CD117-Amanitin Antibody Drug Conjugates Effectively Deplete Human and Non-Human Primate HSCs: Proof of Concept as a Targeted Strategy for Conditioning Patients for Bone Marrow Transplant

Bradley R Pearse, Sean M McDonough, Jennifer L Proctor, Rajiv Panwar, Ganapathy Sarma, Molly A McShea, Lena Kien, Junia Dushime, Hillary L Adams, Sharon L Hyzy, Melissa Brooks, Rahul Palchaudhuri, Qing Li, Nate Kallen, Patrick C Falahee, Pranoti Sawant, Charlotte McDonagh, Anthony E Boitano, Michael P Cooke
Magenta Therapeutics, Cambridge, MA

BACKGROUND

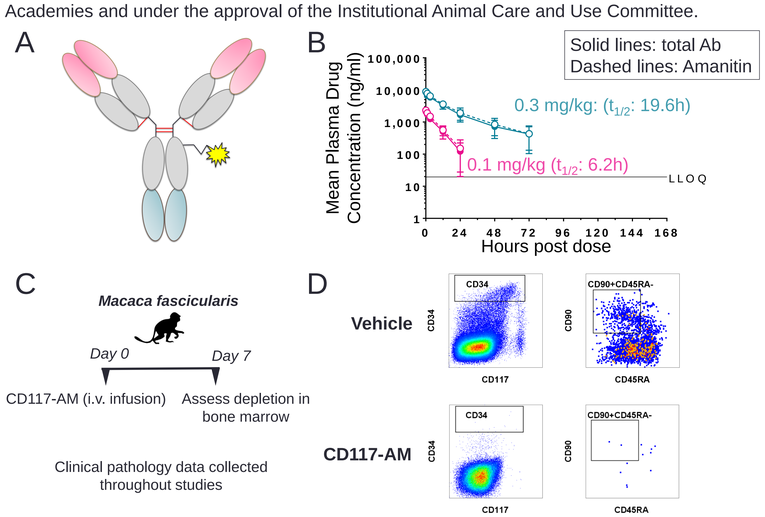
Bone marrow transplant (BMT) is a potentially curative approach for patients with hematologic malignancies, autoimmune diseases or genetic diseases. Prior to transplant, patients are prepared with non-specific genotoxic chemotherapy alone or in combination with total body irradiation, which are associated with early and late morbidities, including organ toxicities, infertility, secondary malignancies, and substantial risk of mortality. As a result, many eligible patients do not consider transplant. CD117, which is specifically expressed on hematopoietic stem cells (HSCs) and progenitors, is rapidly internalized, and is an ideal target for a targeted non-genotoxic antibody drug conjugate (ADC) approach to conditioning. We have previously shown that a single dose of an anti-CD117 ADC depleted >95% of bone marrow HSCs in a humanized mouse model and reduced disease burden while extending survival in an AML tumor model (Hartigan et al., *Blood* 2017 130:1894). The aim of this translational study was to develop a potent, non-genotoxic anti-CD117 ADC highly effective in eliminating host HSC with an engineered half-life (<24h) and minimal adverse side effects in a non-human primate (NHP) model. An anti-CD117 antibody conjugated to the non-genotoxic RNA polymerase II inhibitor amanitin (AM), capable of killing quiescent and dividing cells, could improve patient preparation for allogeneic transplant in malignant settings and autologous transplant for gene therapy applications.

METHODS

CD117-ADC
A fully human antibody to CD117 was conjugated to amanitin, obtained under license from Heidelberg Pharma (Heidelberg, Germany). The isotype control ADC is a non-targeted monoclonal human IgG antibody conjugated to amanitin. The control unconjugated CD117 antibody is the fully human antibody. Both wild type and engineered half-life variants of the CD117-AM were evaluated.

***In Vitro* Cell Culture**
Kasumi-1 cells were grown according to ATCC guidelines. Human HSCs (CD34+ selected BM cells) were cultured in the presence of IL-6, TPO, and FLT-3 ligand, with or without SCF. For *in vitro* killing assays, Kasumi-1 cells or CD34$^+$ cells were cultured with CD117-ADC for 3 or 5 days, respectively, and viability was assessed by CellTiter Glo or flow cytometry.

***In Vivo* HSC Depletion**
Humanized NSG mice were purchased from the Jackson Laboratories. Mice were given a single administration as indicated. Peripheral blood was analyzed weekly and bone marrow was analyzed by flow cytometry at 21 days post injection. NHP HSC depletion was evaluated in male cynomolgus monkeys after a single administration of ADC (3 animals per group). HSC content in the bone marrow was monitored by flow cytometry and colony-forming unit (CFU) analysis on day 7. Hematology and clinical chemistries were evaluated throughout the studies. 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.



