AMD3100 re-dosing fails to repeatedly mobilize hematopoietic stem cells in the non-human primate and humanized mouse

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Abstract

AMD3100 (plerixafor) is a vital component of many clinical and pre-clinical transplant protocols, facilitating harvest of hematopoietic stem and progenitor cells through mobilization into the peripheral blood circulation. Repeat mobilization with AMD3100 is also necessary for many patients with suboptimal first stem cell collection or those requiring repeat transplantation. In this study we investigated the mobilization efficacy of repeated AMD3100 dosages in the non-human primate and humanized mouse models. In non-human primates we demonstrate effective mobilization after the first AMD3100 administration but significantly poorer response in CD34$^+$ and hematopoietic stem cell-enriched CD90$^+$ cells with subsequent doses of the drug. A similar loss of efficacy with repeated administration was found in immunodeficient mice engrafted with human CD34$^+$ cells, in whom the total human white cell population and particularly human hematopoietic stem and progenitor cells mobilized significantly less effectively following a second AMD3100 administration when compared to first dose. Together, our results are expected to inform future mobilization protocols for the purposes of peripheral blood hematopoietic stem cell extraction or for applications in which hematopoietic stem cells must be made accessible for *in vivo*-delivered gene targeting agents.

Keywords

Hematopoietic Stem Cells; Hematopoietic Stem Cell Mobilization; Hematopoietic Stem Cell Transplantation; Anemia; Sickle Cell

Introduction

Hematopoietic stem and progenitor cell (HSPC) transplantation constitutes the gold standard treatment for many malignant and non-malignant conditions.[1] Mobilization of HSPCs from

the bone marrow (BM) niche into the peripheral blood (PB) circulation is a critical part of most autologous and allogeneic HSPC transplant protocols, facilitating HSPC collection from the circulation by apheresis.[2,3] This avoids invasive BM harvest, with inherent risks of infection, bleeding, structural damage, and the need for a general anaesthetic.[4,5] A further advantage to transplantation of PB HSPCs rather than BM-derived cells is more rapid hematopoietic reconstitution.[5,6]

The most frequently administered HSPC mobilization agent is the pro-inflammatory cytokine granulocyte colony stimulating factor (G-CSF).[2,7,8] AMD3100 (Plerixafor) was first approved for clinical use as a second mobilization agent in 2008, providing an additional or alternative option for patients and donors. AMD3100's mechanism of action is primarily through reversible, competitive antagonism of the cytokine receptor CXCR4 expressed on the HSPC cell surface, interrupting the bond with its ligand CXCL12 in the BM. Interaction with a second receptor, CXCR7, for which AMD3100 acts as an allosteric agonist, is also likely to contribute to its mobilization property.[9,10] In addition, AMD3100 administration has been demonstrated to reverse the CXCL12 gradient across the BM niche in mice, which may contribute to the egress of HSPCs from the BM into the PB circulation. [11] AMD3100 is most frequently used alongside G-CSF, since both molecules synergize to increase numbers of circulating HSPCs and offer better chance of successful harvest.[12,13] AMD3100 has also been used as a single mobilization agent in patients and healthy donors, thereby circumventing the side-effects and potentially severe complications associated with G-CSF administration.[14–17] These include bone pain, 'flu-like symptoms, and splenic rupture.[18,19] AMD3100 administration, on the other hand, is usually associated with minimal side-effects and a more rapid mobilization profile than G-CSF.[15,20,21] In sickle cell disease patients, single agent AMD3100 is the only safe and effective mobilization regimen available to facilitate *ex vivo* genome modification strategies, since G-CSF can precipitate life-threatening sickle cell crises.[22–24]

Mobilization of HSPCs into the PB is increasingly important in the field of *ex vivo*- as well as *in vivo*-genome engineering. Since hematopoietic stem cells are responsible for long-term repopulation of all hematopoietic cell lineages, targeting these cells for genetic manipulation by genome editing or gene transfer therapy results in modification of their entire progeny.[25] This approach offers the promise of long-term therapeutic alteration within all blood cell types and hence cure of a vast array of hematological and immunological disorders.[26] *Ex vivo* genetic manipulation of autologous HSPCs prior to reinfusion is being trialled in many preclinical applications and is already under clinical investigation for the treatment of various conditions including sickle cell disease, β-thalassemia and severe combined immunodeficiency.[27–29] There is now growing interest in *in vivo* genome engineering of HSPCs, which in most cases also relies on mobilization of these cells into the periphery to facilitate exposure to agents such as retroviral vectors or nanoparticles.[30–33]

A single mobilization episode results in inadequate HSPC collection in many patients, requiring remobilization at a future date.[2] Sickle cell disease patients mobilized for HSPC collection and gene therapy treatment are predicted to require repeat mobilization in one third of cases.[22] Remobilization may also be employed at the point of disease relapse for

second or subsequent autologous transplants.[1] It is therefore important to establish the efficacy of repeated AMD3100 administration for HSPC mobilization.

