Anti-human CD117 antibody-mediated bone marrow niche clearance in non-human primates and humanized NSG mice.

Authors:

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Materials and Methods

Non-human primate animal care, study design, and clinical observation

Eight juvenile 37-39 months old male cynomolgus macaques (3.4–4.0 kg) were purchased from Charles River Laboratories and housed at the SRI International (Menlo Park, CA) animal facility in accordance with the National Research Council (NRC) Guide for the Care and Use of Laboratory Animals, 8th edition (2011) and the Animal Welfare Standards incorporated in 9 CFR Part 3, 1991. A series of three tuberculin (TB) tests, blood tests, and a complete physical examination was performed on each NHP before quarantine release. Two randomly assigned animals were grouped to receive increasing doses of AMG 191 (0.1 mg/kg, 1.0 mg/kg, 5.0 mg/kg, 25 mg/kg). Assigned dose levels of AMG 191 were administered into animals via a single intravenous bolus injection. Animals were monitored at least once daily and clinical observations were recorded once daily on days 1 through 21 and days 41 through 45, and immediately post-dose on treatment days, or more often as clinical signs warranted. Animals were examined for any altered clinical signs, including gross motor and behavioral activity, and observable changes in appearance. Body weights were recorded once weekly during days 1 through 21 and days 42 through 46.

Collection of NHP blood for hematology

Blood were collected using K_3EDTA as the anticoagulant for hematology analysis on day 1 prior to dose administration and days 4, 7, 10, 15, 21 and 42. Animals were fasted before blood collection.

Preparation of serum and pharmacokinetics of AMG 191 in serum from NHP

2 ml of whole blood was collected from femoral, cephalic, or saphenous vessel into tubes without anticoagulant to process serum on days 1 (pre-dose and 5 min post-dose), 4, 7, 10, 15, 21, and 42. The level of AMG 191 in serum was analyzed at Eurofins Pharma Bioanalytical Services US Inc. (St. Charles, MO) using the electrochemiluminescent method developed by Amgen Inc. Briefly, microplates were coated with histidine tagged human CD117 protein as the capture reagent. Diluted serum samples along with standards were incubated in coated microplates. After a series of thorough washing, AMG 191 were detected by a biotin conjugated monoclonal anti-AMG 191 antibody and ruthenium labeled streptavidin. Electrochemiluminescence signals were measured on the Sector Imager 6000 (MSD®) at 620 nm. Pharmacokinetics of AMG 191 was determined using Phoenix® WinNonlin® (version 6.3) software to perform non-compartmental modeling. Maximal serum concentration (Cmax), time to maximum serum concentration (Tmax), area under the serum concentration-time curve (AUC), and terminal elimination half-life ($t_{1/2}$) were determined.

Collection of bone marrow aspirates from NHP

Bone marrow aspirates from NHP were collected on days 0 (pre-treatment), 4, 7, 10, 15, 21, and 42. Animals were anesthetized for bone marrow collection. A small incision was made on the skin using a scalpel, following which an 18-gauge 1-inch bone marrow needle (or an appropriate size needle based on the size of the NHP) was pushed with firm pressure and a twisting motion into the marrow cavity. The stylet was removed from the needle, a heparin-coated 10-ml syringe was attached, and the plunger was pulled back to collect up to 2 ml of bone marrow. Immediately after collection, bone marrow aspirates were re-suspended with ~10 ml PBS (pH 7.2 to 7.4) and heparin

(40 unit/ml) at room temperature followed by filtration with 70 µm cell strainer. Cell numbers in bone marrow aspirates were counted by Cellometer Auto 2000 cell counter using acridine orange stain to enumerate nucleated cells and propidium iodide to determine viability. Mononuclear cells were isolated by Ficoll density gradient centrifugation followed by red blood cell lysis.

AMG 191 treatment of humanized NSG mice

Human $CD34^+$ hematopoietic stem cell-engrafted humanized NSG™ mice (hu-CD34 NSG, 10-11 weeks old) were purchased from Jackson Laboratory. Humanized mice had 35-95% human cell ($hCD45^+$ cells) chimerism in BM when received. Femoral BM aspirates from humanized NSG mice were collected before AMG 191 administration to monitor the baseline level of human cell composition and HSPCs in BM. 0.3 and 1.0 mg/kg of AMG 191 was injected into humanized NSG mice via a single intravenous bolus injection and the second BM aspirates were collected 2-weeks post AMG 191 injection from the opposite femur to monitor the depletion of HSPCs. The BM aspirate was collected in 500 µl PBS containing 10 mM EDTA and 2% fetal bovine serum. Cells were centrifuged and stained with antibody cocktail (PacOrange-hCD45, FITC-mCD45, APC-hCD34, BV421-hCD117 (104D2), PE-Cy7-hCD38, PE-hCD13/hCD33, APC-Cy5.5-hCD19, APC-Cy7-hCD3, PE-Cy5-mTer119, Propidium Iodide). The procedure of BM aspirate collection from mouse was approved by the Administrative Panel on Laboratory Animal Care (APLAC) at Stanford University.

