Antibody Drug Conjugates Targeted to CD45 or CD117 Enable Allogeneic Hematopoietic Stem Cell Transplantation in Animal Models

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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. Prior to BMT, patients are prepared with high-dose chemotherapy alone or with total body irradiation, and both are associated with early and late morbidities, organ toxicities, infertility, secondary malignancies and substantial risk of mortality. 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).

ADCs targeted to mouse CD45 or mouse CD117 have recently been shown to effectively condition immunocompetent mice for BMT (Palchaudhuri *et al*. Nature Biotech 2016 34:738–745; and Czechowicz *et al.* Blood 2016 128:493). These ADCs were created using saporin (SAP), a ribosome-inhibiting protein, which once internalized elicits cytotoxicity in a cell cycle-independent manner. Both anti-CD45-saporin (CD45-SAP) and anti-CD117-saporin (CD117-SAP) effectively depleted bone marrow HSCs as single dosed agents, and enabled efficient autologous HSC engraftment (>95% long-term donor chimerism). These ADCs have also enabled BMT in murine models of Fanconi Anemia (Poster 2041).

To further investigate the utility of these murine tool ADCs, we explored CD45-SAP and CD117-SAP in the context of allogeneic minor mismatch transplant. Using the Balb/c donor into DBA/2 transplant model we sought to determine whether CD45-SAP or CD117-SAP could enable allogeneic transplant as single entity agents or needed to be combined with additional immunosuppressive agents (e.g. Cytoxan, ATG-mimic).

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. Similarly, to create CD117-SAP, biotinylated anti-CD117 (clone 2B8) mAb was combined with streptavidin saporin). 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.

Immunosuppressants

To mimic ATG, we used a naked anti-CD45 mAb (clone 30F11, 25 mg/kg IP) which relies on effector function to potently deplete peripheral lymphocytes without affecting bone marrow HSCs. Cytoxan was administered at 200mg/kg IP 3 days post-transplant to prevent GvHD from the donor T cells, as shown in the schemes. Total body irradiation (2Gy or 9Gy) was performed using an X-ray irradiator.

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.

MURINE HSC DEPLETION BY CD45-SAP

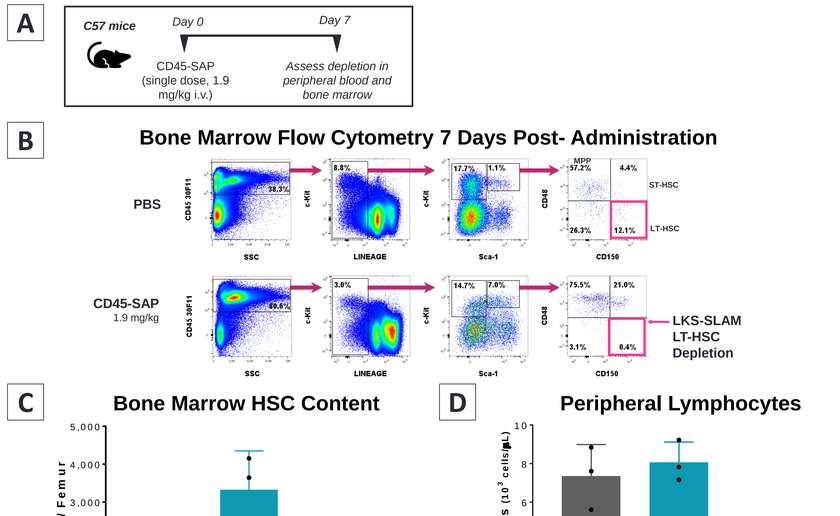
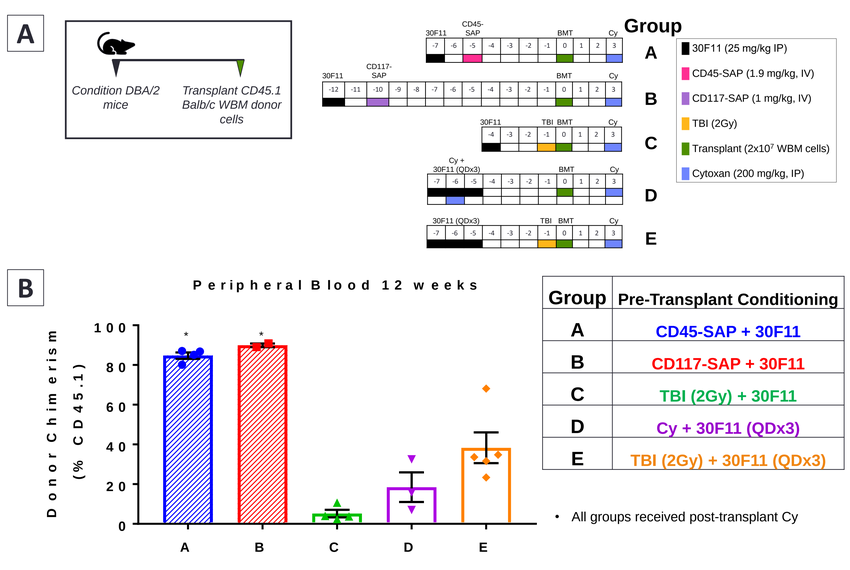


