Single Dose CD117-targeted Antibody Drug Conjugate (ADC) in Combination with Lymphodepleting Antibodies Enables Allogeneic Hematopoietic Stem Cell Transplantation in Mice without Chemotherapy or Radiation

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#1682

BACKGROUND

Hematopoietic stem cell transplant (HSCT) is a highly effective and potentially curative treatment for malignant and non-malignant blood disorders. However, patient eligibility for this procedure can be limited due to the mortality and morbidity risks associated with current conditioning regimens, including organ toxicity, infertility, and secondary malignancies. We have generated an anti-mouse (anti-m) CD117-targeting antibody conjugated to pyrrolobenzodiazepine (PBD), designated anti-mCD117-PBD, which demonstrated the ability to condition mice effectively for hematopoietic stem cell transplantation in combination with lymphodepleting anti-mCD4 and anti-mCD8 antibodies for a full allogeneic (allo) transplant.

Our tool ADC was engineered for rapid clearance to enable a timely HSCT following conditioning. A single dose of 1 mg/kg robustly depleted long-term hematopoietic stem cells (LT-HSC) by 97% compared to PBS controls in C57BL/6 mice. We first evaluated the ability of single doses of 1 and 3 mg/kg anti-mCD117-PBD to condition for transplant in a congenic mouse model (C57BL/6 hosts [CD45.2+] with B6.SJL-Ptprca Pepcb/boyJ donors [CD45.1+]). We then evaluated conditioning with a single dose of 3 mg/kg anti-mCD117-PBD, in combination with 250 µg/mouse each of anti-mCD4 (GK1.5) and anti-mCD8 (YTS 169.4) antibody, in a fully mismatched allo-HSCT model (C57BL/6 hosts [CD45.2+] with CByJ.SJL(B6)-ptprca/J donors [CD45.1+]). In both studies, a dose matched non-targeted isotype-PBD (iso-PBD) was used as a negative control, while total body irradiation (TBI) was used as a fully myeloablative positive control. Rat IgG (rIgG, LTF-2) was used as a negative lymphodepletion control antibody in the allo-HSCT study. Conditioned mice were transplanted with $2x10^7$ whole BM donor cells. Lymphodepleting antibodies were dosed daily for three consecutive days before allo-HSCT. Peripheral blood chimerism was assessed over 16 weeks (congenic model) to 24 weeks (allo model), at which time donor HSC chimerism was evaluated in the terminal bone marrow.

In the congenic HSCT model, conditioning recipient mice with a single dose of 3 mg/kg anti-mCD117-PBD enabled robust donor chimerism in the peripheral blood and bone marrow, as well as reconstitution of the T-, B- and myeloid cell compartments, that was comparable to the 5 Gy TBI positive control. Treatment with the isotype-PBD control at 3 mg/kg was ineffective at enabling HSC engraftment. In the fully mismatched allo-HSCT model, recipient mice conditioned with 3 mg/kg anti-mCD117-PBD in combination with 250 µg/mouse each of lymphodepleting anti-mCD4 + anti-mCD8 antibodies enabled full donor chimerism, achieving >90% engraftment by week 12 in the peripheral blood which was sustained through the end of the study at week 24. Multilineage reconstitution of immune cell subsets was also observed in this study, with >90% donor chimerism seen in the B cell and myeloid compartments and T cell reconstitution above 75%. There was >90% donor LT-HSC engraftment in the bone marrow at study termination. These results were comparable to the chimerism observed in the 9 Gy TBI positive control mice. Groups conditioned with the non-targeting iso-PBD or anti-rIgG isotype antibody controls did not support donor engraftment in the model.

In conclusion, conditioning with 3 mg/kg anti-mCD117-PBD, in combination with lymphodepleting antibodies anti-mCD4 and anti-mCD8, enables complete donor chimerism in a fully mismatched allo-HSCT murine model. This targeted conditioning approach could offer a more favorable risk-benefit profile over currently available conditioning regimens and could extend the curative potential of allo-HSCT to more patients with malignant and non-malignant diseases who otherwise would not be eligible for HSCT.

