A SINGLE DOSE OF SHORT HALF-LIFE CD117 ANTIBODY DRUG CONJUGATE ENABLES HEMATOPOIETIC STEM CELL BASED GENE THERAPY IN NONHUMAN PRIMATES

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INTRODUCTION

Autologous hematopoietic stem cell transplantation (auto-HSCT) has tremendous potential to cure many genetic diseases in the clinic, but its broad curative potential, may be limited because of morbidity/mortality from cytotoxic chemotherapy-based conditioning. To overcome these limitations, we developed antibody drug conjugates (ADC) targeting CD117 (C-KIT) to specifically deplete hematopoietic stem and progenitor cells (HSPC). To validate CD117-ADC mediated depletion prior to HSCT pre-clinically, we developed an optimized tool non-human primate (NHP) short half-life anti-CD117-ADC and evaluated it in an auto-gene modified HSCT in rhesus.

METHODS AND RESULTS

The tool CD117-ADC has potent depletion of human and NHP CD34+ cells in vitro. Humanized NGS mice treated with a single dose had full depletion of human HSPCs in the bone marrow, while maintaining peripheral immune cells. To facilitate use in HSCT, the CD117-ADC was engineered to have a fast clearance and the half-life was 10 hours in NHP.

We next explored whether the tool CD117-ADC could enable auto gene modified HSCT in the rhesus model. Two rhesus NHP were mobilized with G-CSF and plerixafor. The selected CD34+ cells were transduced with β-globin encoded lentivirus and cryopreserved. The animals the received a single dose of the CD117-ADC which did not bind only to CD34+ cells expressing Fc receptors, and for rapid clearance to prevent elimination of incoming transplanted cells.

Figure 1. Tool CD117-ADC as a HSC depleting agent. A) Overview of the Anti-CD117 ADC features. The ADC has a partial payload capability of killing of quiescent and dividing cells. It is engineered with Fc silencing to prevent killing of quiescent and dividing cells expressing Fc receptors, and for rapid clearance to prevent elimination of incoming transplanted cells. A) Anti-CD117 ADC potently depletes primary human and NHP CD34+ cells in vitro as assessed by flow cytometry after 7 day incubation. B) Humanized NGS mice were treated with a single dose of the CD117 ADC which did not bind only to CD34+ cells expressing Fc receptors, and for rapid clearance to prevent elimination of incoming transplanted cells. B) Assess HSC depletion in humanized NGS bone marrow.

Figure 2. Single Dose of short half-life engineered CD117-ADC selectively depletes HSCs in NHPs. A) Study design for single dose CD117-ADC administration in rhesus followed by assessment of HSC depletion in bone marrow at day 6. B) Robust depletion of bone marrow colony forming units was also observed on Day 6 in an ex vivo assay of the bone marrow aspirate. C) Single dose of the CD117-ADC was well tolerated and showed potent depletion of CD34+ cells compared to baseline as assessed by flow cytometry. D) CD117-ADC was engineered for rapid clearance to allow for optimal timing for graft infusion. The engineered CD117-ADC has a half-life of 10 hours and drops below the lower limit of detection of ELISA after 48 hours. Molecular pharmacokinetics shows in grey predict the ADC will be below a cytotoxic concentration after 5 days which should enable safe graft infusion as opposed to a wild type ADC.

Figure 3. Treatment Schema For Gene-Marked Autologous Transplantation in Rhesus Primates. A) CD117 ADC schema. 2 animals were mobilized with G-CSF and plerixafor, their CD34+ cells enriched and transduced with a lentiviral vector encoding beta-globin vector and cryopreserved. The animals the received a single dose of the CD117-ADC at 0.3 mg/kg IV and the transplanted cells were thawed and infused 6 days later. B) Historically published (multi-dose busulfan) cohort of animals in which animals received lentiviral vector transduced CD34+ cells after conditioning with a 4-day dosing regimen of busulfan that was pharmacokinetically dosed to achieve myelosuppression.

CONCLUSIONS

• The engineered tool CD117-ADC shows potent activity on human and NHP CD34+ cells in vitro
• The tool CD117-ADC robustly depletes rhesus HSCs in vivo, has a favorable safety profile, spares the immune system and is cleared rapidly to allow for optimal timing of graft infusion.
• A single dose of the tool CD117-ADC enables engraftment of β-globin gene modified HSCs in a primate model without chemotherapy, achieving similar VCN levels to busulfan.
• These results validate the use of CD117-ADC for targeted stem cell depletion prior to transplantation and supports development as a new conditioning agent for autologous HSCT based gene therapy approaches.
• This targeted approach for safer conditioning could improve the risk benefit profile for patients undergoing transplant and enable more patients to benefit from potentially curative therapies including gene therapy for many genetic diseases including sickle cell disease.