(A) The CD117-AM ADC is a full length fully human IgG1 with modifications to support conjugation and optimize the pharmacokinetic properties. **(B)** The engineered ½ life anti-CD117-AM demonstrates rapid clearance in cynomolgus monkeys with a half-life suitable for patient preparation for transplant (n=3/group). Clearance rates are similar for the total ADC and amanitin detection. **(C)** Dosing and sample collection scheme for the analysis of ADC in non-human primates. **(D)** Representative flow plots for a single cynomolgus monkey treated with vehicle or anti-CD117-AM.

RESULTS

In Vitro Killing

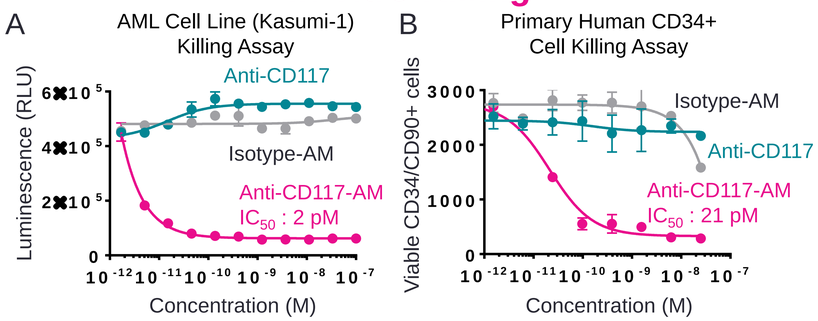


Figure 1: Anti-CD117-amanitin is highly effective at killing CD117 expressing cell lines or primary human CD34$^+$ cells *in vitro*. **(A)** Kasumi-1 cells were cultured for three days in the presence of anti-CD117-AM or controls. Cell viability was measured by Celltiter Glo. **(B)** Isolated primary human CD34$^+$ bone marrow cells were cultured for 5 days with anti-CD117-AM or controls. Live cell counts were determined by flow cytometry.

In Vivo Human HSC Depletion – WT vs. engineered half-life ADC

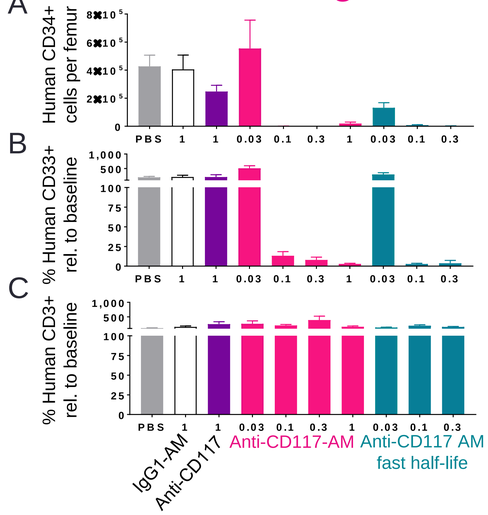


Figure 2: **The anti-CD117-amanitin conjugate selectively depletes human stem and progenitor cells (CD34+) in humanized NSG mice.** CD117-AM or controls were dosed on day 0. Peripheral blood and bone marrow were collected on day 21 and analyzed by flow cytometry. **(A)** The absolute number of CD34$^+$ cells in the bone marrow of CD117-AM or control treated mice 21 days after a single administration. **(B)** Percent of human myeloid, **(C)** and T cells present in the peripheral blood of CD117-AM or control treated mice, expressed as a percent of that cell population prior to treatment (normalized to baseline).

