Targeted CD45 Antibody Drug Conjugate Enables Full Mismatch Allogeneic Hematopoietic Stem Cell Transplantation in a Murine HSCT Model as a Single Agent

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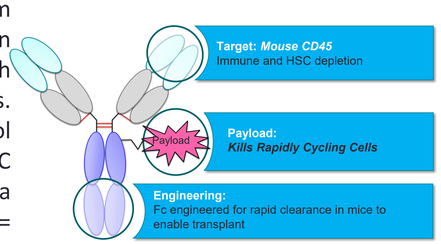
BACKGROUND

- Hematopoietic stem cell transplant (HSCT) is a potentially curative treatment for malignant and non-malignant disorders (e.g. genetic disorders and autoimmune disease).
- Current regimens for patient preparation, or conditioning, prior to HSCT limit the use of this curative procedure due to regimen-related mortality and morbidities, including risks of organ toxicity, infertility, and secondary malignancies.
- To address these issues, we are developing novel antibody drug conjugates (ADCs) to provide the benefit of full-intensity conditioning to remove disease-causing cells while reducing the severity of conditioning-related adverse events in HSCT.
- We have developed a novel anti-CD45 ADC targeting HSC and immune cells in mice engineered to have rapid clearance. The aim of this study was to determine if it can be used to enable full mismatch allogeneic HSCT in a murine model.

METHODS

ADCs

Magenta's platform enables the generation of targeted ADCs with customizable profiles. We developed a tool anti-mouse CD45 ADC engineered to have a short half-life ($t_{1/2}$ = 1.7hr, Figure 1 B) to enable HSCT.

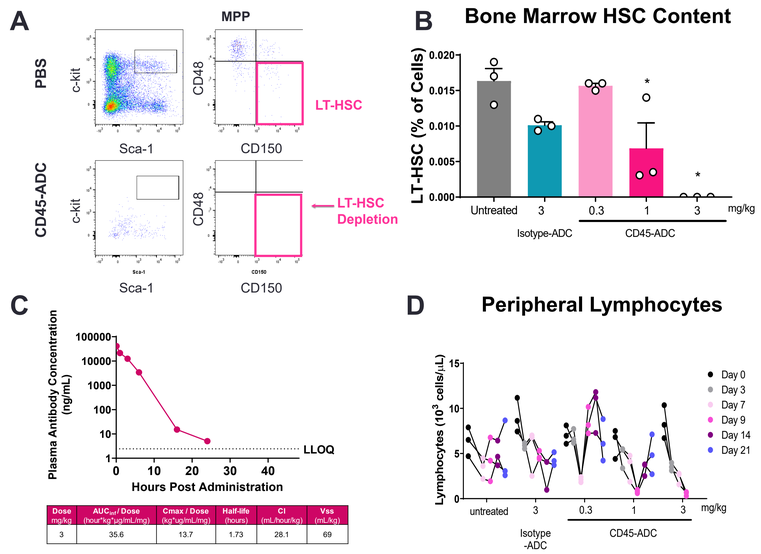


Animal studies

C57BL/6, DBA/2, B6.SJL (B6 CD45.1+), and CbyJ.SJL (Balb/c CD45.1+) mice were used in these studies.

The CD45-ADC was evaluated in unmanipulated C57BL/6 mice to determine a myeloablative dose and to establish pharmacokinetics. The optimal dose of CD45-ADC was evaluated for the ability to condition for transplant in a congenic autologous mouse transplant model. Then, CD45-ADC was evaluated in an allogeneic minor mismatch HSCT model in which conditioned DBA/2 mice were transplanted with 2 x 10^7 whole bone marrow cells harvested from pooled Balb/c CD45.1+ donors. Finally, we evaluated whether a single dose of the tool CD45-ADC was sufficient to enable chimerism in a full mismatch allo-HSCT model in which conditioned C57BL/6 mice (H2-b) were transplanted with 4 x 10^7 whole bone marrow cells from pooled Balb/c CD45.1+ (H-2d) donors. 9 Gy TBI served as the conventional conditioning positive control in all experiments. Peripheral blood chimerism assessed over 16 weeks. Terminal chimerism in spleen and thymus was evaluated at week 22.

MURINE HSC DEPLETION BY CD45-ADC



Dose (mg/kg)	AUC_{inf} / Dose (hour*kg*μg/mL/mg)	Cmax / Dose (kg*μg/mL/mg)	Half-life (hours)	Cl (mL/hour/kg)	Vss (mL/kg)
3	35.6	13.7	1.73	28.1	69

Figure 1 CD45-ADC effectively depletes murine HSCs and lymphocytes. CD45-ADC was dosed on day 0. Bone marrow was collected on day 2 and HSC depletion assessed by flow cytometry. **(A)** Phenotypic long-term HSC (LT-HSC, Lin− cKit+ Sca1+ CD150+ CD48-) depletion 2 days after single dose of CD45-ADC (3 mg/kg) administration. **(B)** % LT-HSC depletion. **(C)** CD45-ADC (3 mg/kg) half-life in C57Bl/6 mice is 1.7 hours. **(D)** Peripheral lymphocytes reach nadir by day 9 post administration of CD45-ADC (3 mg/kg), indicating effective depletion by CD45-ADC. *p <0.05 when comparing CD45-ADC treated mice versus untreated mice.