MURINE HSC DEPLETION

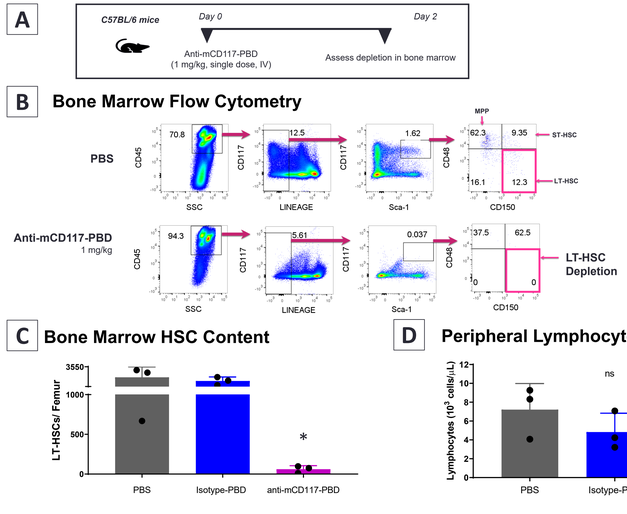
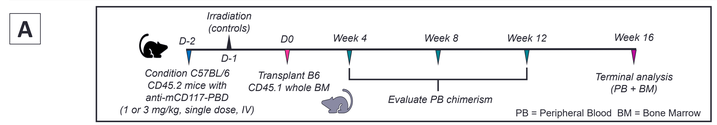
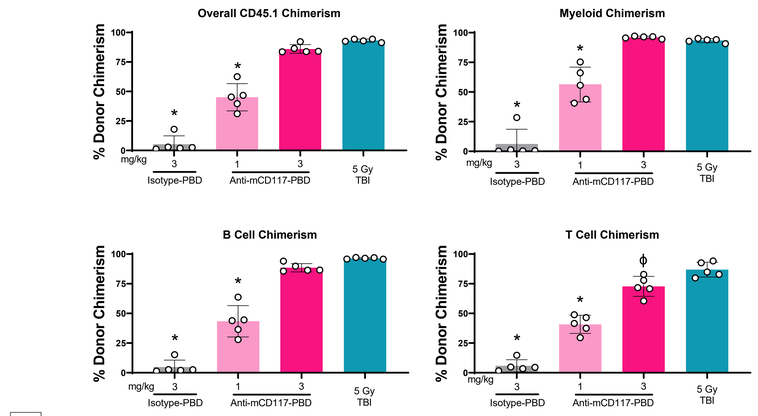


Figure 1: Anti-mCD117-PBD effectively depletes murine HSCs
(A) Schematic of *in vivo* study. A dose of 1 mg/kg anti-mCD117-PBD, Isotype-PBD or PBS was dosed on day 0. Bone marrow was collected on day 2 and examined by flow cytometry. **(B)** Flow cytometry gating strategy and results show depletion of long-term HSCs (LT-HSCs) by anti-mCD117-PBD. **(C)** Bone marrow LT-HSCs 2 days post dosing of PBS, isotype-PBD or anti-mCD117-PBD. Anti-mCD117-PBD depleted LT-HSCs 97% compared to PBS control. **(D)** Peripheral lymphocytes 2 days post dose shows no depletion with 1 mg/kg anti-mCD117-PBD. * p <0.05 when comparing anti-mCD117-PBD against any PBS or Isotype-PBD control groups; ns = not significant

MURINE CONGENIC TRANSPLANT





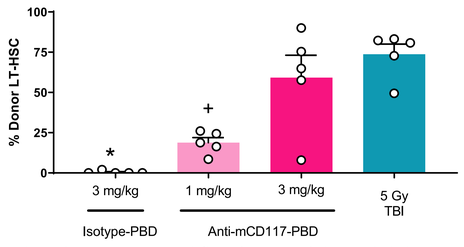
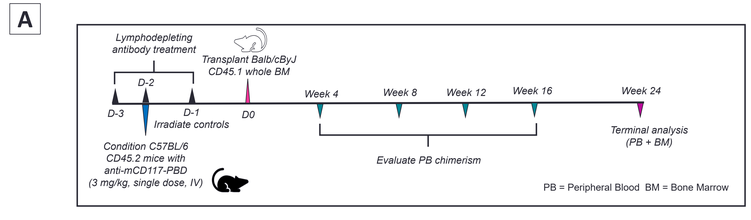


Figure 2: Congenic transplant of B6.SJL-Ptprca Pepcb/boyJ (B6 CD45.1) donor whole bone marrow cells into C57BL/6 recipients
(A) Schematic of *in vivo* model. Recipient mice were conditioned with 5 Gy TBI, 3 mg/kg Isotype-PBD, 1 or 3 mg/kg anti-mCD117-PBD via IV injection prior to transplant. C57BL/6 (CD45.2) recipient mice received $2x10^7$ whole bone marrow cells from B6.SJL-Ptprca Pepcb/boyJ (CD45.1) donor mice. Peripheral blood chimerism was analyzed monthly by flow cytometry to week 16 post-transplant, after which terminal bone marrow was collected. **(B)** Peripheral donor chimerism 16 weeks post-transplant. A dose of 3 mg/kg anti-mCD117-PBD enabled >86% overall donor engraftment, and multilineage reconstitution of the myeloid (96% donor), B cell (88% donor) and T cell (72% donor) compartments. **(C)** Engraftment of donor LT-HSCs in the bone marrow analyzed by flow cytometry at 16 weeks post-transplant. A dose of 3 mg/kg anti-mCD117-PBD had similar engraftment to 5 Gy TBI positive control mice (59% vs 73%, respectively). $^{\Phi}$p<0.05, *p <0.01 and *p<0.0001 when comparing to TBI control.