In this study, we investigated the effect of repeated AMD3100 administration on HSPC mobilization in two key preclinical transplant models: the non-human primate (NHP) and the humanized mouse model. The NHP model has been extensively established by our group and has the advantage of a high degree of systemic uniformity and genetic homology to humans, allowing more direct translation to the clinic.[34–36] In this model we further described the hematopoietic stem cell-enriched CD34⁺CD90⁺CD45RA⁻ (CD90+) subset as a novel target for genome editing to reactivate fetal hemoglobin for the cure of β-hemoglobinopathies.[27,34,37] While these studies used G-CSF-primed BM-derived HSPCs for gene modification and transplantation, we sought to implement a mobilization regimen based on AMD3100 alone followed by PB stem cell collection, since this more closely reproduces standard clinical practice and is currently the gold standard for sickle cell disease patients.[22,24]

To complement these findings in NHPs and directly examine the response of first and subsequent dosages of AMD3100 in human HSPCs, we additionally used the mouse xenotransplantation model. Non-obese diabetic (NOD)-severe combined immunodeficiency (SCID)-common γ chain−/− (NSG) mice engrafted with human CD34⁺ cells are commonly used as a preclinical model to study HSPC transplantation and multilineage engraftment. [38–40] In addition, this model has been employed for the evaluation of *in vivo* gene therapy strategies involving HSPCs mobilized with G-SCF and AMD3100.[41,42] Together our data demonstrate reduced mobilization of HSPCs with repeated AMD3100 administration in both the NHP and humanized mouse models.

Results

Reduced CD34⁺ HSPC and hematopoietic stem cell (HSC)-enriched CD90⁺ PB mobilization upon repeat ADM3100 mobilization in NHPs

We first investigated kinetics of mononuclear cell (MNC) mobilization (taken to be reported total white cell count) in healthy adult rhesus macaques (*Macaca mulatta*) receiving a first dose (n=5), a second dose (n=3) and a third dose (n=1) of AMD3100. Peak MNC count in the PB was found at 2–3 hours post AMD3100 dose, a little earlier than previously reported (Fig 1A).[43]

Mean baseline MNC count prior to the first dose of AMD3100 was 10.5×10^9/l (within normal range) and rose significantly to 30.5×10^9/l after treatment (n=5, p=0.0003). Mean baseline MNC count prior to the second or third dose of AMD3100 was 9.6×10^9/l, and rose to 24.3×10^9/l after treatment (n=4, p>0.05). There was no significant difference between baseline MNC counts before first vs subsequent dose AMD3100, or between peak MNC count following first vs subsequent doses (Fig 1B). Where analysis was restricted to the results pertaining to first vs second dose AMD3100 only in animals receiving at least 2 doses (n=3), mean baseline MNC count prior to first dose AMD3100 was 11.3×10^9/l and rose significantly to 31.0×10^9/l (p= 0.0152). Prior to second dose AMD3100 baseline MNC count was 10.3×10^9/l and rose to 20.0×10^9/l (p>0.05). Again, there was no significant

difference between baseline or peak MNC count when comparing first to second dose AMD3100. In summary, these data demonstrate successful increase in the MNC count in NHPs following first AMD3100, with non-significantly reduced response on repeated treatments.

Kinetics of $CD34^+$ cell mobilization with first dose AMD3100 are consistent with those reported by other groups (Fig 1C, Fig 1D). In contrast to MNCs, peak $CD34^+$ count in PB was significantly reduced with repeated AMD3100 dosages. Mean $CD34^+$ cell count following the first dose of AMD3100 for all animals was 38.3×10^6/l (n=5), whereas $CD34^+$ count measured after each subsequent dose was significantly reduced by nearly 7-fold with a mean of 5.7×10^6/l (n=4, p=0.0005, Fig 1E). Where results for animals receiving first and second dose AMD3100 only were taken, peak $CD34^+$ cell count after first dose was 45.2×10^6/l and after second dose was 5.5×10^6/l (n=3, p=0.0011).

