CD45-Targeted Antibody Drug Conjugate Plus Post Transplant Cytoxan is Sufficient to Enable Allogeneic Bone Marrow Transplant in a Minor Mismatch Mouse Model

Sharon L. Hyzy, Rahul Palchaudhuri, Jennifer L. Proctor, Brad R. Pearse, Ganapathy N. Sarma, Hillary L. Adams, Sharon Aslanian, Geoff O. Gillard, Tahirih L. Lamothe, Olga Burenkova, Melissa Brooks, Andrew D. Gabros, Charlotte F. McDonagh, Anthony E. Boitano, Michael P. Cooke

Magenta Therapeutics, Cambridge, MA

BACKGROUND

Bone Marrow Transplant (BMT) 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 BMT 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 BMT in malignant and non-malignant conditions. To address these issues, we are developing antibody drug conjugates (ADCs) targeting hematopoietic stem cells (HSCs) and immune cells to safely condition patients for allogeneic BMT (35% of all transplants, CIBMTR) and autologous BMT (for autoimmune disease).

Targeted preparation using antibody drug conjugates (ADCs) to mouse CD45 has previously been shown to be sufficient to enable bone marrow transplant (BMT) in syngeneic immune competent mice (Palchaudhuri et al. Nature Biotech 2016 34:738–745), and this approach to preparation has the potential to expand the utility of BMT if it can be successfully translated to patients. This ADC were created using saporin (SAP), a ribosome-inhibiting protein, which once internalized elicits cytotoxicity in a cell cycle-independent manner. Anti-CD45-saporin (CD45-SAP) effectively depleted bone marrow HSCs as single dosed agents, and enabled efficient autologous HSC engraftment (>95% long-term donor chimerism).

To further investigate the utility of this tool ADC in murine transplant models, we explored anti-CD45-saporin (CD45-SAP) in an allogeneic minor mismatch transplant model (Balb/c donor into DBA/2 recipients). The goal of the work was to identify the level of immune suppression, if any, that needs to be used in combination with CD45-SAP to enable high donor chimerism in the allogeneic setting.

METHODS

Saporin (SAP)- based Immunotoxins

To create CD45-SAP, commercially available biotinylated anti-CD45.2 (clone 104) mAb was combined with streptavidin-saporin (ATS Bio, Catalog IT-27) in a 1:1 molar ratio just prior to injection. Dosing was calculated based on the amount of antibody used to create the immunotoxin. The isotype-SAP was created by using a biotinylated mIgG2a isotype mAb.

Animal studies

C57Bl6, DBA/2 and CD45.1 Balb/c mice were purchased from the Jackson Laboratories. DBA/2 mice were transplanted with 2 x10^7 whole bone marrow cells harvested from pooled Balb/c CD45.1 congenic donors. All in vivo research was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Research Council of the National Academies and under the approval of the Institutional Animal Care and Use Committee.

CD45-SAP (1.9 mg/kg, iv) was evaluated alone or in combination with additional immune modulating agents: clone 30F11 (25 mg/kg, IP), a naked anti-CD45 antibody that mimics ATG by relying on effector function to enable potent peripheral B- and T -cell depletion; pre-transplant Cytoxan (PreTCy, 200 mg/kg, IP), 2 Gy total body irradiation (TBI), and post-transplant Cytoxan (PTCy, 200 mg/kg, IP) to prevent graft versus host disease as well as block host versus graft rejection. 9 Gy TBI was used as the conventional conditioning positive control. Conditioned mice were transplanted with 2x10^7 whole bone marrow cells, and chimerism assessed over 12 weeks.

MURINE HSC DEPLETION BY CD45-SAP

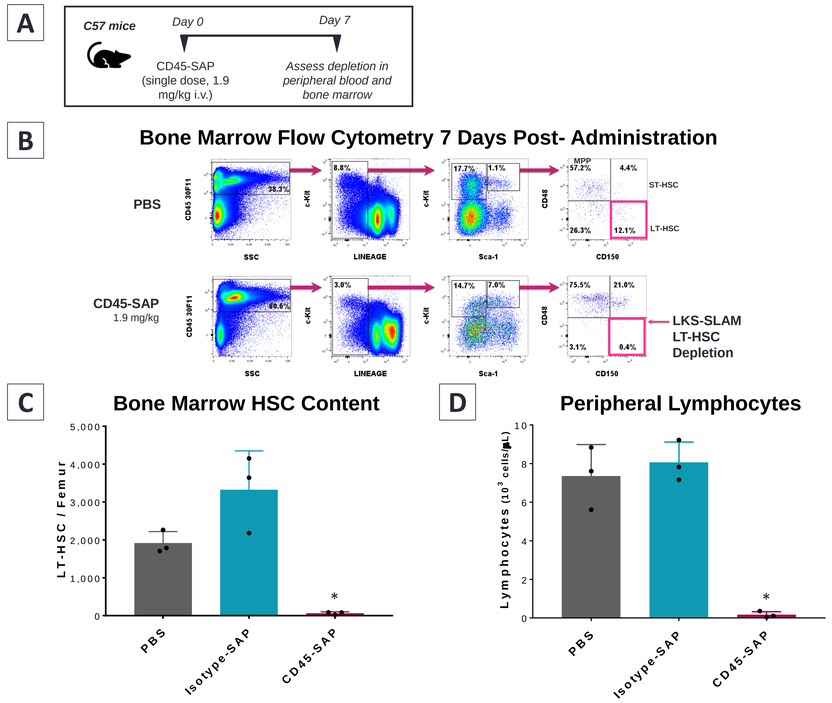
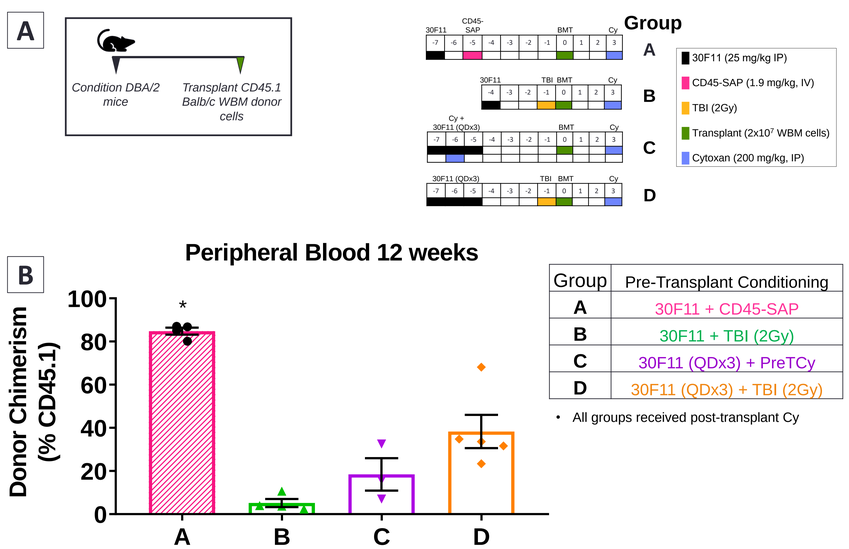


