200, 202, 204, 213, 214, 226, 227, 231, 250, 263, 268, 276, 280, 319–321, 324, 325, 330, 331, 337, 338, 350, 352, 354, 357, 364, 371
 Soluble B cell activating factor (sBAFF) 77, 80
 S100 protein 74

- Staining 174, 180, 181, 187,
197, 199, 203, 227, 230, 241, 245, 247, 248,
254, 262, 263, 265–267, 273–275, 281, 283,
284, 293, 294, 296, 297, 306, 307, 311, 312,
319, 324, 325, 338, 356, 362–365, 368, 370, 373
- Statins 6, 110
- Stem cells 16, 57, 71, 72, 91,
95, 96, 98, 99, 102, 104, 105, 107, 109, 112,
113, 115, 117, 127, 142, 143, 161–181, 183,
210–212, 220–225, 232, 233, 238, 259, 315,
316, 333, 360, 361
- Steroids 7, 74, 85, 97, 110, 113, 143
- Suppression of tumourigenicity 2 (ST2) 77
- Surgeries 103, 210, 222, 227, 231–233
- Survivorship 92, 93, 101, 116, 117
- T**
- T cell depletion (TCD) 6, 8, 66, 96,
118, 142, 146, 151–152, 156
- T cell immunoglobulin and mucin domain 3
(TIM-3) 77, 78, 80, 267
- T cell proliferation 6, 7, 297, 308, 311, 312
- T cells
alloreactive 2, 6, 9, 16, 238,
260, 300, 333, 334
CD3 280
CD4 60–64, 66, 128, 131,
249, 251, 255, 260, 283, 287–297, 299–312,
316, 317
CD8 8, 59, 85, 128, 143,
163, 184, 237, 249, 279, 316, 339
conventional 238, 260, 281
cytotoxic 237, 260, 317
effector 142, 162, 253, 254, 262, 296
helper 2, 202, 205, 260, 300
memory 287–297
regulatory (Treg) 6, 58, 62, 131,
142, 196, 238, 239, 245, 248–250, 254, 260,
262, 279–281, 283, 317, 352
- Thrombocytopenia 59, 65, 66
- Thrombotic microangiopathy (TAM) 86, 87, 105
- Tibia 148, 156, 169, 170, 244, 269
- Tissue damage 2, 17, 58, 62,
73, 85, 128, 142, 155, 229, 238, 260
- TNF receptor 6, 77, 142
- Tocilizumab 6
- Toll-like receptor (TLR) 7, 74, 299, 304
- Total body irradiation (TBI) 17, 94–100,
102, 104–110, 112–117, 120–122, 128, 129,
134, 137, 143, 155, 162, 165, 167, 168, 212,
223, 224
- Transcription factors 241, 254, 262, 266
- Transplantation
allogeneic 130, 131, 134, 237–248
autologous 127
haploidentical 15, 19, 207, 238
matched-related donor (MRD) 15, 19
matched-unrelated donor (MUD) 15, 19,
162, 238
semi-allogeneic 131
syngeneic 134
- Transplantation-related mortality (TRM) 73, 77
- Trypan blue 132, 135, 165,
170, 179, 232, 264, 265, 301, 303, 309, 318,
320, 322, 324, 326, 331, 362, 364
- Tumorigenesis-2 17
- Tumour necrosis factor (TNF) 268, 288,
289, 292, 296
- Typing 15, 208–210, 215–220
- U**
- Umbilical cord blood (UCB) 15, 16, 63
- Univariate analysis 73
- Uric acid 74, 80
- Ursodiol 212, 223, 225, 227
- V**
- Vacu-tainer tubes 184, 189, 202, 209,
210, 212–214, 216, 221, 225, 227, 289, 301,
302, 319, 325, 335, 340, 354
- Vedolizumab 8
- Viability dye 146, 152, 166, 305–307, 357
- Von Willebrand factor (vWF) 75, 80
- Vorinostat 6
- W**
- Weight loss 59, 60, 65, 100,
137, 138, 154, 158, 173, 174, 179, 180, 184,
192, 193, 201, 203, 234, 235, 250, 283, 323, 346
- X**
- Xenotransplantations 360
- Z**
- Zombie dye 204, 247, 250, 254