NHP Depletion – WT half-life ADC

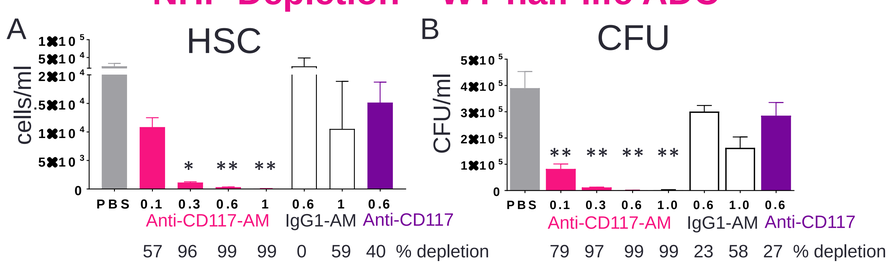
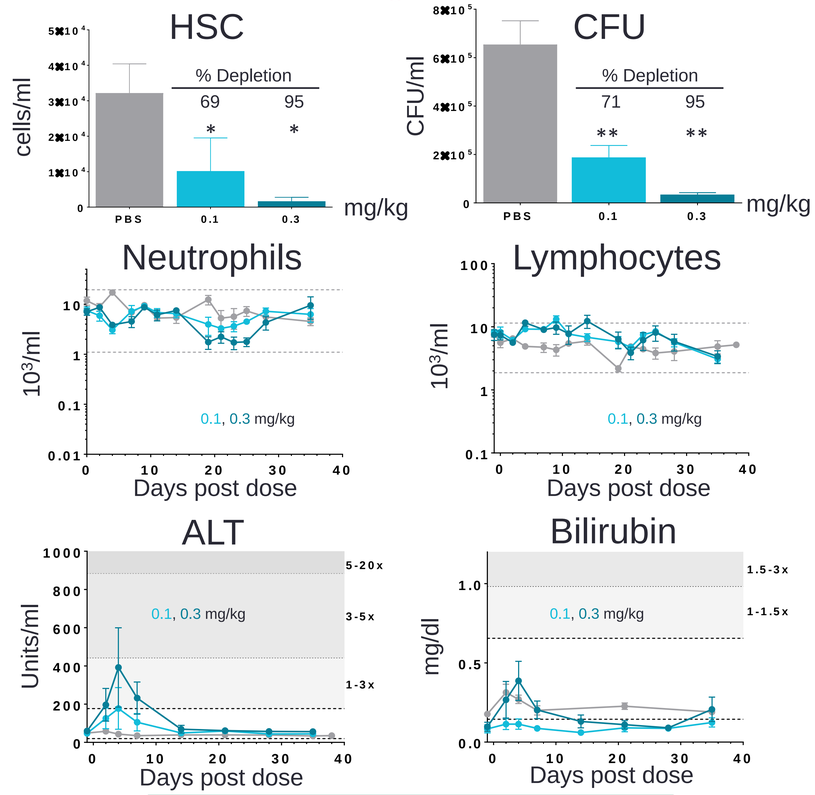


Figure 3: **A single dose of amanitin conjugated anti-CD117 eliminates bone marrow HSCs in cynomolgus monkeys.** Male cynomolgus monkeys received a single i.v. dose of anti-CD117-AM, isotype-AM (IgG1-AM), or unconjugated anti-CD117 antibody. Bone marrow HSC counts were determined by flow cytometry 7 days post administration. The fraction of HSC and CFU depleted after treatment with anti-CD117-AM, IgG1-AM, or unconjugated anti-CD117 was calculated relative to the PBS group. * p <0.05, ** p<0.01 when comparing CD117-AM against vehicle.

NHP Depletion – Engineered half-life ADC



No change above upper limit normal (ULN) for the additional parameters:			
GGT	Albumin	BUN	LDH
ALP	Creatinine	Glucose	PT/PTT

Figure 4: **The engineered half-life anti-CD117-amanitin conjugate selectively depletes target cell populations in cynomolgus monkeys.** CD117-AM or control were dosed on day 0 as a single administration (0.1, 0.3 mg/kg). Bone marrow aspirates were collected on day 7 post-dose. Peripheral blood was collected through the course of the study. Phenotypic HSCs were quantified by flow cytometry and assessment of colony forming units (CFU) from the bone marrow aspirate. On-target dose-dependent depletion of HSC and CFU was observed. Counts of neutrophils, and lymphocytes in treatment groups are depicted. On-target dose-dependent depletion is observed for neutrophils with lymphocytes spared. A transient dose-dependent mild elevation of liver enzymes was observed in groups treated with the highest doses of isotype-AM (not shown) and anti-CD117-AM. The fraction of HSC and CFU depleted after treatment with anti-CD117-AM was calculated relative to the PBS group. * p <0.05, ** p<0.01 when comparing CD117-AM against vehicle.

CONCLUSIONS

- Anti-CD117-AM exhibits potent elimination of NHP HSCs and progenitors *in vivo*. The potency of fast half-life anti-CD117-AM was comparable, providing a model for target cell depletion and rapid clearance prior to bone marrow transplant. Preliminary data suggests that the ADC was well tolerated at the efficacious doses.
- This strategy preserves the adaptive immune system with delayed onset of neutrophil nadir, potentially shortening the period of neutropenia.
- Targeted depletion of hematopoietic stem cells could provide a significant improvement in standard-of-care approaches to patient preparation prior to allogeneic and autologous bone marrow transplant including HSC-based gene therapy and broaden patient access to this potentially curative therapy.

Next steps: Begin IND-enabling studies in 2019

Additional work from Magenta's targeted conditioning programs can be viewed in posters 3316, 4526, 3324 and 2041