We were also interested to determine if similar results apply to our recently described HSC-enriched immunophenotype $CD34^+CD90^+CD45RA^-$.[37] The timing of $CD90^+$ mobilization appears to follow a similar pattern to that of the total $CD34^+$ population (Fig 1C, Fig 1F). [43,44] Similarly to bulk $CD34^+$ counts, we observed a significant decrease in the number of $CD90^+$ cells mobilized after the second or third AMD3100 doses relative to the first dose. PB $CD90^+$ count at time of peak $CD34^+$ mobilization was 12.9×10^6/l (n=5) after the first dose and was reduced to 0.9×10^6/l (n=4) after subsequent dosages (p=0.0234, Fig 1G). Where results for animals receiving first and second dose AMD3100 only were taken, peak $CD90^+$ cell count were 15.0×10^6/l and 0.5×10^6/l respectively (p>0.05).

Therefore, while total MNC count demonstrated only a non-significant trend towards reduced response with repeated doses of AMD3100, HSPC mobilization (both bulk $CD34^+$ cells and HSC-enriched $CD90^+$ cells) was significantly poorer with second and subsequent dosages in comparison to first AMD3100 administration.

Reduced human HSPC mobilization with repeated AMD3100 administration in the humanized mouse model

Our observation that HSPCs fails to re-mobilize with repeat AMD3100 in NHPs was unexpected, so we sought to further investigate this phenomenon in human HSPCs. For this, we used the NSG mouse transplantation model. NSG mice were engrafted with human $CD34^+$ for 20 weeks, and subsequently received a first, then a second dose of AMD3100, and the effect on human HSPC mobilization was measured by PB analysis at 1.5 hours after dosing. Among these, a selected number of animals also received a third dose of AMD3100. Human engraftment, as reflected by the frequency of human $CD45^+$ ($hCD45^+$) cells, remained stable during the course of the experiment (Fig 2A–B). Time between each AMD3100 administration episode was 3–4 weeks.

The response of total human MNCs was assessed first, as defined by the $hCD45^+$ cell population, by comparing baseline to peak levels, and a reduced count in PB was found after each sequential AMD3100 dosing. After the first dose, mean human MNC count rose from 0.19×10^9/l to 0.50×10^9/l (n=8, p=0.0002). After second dose, the increase was less significant, from 0.17×10^9/l to just 0.27×10^9/l (n=8, p=0.0349). Following a third dose of

AMD3100, there was no significant increase in circulating human MNCs when comparing baseline 0.24×10^9/l (n=8) to peak count 0.33×10^9/l (n=4, p>0.05). Furthermore, we noted a significant decrease in the amplitude of mobilized hCD45$^+$ cell count between first and second doses (n=8, p=0.0349) (Fig 2C).

Even more importantly, the response of human HSPCs, as defined by CD34$^+$ cells, was also reduced with repeated doses of AMD3100. With a first dose, mean CD34$^+$ cell count rose from 0.24×10^6/l to 1.97×10^6/l (p=0.0135) whereas counts with the second and third doses did not change significantly (from 0.59×10^6/l to 0.51×10^6/l with second dose and from 0.19×10^6/l to 0.07×10^6/l with third dose). The amplitude of mobilization of human HSPCs following second or third dose AMD3100 was also significantly lower than after first dose (p=0.0034 and p=0.0219 respectively) (Fig 2D).

A second humanized NSG mouse cohort was used to confirm the loss of efficacy of repeated AMD3100 dosages in this model. Results confirmed findings of poorer hCD45$^+$ and CD34$^+$ cell mobilization with a second dose of AMD3100 when compared to first dose (n=7, p=0.0065 and p=0.0225 respectively). (Suppl figure 1).

In summary, total human MNCs mobilized more poorly in response to subsequent doses of AMD3100 in comparison to first dose and CD34$^+$ cells also failed to mobilize with repeat doses after a good response to first dose was demonstrated. Therefore, the reduction in AMD3100 efficacy with repeated dosing first noted in the NHPs was confirmed to also apply to human HSPCs.

AMD3100 reverses the CXCL12 gradient across the BM in humanized mice and is not impacted by repeated dosing

One hypothesis for the mechanism behind the loss of AMD3100 efficacy with repeated dosing is a failure to re-establish the CXCL12 gradient reported in mice following the first AMD3100 exposure, after each subsequent dose.[11] To investigate this hypothesis, we measured plasma CXCL12 pre- and post-AMD3100 treatment in several engrafted mice after the first and second dosing. Consistent with AMD3100 reversing the CXCL12 gradient across the BM, we found that plasma CXCL12 levels increased after administration (Fig2E). There was no statistical difference in this increase in CXCL12 after repeat AMD3100 dosage when compared to first dose, thus ruling out this possible mechanism to explain the reduced AMD3100 efficacy in this context.