MURINE ALLOGENEIC TRANSPLANT



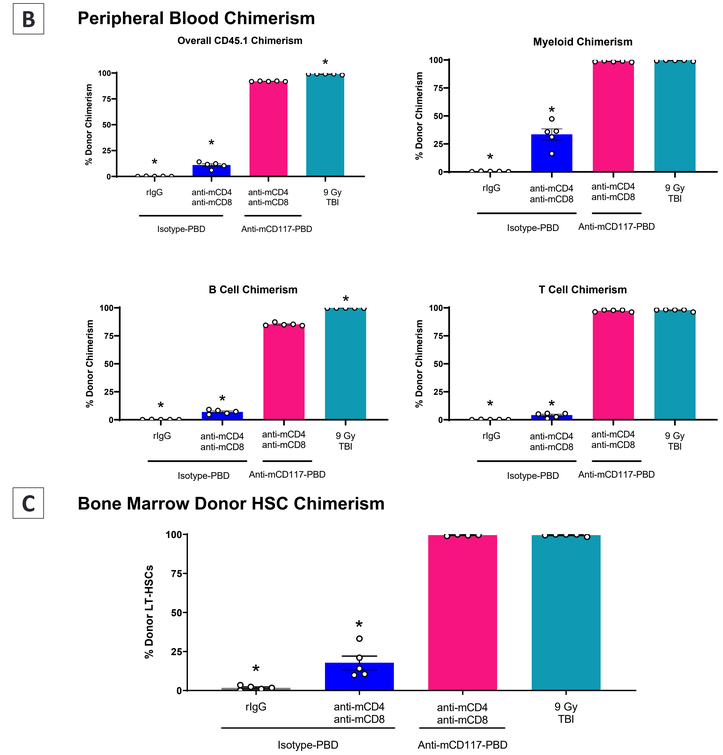


Figure 2: Fully mismatched allogeneic transplant of CByJ.SJL(B6)-Ptprca/J (Balb/cByJ CD45.1) donor whole bone marrow cells into C57BL/6 recipient mice.
(A) Schematic of *in vivo* model. Recipient mice were conditioned prior to transplant with a 3 mg/kg single dose of anti-mCD117-PBD or isotype-PBD in combination with either 250 µg/mouse QDx3 each of anti-mCD4 and anti-mCD8 lymphodepleting antibodies or rIgG control via IV injection. Total body irradiation at 9 Gy was used as a positive control. C57BL/6 (CD45.2) recipient mice received $2x10^7$ whole bone marrow cells from CByJ.SJL(B6)-Ptprca/J (CD45.1) donor mice. **(B)** Overall peripheral donor chimerism 12 weeks post transplantation. Anti-mCD117-PBD in combination with lymphodepleting anti-mCD4 and anti-mCD8 antibodies enable >90% donor engraftment in the periphery. Multilineage reconstitution of myeloid (99% donor), B cell (85% donor) and T cell (97% donor) compartments was observed. **(C)** Mice conditioned with anti-mCD117-PBD in combination with lymphodepleting anti-mCD4 and anti-mCD8 antibodies had similar donor engraftment of LT-HSCs in the bone marrow as 9 Gy TBI conditioned mice (both >99%). *p< 0.001 when comparing anti-mCD117-PBD + anti-mCD4/CD8 to rIgG-treated or isotype-PBD treated controls. The rIgG + anti-mCD117-PBD conditioned mice succumbed to body weight loss/morbidity around day 11-14 post-transplant; therefore week 12 peripheral blood chimerism could not be calculated and terminal BM analysis was conducted at week 2 post-transplant and was not included.

CONCLUSIONS

We have demonstrated that our anti-mCD117-PBD ADC effectively depleted host HSCs and enabled successful engraftment across minor histocompatibility antigens as well as fully mismatched allogeneic transplant when in combination with a lymphodepleting agent. This supports the use of CD117-targeting ADC to:

- **Condition for autologous HSCT in gene therapy settings as a monotherapy**
- **Condition for allogenic HSCT in combination with lymphodepleting agents in malignant and non-malignant settings**

This targeted conditioning approach could not only offer a more favorable risk-benefit profile over currently available conditioning regimens but could also provide patients who otherwise would not be eligible for HSCT, the opportunity to receive this curative treatment.

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