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

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Abstract:

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 developed a novel anti-CD117 antibody drug conjugate (ADC) that, in combination with lymphodepleting antibodies, can effectively condition mice to support a full allogeneic (allo) transplant. Specifically, we used a tool anti-mouse (anti-m) CD117-PBD ADC in combination with anti-mCD4 and CD8 depleting antibodies and assessed the ability of the combination to successfully condition in a murine model of allo-HSCT.

Our tool ADC, anti-mCD117-PBD, 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 anti-mCD4 (GK1.5) and anti-mCD8 (YTS 169.4) antibody, in a fully mismatched allo transplant 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 9 Gy total body irradiation (TBI) was used as a fully myeloablative positive control. Anti-rat (anti-r) IgG isotype (LTF-2) was used as a negative lymphodepletion control antibody in the allo-HSCT study. Conditioned mice were transplanted with 2e7 whole BM cells. Lymphodepleting antibodies were dosed daily for three consecutive days before transplant. 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 9 Gy TBI positive control for myeloablative conditioning. Treatment with the iso-PBD control at 3 mg/kg was not effective 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 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 (Figure 1A). 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% (Figure 1B-D). There was 99% donor HSC engraftment in the bone marrow at study termination (Figures 1E & F). 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.

Figure:

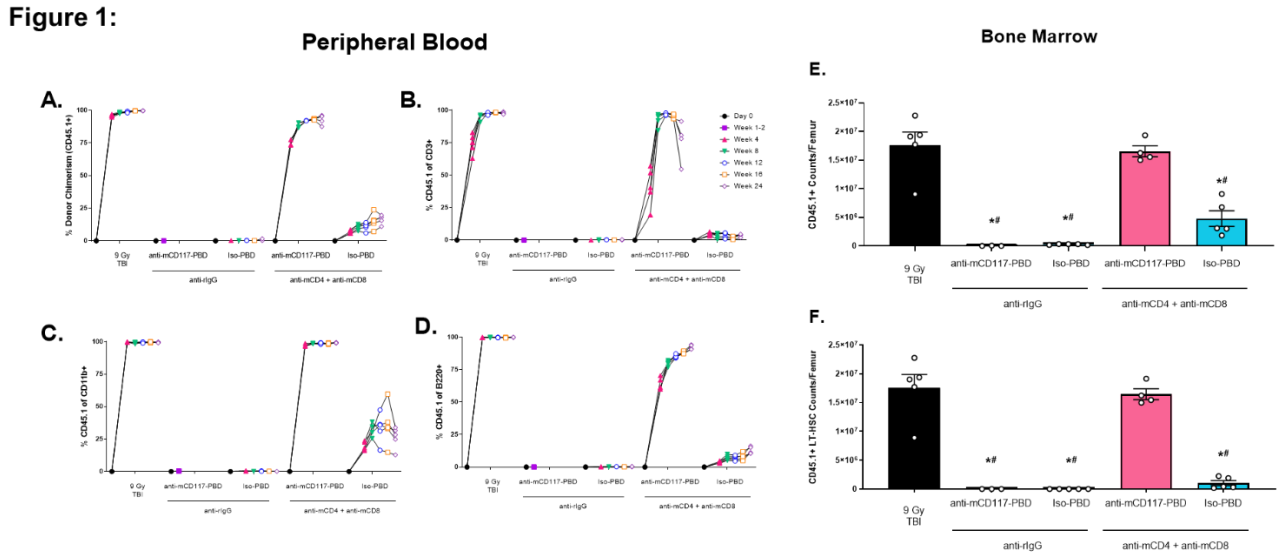

Figure 1. A single dose of 3 mg/kg anti-mCD117-PBD enables full, multilineage chimerism in a murine allo transplant model. C57BL/6 (CD45.2+) mice were conditioned with 3 mg/kg isotype-PBD or anti-mCD117-PBD in combination with 250 µg/mouse anti-mCD4 and anti-mCD8 or anti-rIgG antibodies 48 hours prior to transplantation with 2e7 CByJ.SJL(B6)-ptprca/J (CD45.1+) whole bone marrow cells. Donor cells were detected in the peripheral blood at 8 weeks post-transplant using the CD45.1+ antigen and persisted through week 24 (A), and reconstitution was multilineage (B-D). Terminal bone marrow chimerism in mice conditioned with anti-mCD117-PBD + anti-mCD4/CD8 lymphodepletion was similar to TBI controls (E-F). *p<0.0001 versus TBI; #p<0.0001 versus anti-mCD117-PBD + anti-mCD4/anti-mCD8; ANOVA with post hoc Šídák's multiple comparisons test.