Discussion

We have presented results demonstrating significantly poorer mobilization of HSPCs in NHPs following repeated administration of AMD3100 in comparison to first dose. This is the case for the CD34$^+$ cell population as a whole, the most commonly used marker for HSPCs and therefore the measure of mobilization adequacy employed routinely in the clinic and in preclinical NHP transplant protocols. Arguably of even greater consequence, we also observed reduced mobilization of the HSC-enriched CD90$^+$ subset with repeat AMD3100 dosage. Single dose AMD3100 mobilization was previously documented in NHPs,[45,46] this data adds to the available literature by characterizing the response to repeat AMD3100

administration in a cohort of rhesus macaques. Given the importance of the NHP model in preclinical investigation, including HSPC transplant and genome engineering studies, these results are noteworthy.

Failure of human HSPCs to remobilize with repeated AMD3100 doses in the humanized mouse model was demonstrated in 2 separate animal cohorts. Response of the total human mononuclear cell population was also reduced with subsequent doses. Since this is the most commonly used pre-clinical animal model for the study of human hematopoiesis, the inability to effectively remobilize HSPCs in these experimental animals with AMD3100 is also highly significant and will impact upon preclinical protocols for the investigation of a multitude of conditions.

The loss of AMD3100 activity on repeated administration in these 2 valuable preclinical models is of greatest significance where therapeutic interventions for sickle cell disease are being investigated. This is because AMD3100 is the only clinically available mobilization agent which can safely be used in this patient population. Limitations imposed by an inability to reuse this drug effectively in experimental animals may result in protocols utilizing AMD3100 alone being abandoned. This would introduce a further level of variance between preclinical and clinical protocols for the treatment of sickle cell disease including modified autologous transplantation and *in vivo* genome engineering.

It was previously documented that the clinical response to G-CSF may be reduced on repeated exposure. Whilst there are some conflicting data relating to remobilization in patients, where healthy donors were given repeated doses of G-CSF with up to a year between doses, efficacy of remobilization was reduced, demonstrating a long-lasting deficit in remobilization.[47,48] This is the first study to demonstrate a similar deficit in remobilization with repeat AMD3100 dosing, many weeks after the initial exposure. This loss of efficacy is highly significant in practical as well as statistical terms.

One human study in healthy volunteers reported adequate $CD34^+$ cell mobilization with a repeated dose of AMD3100, at least 2 weeks after initial dose.[49] One significant difference between this study and ours is the AMD3100 dosage used. This clinical study used doses of only 0.24 mg/kg or 0.48 mg/kg whereas significantly higher doses of 1mg/kg (NHPs) and 5mg/kg (immunodeficient mice) were administered in our experiments. It is likely therefore that a higher proportion of the potentially AMD3100-sensitive cells were mobilized in our preclinical models than in the clinical study of healthy donors. We hypothesize therefore that the reservoir of potentially AMD3100-sensitive cells remaining in the BM after first mobilization was greater in the healthy donor study than in the NHPs or mice, allowing for successful mobilization of a different cell population upon second AMD3100 administration in the volunteer donors. This reservoir of mobilizable cells was proportionally more depleted in the preclinical models leaving fewer potentially AMD3100-sensitive cells to be mobilized upon repeat dosing of this drug, resulting in failure of HSPC mobilization on subsequent administration. It may be that if higher AMD3100 doses are used in the clinic then a similar depletion of potentially mobilizable cells would occur after first administration, and failure of remobilization on repeated dosing would also be seen.

Furthermore, pathological BM such as that of sickle cell disease or thalassemia patients cannot be assumed to respond to repeated AMD3100 administration in the same way as that of a healthy volunteer. Defective hematopoietic support of BM stromal cells from β-thalassemia patients has been demonstrated,[50] and, anecdotally, patients with β-thalassemia have been found to remobilize poorly with repeated AMD3100 dosing (C Samuelson, personal communications). Further clinical studies are required to investigate the response of patients in this scenario, particularly those with the types of hematological pathology most likely to require repeated AMD3100 administration, such as the β-hemoglobinopathy populations.