Figure 1: **CD45-SAP ADC effectively depletes murine HSCs and lymphocytes.** **(A)** Schematic of *in vivo* study. CD45-SAP or controls were dosed on day 0. Peripheral blood and bone marrow were collected on day 7 and examined by CBC and flow cytometry, respectively. **(B)** Flow cytometry gating strategy and results show depletion of long-term HSCs by CD45-SAP. **(C)** Bone marrow long-term HSCs 7 days post dosing of PBS, isotype-SAP or CD45-SAP. **(D)** Peripheral lymphocytes 7 days post-dosing shows effective depletion by CD45-SAP. * p <0.05 when comparing CD45-AM against any control group.

MURINE MINOR MISMATCH TRANSPLANT



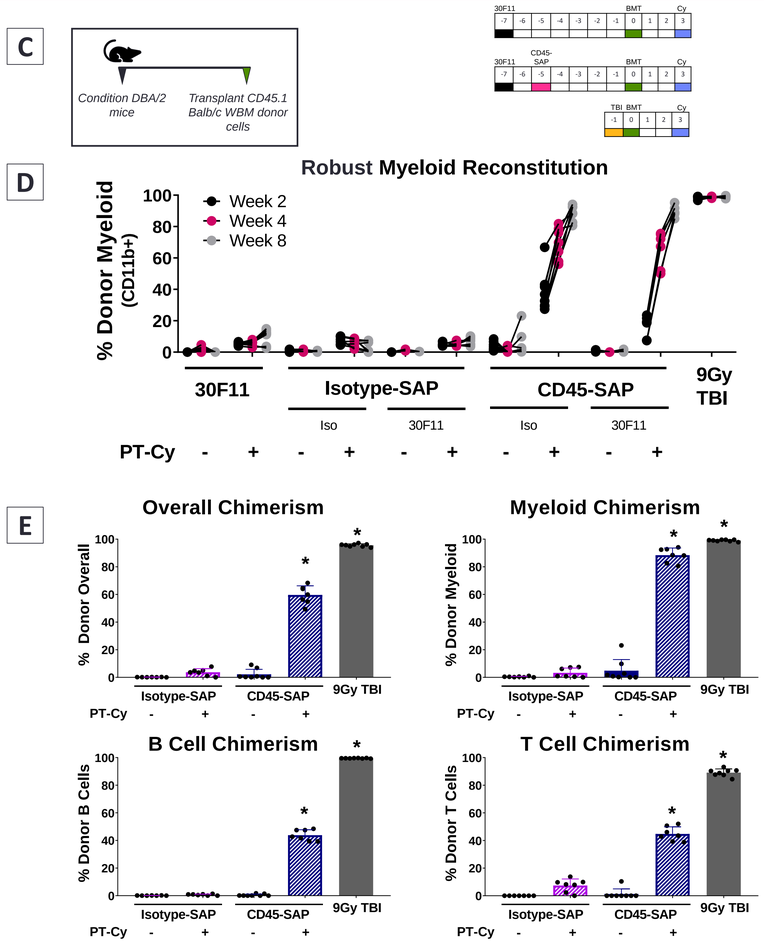


Figure 2: **Minor mismatch transplant of Balb/c donor cells into DBA/2 recipients.** **(A)** Schematic of *in vivo* model with dosing schedule for the various experimental groups **(B)** Overall peripheral donor chimerism 12-weeks post transplantation. CD45-SAP in combination with immunosuppressants enable >80% donor minor mismatch engraftment. **(C)** Experimental design to delineate combination required to enable engraftment. **(D)** Kinetics of myeloid reconstitution post-transplant. CD45-SAP plus post-transplant Cytoxan is sufficient to enable minor mismatch engraftment. **(E)** Peripheral donor engraftment at 8 weeks is multilineage. * p <0.05 when comparing against control groups.

CONCLUSIONS

- **Conditioning with a single dose of CD45-SAP with post-transplant Cytoxan to prevent GvHD enables successful engraftment across minor histocompatibility antigens in the minor mismatched setting without the need for additional immune suppression.**
- **The antibody-drug conjugate was more effective than sublethal irradiation, a naked depleting anti-CD45 antibody, or pre-transplant Cytoxan.**

Next steps

Progress to Development Candidate for selective, non-DNA damaging, targeted depletion of stem and immune cells for transplant conditioning:

- **Anti-CD45 alpha-amanitin ADC Poster 129, Talk 40**