THE IMPACT OF CD45-ANTIBODY-DRUG CONJUGATE CONDITIONING ON CLONAL PATTERNING POST HSPC TRANSPLANTATION IN RHESUS MACAQUES

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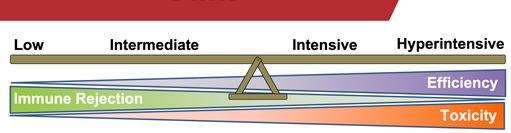
INTRODUCTION

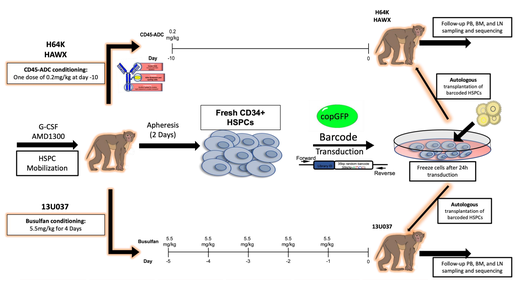
Non-human primate models have been crucial for preclinical development of hematopoietic stem and progenitor cell (HSPC) therapies, since they share many properties with humans(1). Conventional pre-HSPC transplantation conditioning methods such as total body irradiation (TBI) and/or chemotherapy kill dividing cells non-specifically and have significant short and long-term toxicities. Antibody-drug conjugate (ADC) conditioning offers a potentially more targeted and safer approach to clearing the bone marrow (BM) niche for robust HSPC engraftment. Targeting CD45, a cell surface protein expressed only on HSPCs and on all their progeny, including T cells, is of great relevance to the development of HSPC gene therapies and allo-transplantation, because this approach may also prevent rejection of cells expressing foreign transgenes or donor antigens.

AIM



We aimed to investigate hematopoietic reconstitution, tolerance to foreign transgene products and HSPC clonal dynamics after transplantation of lentivirally-transduced and barcoded copGFP-expressing autologous HSPCs following conditioning with a toxin-conjugated anti-CD45 ADC as compared to standard busulfan in the rhesus macaque model.

METHODS



Animal	Conditioning	CD34+ collected (X 10^6)	CD34+ infused (X 10^6)	CD34+/kg (X 10^6)	% CopGFP+ infused cells	Last follow up
H64K	CD45-ADC	33.9	24	4.9	45%	14m
HAWX	CD45-ADC	27	50	7.8	43%	12m
13U037	Busulfan	18.9	50	6.3	61.1%	14m

Details on lentiviral HSPC barcoding following total body irradiation (TBI) or busulfan conditioning are available in our prior publications. Busulfan alone at this dose results in engraftment of transduced autologous cells, but not tolerance to foreign proteins such as copGFP(2-5).

RESULTS

1. Complete blood counts (CBC) over time

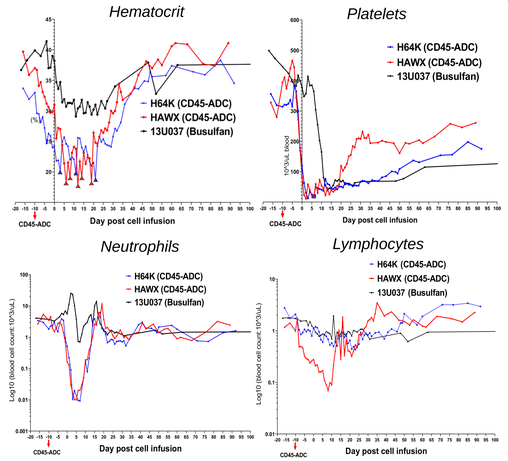


Figure 1. Blood counts over time. CD45-ADC administration occurred on day -10 and infusion of transduced autologous CD34+ HSPCs on day 0. Arrowheads designate whole blood transfusions to treat low hematocrit and the square designates a platelet transfusion for low platelets for the CD45-ADC monkeys. Busulfan monkey 13U037 did not require transfusions post-transplantation.