We have presented data showing poor response to repeated AMD3100 administration in humanized mice up to 4 weeks after initial dose, and in NHPs up to 14 weeks after first exposure. It is unclear how long this effect would last and further studies are recommended to investigate this in the preclinical models described. This study has investigated the effect of repeated AMD3100 when dosed as a single mobilization agent. It must be established whether the same drop in efficacy with repeat AMD3100 dosing is also observed where this drug is combined with G-CSF, as is often the case in clinical practice. This also warrants detailed investigation, as the implications of such would be even more far-reaching. For example, the American Society for Blood and Marrow Transplantation (ASBMT) recommends that in patients failing to mobilize adequately during a first episode, a repeat mobilization attempt can begin only 2–4 weeks following the first.[2] As many of these patients would have received AMD3100 during their first attempt and are then likely to receive it also during repeat treatment, it is important to establish whether a fall-off in AMD3100 efficacy in this scenario is also a concern.

The mechanism for reduced HSPC mobilization in response to repeat AMD3100 has not yet been elucidated. One hypothesis investigated in our studies was that this may be due to a reduction in the plasma CXCL12 response to repeat AMD3100 dosing – a response demonstrated in BALB/c mice by Redpath et al.[11] However, our results have shown no significant difference in the rise in plasma CXCL12 concentration with second as compared to first dose AMD3100, thereby ruling out this hypothesis. Of note, this is the first report of plasma CXCL12 response to AMD3100 in humanized NSG mice. Redpath also presented data showing a simultaneous drop in BM CXCL12 level as the plasma concentration rises, following AMD3100 administration. As measurement of BM CXCL12 levels would have required early necropsy of study animals, this was not carried out in this study but an alternative hypothesis as to the cause of AMD3100 inefficacy with repeated dose is a reduction in the response to AMD3100 of the BM milieu, including a possible deficit of CXCL12 response. Future investigations should also include further cellular analysis of HSPCs exposed to AMD3100, including detailed examination of CXCR4 and CXCR7 activation, endocytosis and recycling.

In conclusion, we have presented evidence of a failure of NHPs and humanized mice to remobilize HSPCs on repeat administration of AMD3100. This has significant implications for pre-clinical transplant and HSPC genome engineering protocols. It remains to be proven whether the pathological BM of patients with hematological disorders will similarly fail to

respond to repeat mobilization attempts with this drug and clinical studies in this area are warranted.

Materials and Methods

Ethics and animal welfare statements

All experimental animal protocols employed in these studies were granted approval by the Institutional Animal Care and Use Committee (IACUC) of the Fred Hutchinson Cancer Research Center and University of Washington. NHPs were treated under protocol #3235-01 and NSG mice under protocol #1864. All animal management conforms to recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.[51]

Rhesus macaques were housed at the UW National Primate Research Center (WaNPRC) under conditions approved by the American Association for the Accreditation of Laboratory Animal Care and in accordance with Animal Welfare Act regulations. Animals were cared for and monitored by accredited animal technicians and veterinarians. They were administered sedation prior to blood draws and were fasted appropriately prior to this. All procedures and routine care were conducted according to WaNPRC standard operating procedures.

NSG mice were bred in-house under approved protocols and in pathogen-free housing conditions. Blood draws (by retro-orbital puncture), tail vein and subcutaneous injections were carried out by appropriately trained animal technicians according to center protocols.

AMD3100 mobilization

NHPs underwent baseline blood sampling prior to AMD3100 (AMD3100 octahydrochloride hydrate, Sigma-Aldrich, MO, US) administration then received a first dose of AMD3100 at 1mg/kg SQ resuspended in sterile H20 at a concentration of 5mg/ml. PB draws were taken at a minimum of 2 time points between 2–3.5 hours post-dose into anticoagulated blood collection tubes. 1 animal was sedated for a longer period of time and had additional blood samples drawn: hourly blood draws from 2–7 hours post dose. Samples were taken for investigation of complete blood count (CBC) and for fluorescent activated cell sorting (FACS).

Some of these same animals were given a second administration of AMD3100 6–14 weeks after the first, with identical dosing protocol. Blood draws were again taken at a minimum of 2 timepoints 2–3.5 hours post dose. 2 animals had additional blood draws taken: 1 also had blood drawn at 4 hours post dose and 1 had hourly blood draws from 2–7 hours post dose. PB samples were processed as described below. One animal received a third dose of AMD3100 4 weeks after the second dose with dosing regimen and post-dose testing carried out as above.

2 cohorts of NSG mice were humanized in adulthood, at the age of 6–8 weeks. Humanization was carried out by a single tail vein injection of human G-CSF-mobilized, CD34$^+$-enriched PB MNCs via the tail vein, at $0.5 – 1.5 \times 10^6$ cells per mouse, resuspended

in phosphate-buffered saline with 1% heparin (APP Pharmaceuticals, IL, US) to a total volume of 200ul, following 275 cGy irradiation.