Figure 1: CD45-SAP ADC effectively depletes murine HSCs and lymphocytes.
(A) Schematic of *in vivo* study. CD45-SAP or controls were dosed on day 0. Peripheral blood and bone marrow were collected on day 7 and examined by CBC and flow cytometry, respectively. **(B)** Flow cytometry gating strategy and 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



Group	Pre-Transplant Conditioning
A	CD45-SAP + 30F11
B	CD117-SAP + 30F11
C	TBI (2Gy) + 30F11
D	Cy + 30F11 (QDx3)
E	TBI (2Gy) + 30F11 (QDx3)

- All groups received post-transplant Cy

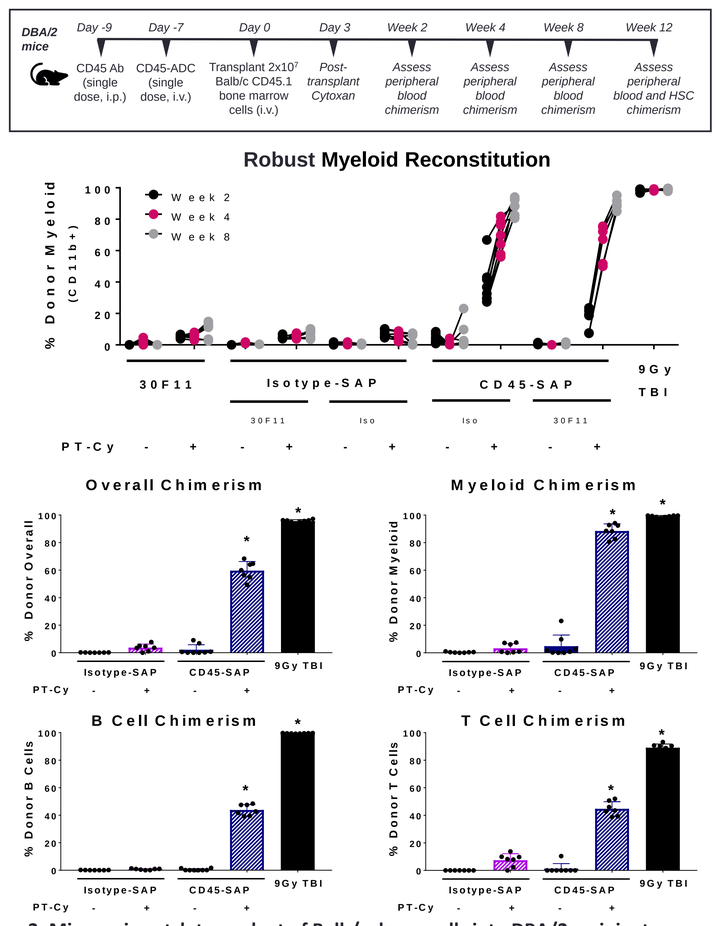


Figure 2: Minor mismatch transplant of Balb/c donor cells into DBA/2 recipients.
(A) Schematic of *in vivo* model with dosing schedule for the various experimental groups **(B)** Overall peripheral donor chimerism 12-weeks post transplantation. CD45-SAP and CD117-SAP in combination with immunosuppressants 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 CD45-SAP or CD117-SAP plus post-transplant Cytoxan to prevent GvHD enables successful engraftment across minor histocompatibility antigens**
- **The antibody-drug conjugates were more effective than a naked depleting anti-CD45 antibody, pre-transplant Cytoxan or sublethal irradiation in combination with post-transplant Cytoxan**

Next steps:

Progress to Development Candidates for selective non-genotoxic targeted depletion of stem and immune cells for transplant conditioning:

- **Anti-CD117 alpha-amanitin ADC Posters 3314 & 3316**
- **Anti-CD45 alpha-amanitin ADC Posters 4526 & 3316**