2. Green fluorescent protein expression and vector copy number in blood cells

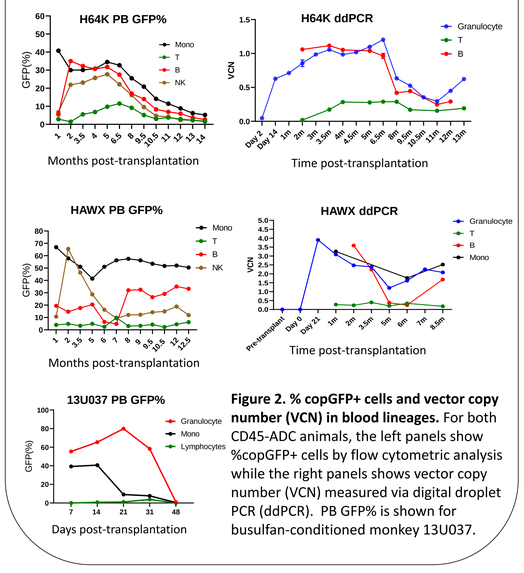


Figure 2. % copGFP+ cells and vector copy number (VCN) in blood lineages. For both CD45-ADC animals, the left panels show %copGFP+ cells by flow cytometric analysis while the right panels shows vector copy number (VCN) measured via digital droplet PCR (ddPCR). PB GFP% is shown for busulfan-conditioned monkey 13U037.

3. Clonality of blood cell lineages post-transplantation

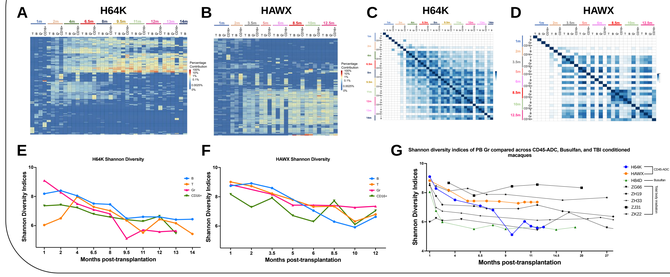


Figure 3. Clonal tracking over time for monkeys H64K and HAWX. Heatmaps showing contributions from the 10 highest contributing clones (barcodes) in each sample mapped across all samples for blood granulocytes (Gr), T, B, and CD16+ NK cells for (A) H64K and (B) HAWX. Each row represents a clone (barcode) and each column a sample. Pearson correlation coefficients heatmaps representing pairwise clonal correlations of contributions from all barcodes retrieved from peripheral blood of (C) H64K and (D) HAWX animals at different time points are shown across timepoints and lineages. Shannon diversity index of (E) H64K and (F) HAWX monkeys shows a decrease in diversity of hematopoietic cellular subsets over time for both animals occurring at time of drop in VCN and %copGFP+ cells. (G) Shannon diversity index of granulocytes isolated from CD45-ADC, busulfan and TBI conditioned monkeys are shown in the same graph(2-5).

4. Anti-copGFP antibody response

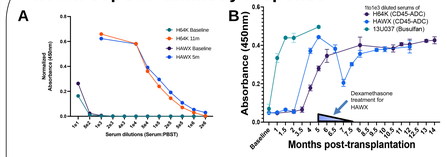


Figure 4. Detection of anti-copGFP antibodies in serum samples. (A) Anti-copGFP detection via ELISA in serially-diluted samples of serum from baseline and for the peak time point in both HAWX and H64K animals. (B) Anti-copGFP detection via ELISA in H64K, HAWX and busulfan conditioned 13U037 NHP serum samples at different time points post-transplantation (all 1:1000 dilutions).

5. Mixed lymphocyte reaction

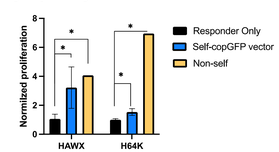


Figure 5. Proliferation rates of lymphocytes after stimulation. Following activation with CD3 and CD28 beads, lymphocytes from 11m and 4m post-transplantation from H64K and HAWX respectively were used as responder cells and cultured for 10 days. Stimulator cells were copGFP-transduced 25 cGY irradiated lymphocytes. "Responder only" included no stimulator cells. "Self-copGFP" added irradiated autologous stimulator cells expressing copGFP. "Non-self" added allogeneic stimulator cells as a positive control.

CONCLUSIONS

- Animals conditioned with 0.2 mg/kg CD45-ADC demonstrated high level short- and medium-term engraftment with barcoded lentivirally-transduced HSPCs.
- CD45-ADC conditioning was well-tolerated, without off-target toxicities
- Highly polyclonal and robust HSPC engraftment persisted for 3-6 months, followed by a decrease in clone number and Shannon diversity, coincident with a drop in copGFP-expressing cells and vector copy number.
- Anti-CopGFP antibodies were detected in both animals at times corresponding to the drop in GFP expression and VCN.
- GFP cell rejection was much slower and less complete with CD45-ADC than following busulfan conditioning and arrested in animal HAWX by a short course of corticosteroids, with residual stable engraftment of copGFP expressing cells.
- A higher dose of CD45-ADC to more potently deplete lymphocytes is being tested, with the goal of achieving stable tolerance to foreign gene products and potentially allo-HSPCs.
- These findings underscore the potential of ADC-conditioning for efficacious, targeted, and non-toxic transplantation conditioning.

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