The first cohort received first dose AMD3100 20 weeks post humanization, followed by second dose and for surviving mice a third dose was also given. The second cohort received first dose AMD3100 9 weeks post humanization followed by a second dose. The time interval between doses was 3–4 weeks in every case. Each dose consisted of SQ injection of 5mg/kg AMD3100 made up to a total of 100ul with sterile H2O. Following each dose blood was drawn 1.5 hours post AMD3100, to coincide with expected peak of mobilization, for CBC and FACS analysis.[52]

CBC and flow cytometric analysis of PB

Aliquots of NHP blood underwent CBC testing by the UW Medicine Laboratories using the Sysmex XN 9000 Hematology Automation Line (Sysmex, IL, US) with MNC reported as total white cell count. Samples for FACS testing first underwent lysis of red blood cells by application of hemolytic buffer (150mM Ammonium chloride, 12mM Sodium bicarbonate, and 500mM EDTA in water). Remaining cells were stained with anti-CD45-V450 (clone D058-1283, BD Biosciences, CA, US); anti-CD34-APC (clone 563, BD Biosciences); anti-CD45RA-APC Cy7 (clone HI100, BD Biosciences) and anti-CD90-PE (clone 5E10, Biolegend, CA, US). FACS was carried out on the BD FACSAria II or BD FACSCanto II flow cytometers.

PB from the NSG mouse cohorts was analysed for total MNC count using the Element HT5 Veterinary Hematology Analyser (Heska, CO, US). Remaining blood was spun down and plasma extracted. Cells then underwent red cell lysis using BD Pharm Lyse™ buffer (BD Biosciences) followed by staining for FACS analysis. Antibody panel utilized for staining included: anti-human CD45 (hCD45)-PerCP or anti-human CD45-PerCP- Cy™5.5 (both clone 2D1); anti-mouse CD45-V500 (clone 30-F11) and anti-CD34-APC (clone 563) (all BD Biosciences). CD34$^+$ gating was informed by results of an FMO sample processed with each cohort of blood samples, stained with anti-hCD45 and anti-mCD45 but not anti-CD34 (clones as above).

Post-acquisition analysis of flow cytometric data was carried out using FlowJo Version 9.

Statistical analysis

For NHP samples, the percentage of CD34$^+$ cells was defined by CD34-high/CD45-low fluorescence within the MNC population (MNCs first being defined by forward and side scatter). Within the bulk CD34$^+$ cell population, the proportion of the hematopoietic stem cell-enriched subpopulation as defined by CD34$^+$CD90$^+$CD45RA$^-$ was determined. At each blood draw timepoint, absolute circulating CD34$^+$ count was calculated by multiplication of CD34$^+$ fraction of MNCs by the total circulating MNC count. Absolute CD90$^+$ cell response was determined by multiplication of CD90$^+$ fraction of CD34$^+$ cells by absolute CD34$^+$ cell number.

For mouse samples, total circulating mononuclear cells and total circulating CD34$^+$ cells were determined at each time point by multiplication of human proportion of CD45$^+$ cells

by total MNC count (taken to be total white cell count reported on CBC), and by multiplication of fraction of $CD34^+$ cells by human $CD45^+$ count, respectively.

Presentation of graphical data utilized GraphPad Prism 7 for Windows (Prism). Excel 2016 for Windows and Prism were used for statistical analysis. Descriptive statistics are presented along with comparison of results related to first and subsequent AMD3100 doses by 2-tailed t-test. Paired t-test is applied where each value has an appropriate paired result; unpaired t-test is applied in every other case. p values of <0.05 are taken to be significant (*) and p values of <0.001 are taken to be highly significant (**).

