INTRODUCTION

Allogeneic hematopoietic stem cell transplant (allo-HSCT) is a potentially curative treatment for malignant and non-malignant blood disorders and has demonstrated impressive outcomes in autoimmune diseases. Current regimens for patient preparation, or conditioning, prior to allo-HSCT limit the use of this curative procedure due to regimen-related mortality and morbidities, including risks of organ toxicity, infertility, and secondary malignancies. This greatly limits the use of allo-HSCT in malignant and non-malignant conditions. To address these issues, we are developing novel antibody drug conjugates (ADCs) to provide the benefit of full-agent approach in combination with CD4 and CD8 depleting antibodies has been shown to be sufficient to enable bone marrow transplant in syngeneic immune competent mice (Pal chaudthuri et al. Nature Biotech 2016), and this approach in combination with CD4 and CD8 depleting antibodies has successfully enabled allo-HSCT in the haploidentical mouse model. The aim of this study was to model our engineered ADC to determine if it can be used to enable full allo-HSCT in mice.

Previous work using an ADC approach to mouse CD45 has been shown to be sufficient to enable bone marrow transplant in syngeneic immune competent mice (Pal chaudthuri et al. Nature Biotech 2016), and this approach in combination with CD4 and CD8 depleting antibodies has successfully enabled allo-HSCT in the haploidentical mouse model. The aim of this study was to model our engineered ADC to determine if it can be used to enable full allo-HSCT in mice.

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 ($t_{1/2} = 1.7$ hr, Figure 1 B) to enable HSCT.

Animal studies

C57BL/6, DBA/2, B6.SJL (B6 CD45.1+), and Cby.C.SJL (Balf/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 10^6 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 chimera 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+ (H2-b) donors. 9 Gy TBI served as the conventional conditioning positive control in all experiments. Peripheral blood chimerism assessed over 16 weeks.

RESULTS

Murne HSC depletion by CD45-ADC

Figure 1: CD45-ADC effectivly 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) depletion 2 days after single dose of CD45-ADC 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.

Murne 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 5 mg/kg Isotype-ADC or CD45-ADC. CD45-ADC enables >95% donor chimerism and peripheral donor engraftment through 16 weeks is multilineage (B-D).

Murne 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 and peripheral donor engraftment at 8 weeks is multilineage (B-D).

CONCLUSION

- A single dose of the tool CD45-ADC is fully myeloablative and enables complete chimera in a full mismatch allo-HSCT model.
- See OS5-4 for additional work with this mouse tool CD45-ADC to enable immune reset with HSCT in mouse models of autoimmune disease.
- This targeted, readily translatable approach for safer conditioning could improve the risk-benefit profile for allogenic and haploidentical HSCT and may extend the curative potential of this modality.