MURINE CONGENIC TRANSPLANT

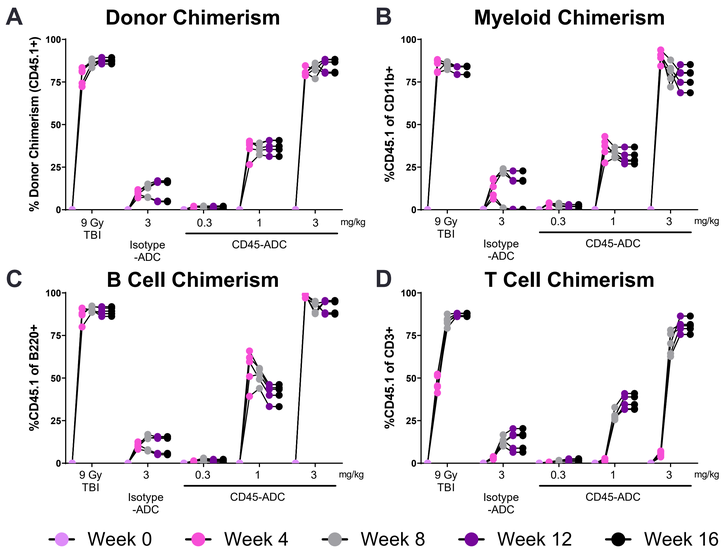


Figure 2: **CD45-ADC enables congenic transplant in murine model.** C57Bl/6 mice were conditioned with 9 Gy TBI, Isotype-ADC, or CD45-ADC and transplanted with whole bone marrow from B6.SJL (B6 CD45.1+) mice. **(A)** Peripheral donor chimerism is >85% in mice conditioned with CD45-ADC (3 mg/kg) through 16 weeks post transplant, comparable to mice conditioned with 9 Gy TBI. **(B-D)** Peripheral donor engraftment is multilineage.

MURINE MINOR MISMATCH TRANSPLANT

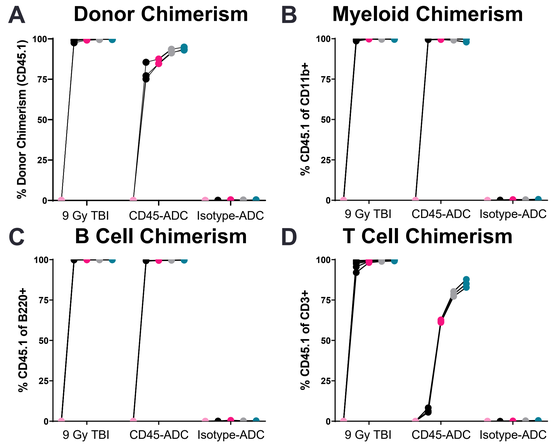


Figure 3: **A single dose of CD45-ADC is sufficient to enable minor mismatch allogeneic transplant of Balb/c CD45.1 donor cells into DBA/2 recipients. (A-D)** C57BL/6 mice were conditioned with 3 mg/kg Isotype-ADC or CD45-ADC. CD45-ADC enables ≥ 95% donor chimerism **(A)** and peripheral donor engraftment through 16 weeks is multilineage **(B-D).**

MURINE ALLOGENEIC TRANSPLANT

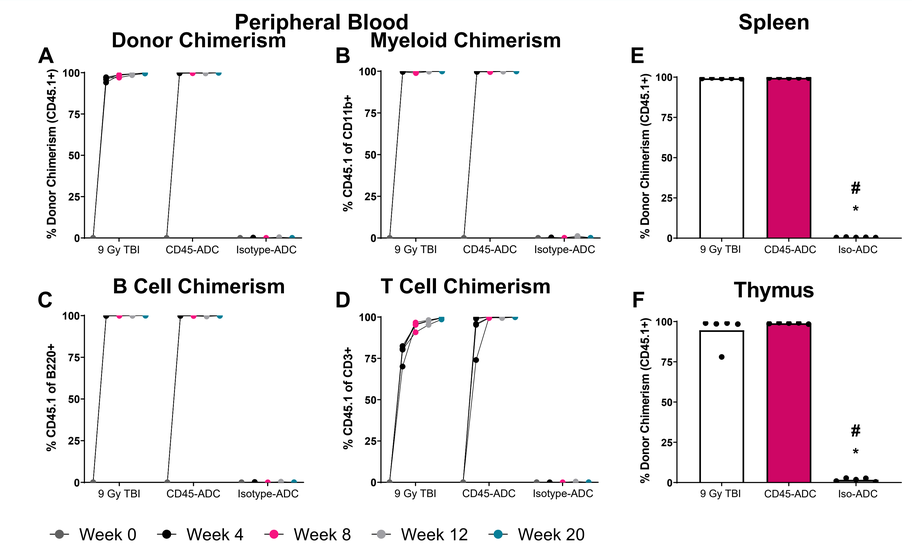


Figure 4: **A single dose of 5 mg/kg CD45-ADC is sufficient to enable allogeneic transplant of Balb/c CD45.1 donor cells into C57BL/6 recipients. (A-D)** C57BL/6 mice were conditioned with 5 mg/kg Isotype-ADC or CD45-ADC. CD45-ADC enables ≥ 95% donor chimerism **(A)** and peripheral donor engraftment is multilineage through week 20. **(B-D)**. Terminal splenic (E) and thymic (F) chimerism in CD45-ADC conditioned mice were similar to TBI. *p<0.05 versus TBI; #p<0.05 versus CD45-ADC; ANOVA with post hoc Tukey's multiple comparisons test.

CONCLUSIONS

- A single dose of the tool CD45-ADC is fully myeloablative and enables complete chimerism in a full mismatch allogeneic HSCT model without the need for additional conditioning agents.
- This targeted approach for safer conditioning could improve the risk-benefit profile for allogeneic and haploidentical HSCT and may extend the curative potential of this modality.