Plasma CXCL12

Plasma CXCL12 levels were measured pre- and post-AMD3100 dosing for first and second doses in the first mouse cohort. Blood samples were spun down at 1700rpm for 15 mins and plasma extracted by pipetting. Plasma from each blood draw was frozen to allow simultaneous testing of all samples for CXCL12 level by ELISA assay using Mouse CXCL12/SDF-1 alpha Quantikine ELISA Kit (R+D Systems, MN, US) with analysis of optical density carried out on the Epoch microplate spectrophotometer (Biotek, VT, US).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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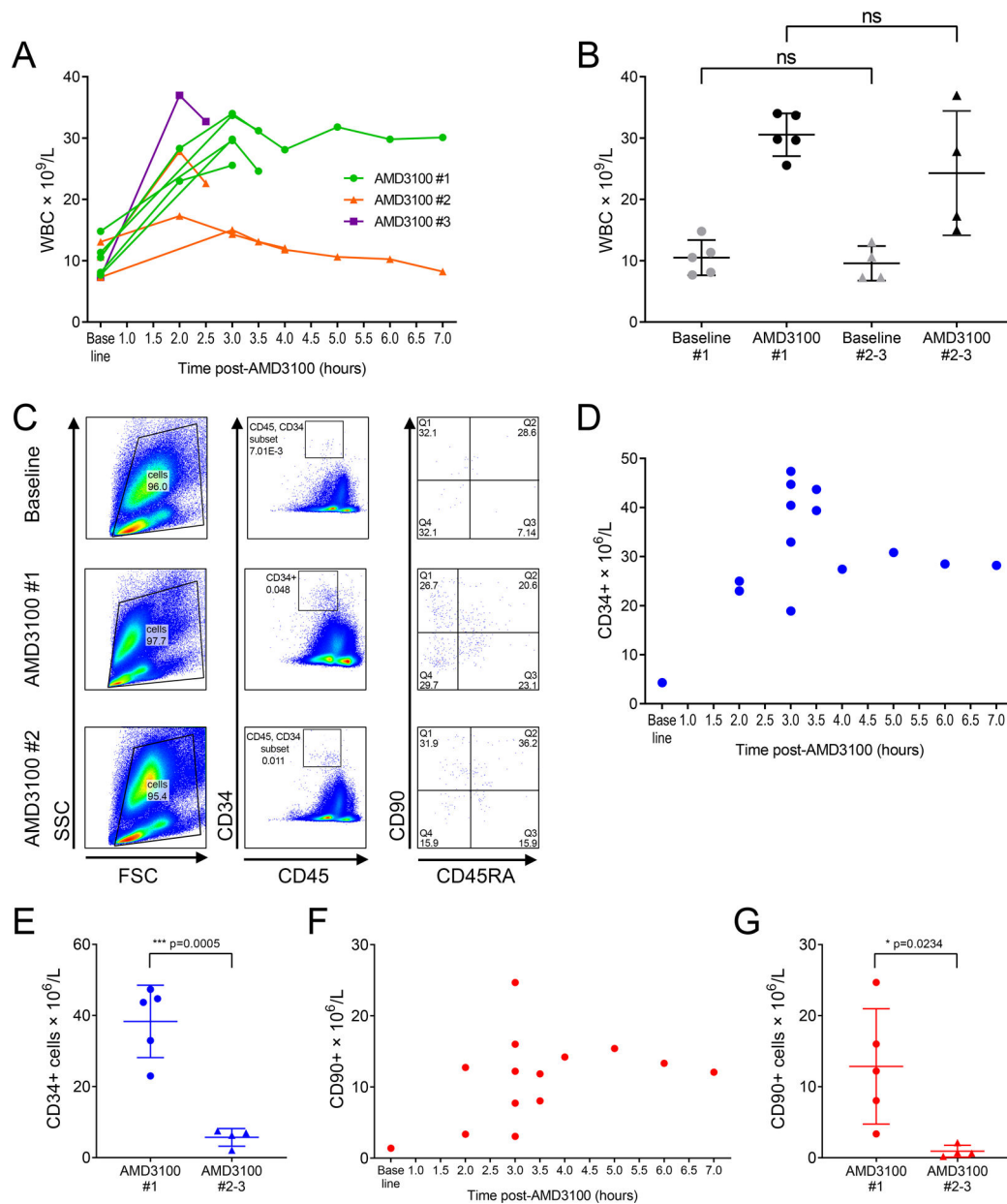
Figure 1. :

Mononuclear cell counts (MNC), CD34$^+$ HSPCs and HSC-enriched CD90$^+$ subpopulation counts in NHPs treated with one or repeated AMD3100 doses.

A: Longitudinal analysis of MNC count in PB of each NHP treated with 1, 2 or 3 AMD3100 doses. B: MNC counts measured pre-AMD3100 (baseline) and at the peak post-AMD3100 administration in all animals following first and subsequent AMD3100 doses (ns = non-significant). C: Representative example of flow cytometric gating strategy for the CD34$^+$ and CD90$^+$ populations. D: Kinetics of CD34$^+$ cell mobilization post first dose AMD3100. E: Peak CD34$^+$ cell mobilization post-AMD3100 in all animals following first and subsequent AMD3100 doses. F: Kinetics of CD90$^+$ cell mobilization post first dose AMD3100. G: CD90$^+$ count within bulk CD34$^+$ population at point of peak CD34$^+$

mobilization in all animals following first and subsequent AMD3100 doses. In B, E and G, bars show mean and SD.
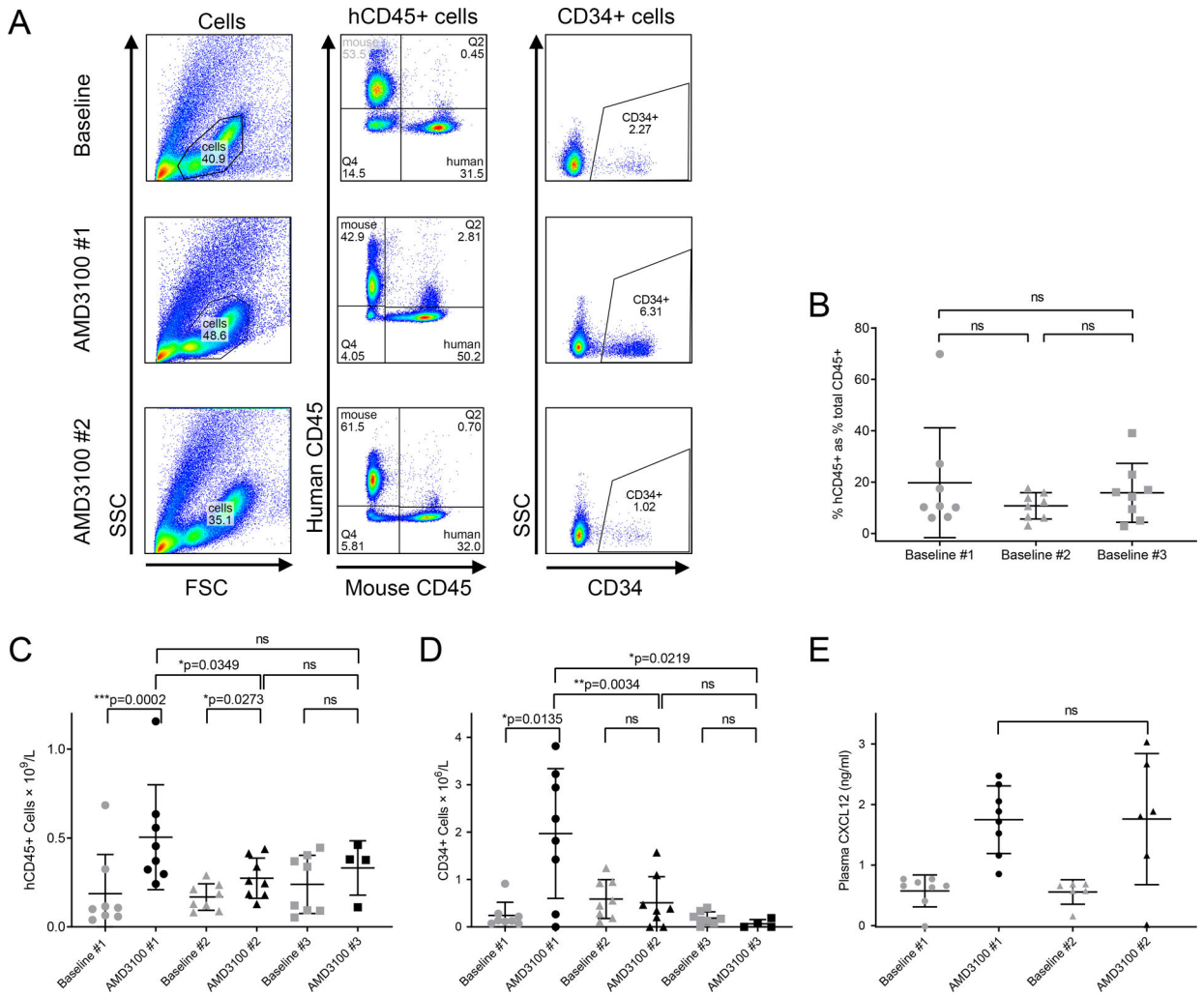
Figure 2. :

Human CD45^{+} and CD34^{+} HSPC counts, and plasma CXCL12 levels in humanized mice treated with one or repeated AMD3100 doses.

A: Representative example of flow cytometric gating strategy utilized in humanized mice. B: Human engraftment, calculated as human CD45^{+} cell percentage of total CD45^{+} cells (mean, SD). C: Absolute hCD45^{+} cell count in peripheral blood pre- and post-AMD3100 doses 1–3 (mean, SD). D: Absolute CD34+ cell count in peripheral blood pre- and post-AMD3100 doses 1–3 (mean, SD). E: Plasma CXCL12 levels measured in peripheral blood pre- and post-AMD3100 doses 1–2 (mean, SD).