Transplantation of mCitrine-transduced $CD34^+$ cells into humanized NSG mice treated with AMG 191

The vector containing CCL-Ubiq-mCitrine-PRE-FB-2XUSE was packaged in a VSV-G pseudotyped lentivirus, concentrated and titered as previously described[1,2]. Pooled cord blood $CD34^+$ cells (Stemcell Technologies) were thawed and plated in non-tissue culture treated 6-well plate coated with retronectin (20 µg/mL, TakaraBio) at a cell density of 5×10^5 cells/mL. Cells were pre-stimulated for 18–24 hours at 37°C, 5% CO2 in transduction medium containing serum free X-vivo 15 medium (Lonza), hSCF (300 ng/mL, PeproTech), hTPO (50 ng/mL, PeproTech), hFlt-3L (100 ng/mL, PeproTech), L-glutamine/penicillin/streptomycin (Gemini Bio-Products). After pre-stimulation, filter-concentrated virus was added to each well at 2×10^7 TU/mL and further incubated at 37°C, 5% CO2 for 24 hours. Transduction efficiency of $CD34^+$ cells with mCitrine expressing lentivirus was ~75% at the time of transplantation confirmed by FACS analysis (data not shown). On 23 and 25 days post AMG 191 injection, humanized NSG mice along with untreated animals were transplanted with unsorted 210,000 and 340,000 of $CD34^+$ cord blood cells transduced with mCitrine expressing lentivirus, respectively. Engraftment level was monitored by quantifying the $mCitrine^+$ cells in each cell subset in BM aspirates of transplanted mice at 6 weeks post-transplantation. BM mononuclear cells were stained with antibody cocktail (Pac Orange-hCD45, FITC-mCD45, APC-hCD34, BV421-hCD117 (104D2), PE-Cy7-hCD38, PE-hCD13/hCD33, APC-Cy5.5- hCD19, APC-Cy7-hCD3, PE-Cy5-mTer119, Propidium Iodide). Cell staining was analyzed on FACSAria™ (BD Biosciences). Data were analyzed using FlowJo 9.5 (Tree Star). Statistical significance (p-value) was determined using paired student *t*-test and Prism software.

HSC profiling of human and NHP hematopoietic cells

Human and NHP BM aspirates were obtained from AllCells and Bioreclamation, respectively. Mononuclear cells were isolated by Ficoll density gradient centrifugation followed by red blood

cell lysis. Cells were stained with PE-Cy7 conjugated human lineage mAbs (anti-CD3, -CD4, -CD8, -CD14, -CD16, -CD20), PE conjugated anti-human CD90, APC conjugated anti-human CD34, and BV421 conjugated anti-CD117 mAbs. PE-Cy7 conjugated human lineage mAbs, anti-CD3 (SP34.2), anti-CD4 (OKT4), anti-CD8 (RPA-T8), anti-CD14 (M5E2) anti-CD16 (3G8), and anti-CD20 (2H7), anti-hCD34-APC (561), anti-mouse IgG1 (RMG1-1) and anti-hCD117-BV421 (104D2) were purchased from Biolegend. FITC-hCD45RA (HI100), PE-hCD13 (WM15), PE-hCD33 (HIM3-4), APC-Cy7-hCD3 (OKT3), APC-Cy5.5-hCD19 (HIB19), FITC-mCD45 (30-F11), PE-Cy7 mCD45 (30-F11) and PE-Cy7-hCD38 (HIT2) antibodies were purchased from eBioscience. Anti-hCD90-PE (PR3) was from Pacific GMP. AMG 191 was kindly provided by Amgen Inc. Cells were analyzed and sorted at FACSAria™ (BD Biosciences). The flow cytometry data were analyzed using FlowJo software (Tristar Inc.).

FITC Conjugation of AMG 191

1 μg/μl of AMG 191 was conjugated with 1 μg/μl FITC (Invitrogen) in 0.1 M sodium carbonate buffer (pH 9.5) for 60 minutes in the dark. Conjugated antibody was separated from unreacted FITC using protein desalting spin columns according to manufacturer's instructions (Pierce). FITC-labeled AMG 191 was titrated on G-CSF mobilized human peripheral blood mononuclear cells (AllCells).

***In vitro* proliferation of human HSCs**

Human bone marrow mononuclear cells (ABM024) were purchased from AllCells. After lysing red blood cells, BMMNC were stained with FITC-hCD45RA, APC-hCD34, PE-hCD90, PE-Cy7-CD38. HSC ($CD34^+CD90^+CD45RA^-CD38^-$) were sorted on the FACSAria™ Cell Sorter to >95% purity. Sorted HSC were re-suspended in StemSpan™ serum-free media (Stemcell Technologies) containing human recombinant stem cell factor (hSCF, 100 ng/mL, PeproTech), thrombopoietin (hTPO, 50 ng/mL, PeproTech), hFlt-3L (100 ng/mL, PeproTech) as a density of 25 cells/100 μL media. 25 cells in 100 μL of media were plated on each well in 96 well U-bottom tissue culture plate and cultured in CO_2 tissue culture incubator at 37°C with 5% CO_2 for 7 days in the absence or presence of increasing concentrations of AMG 191 (0.01, 0.1, 1.0, and 10 μg/mL). Total cell numbers were assessed from triplicate wells on days 2, 4, 6, and 7 by counting cells under the inverted light microscope.

Methylcellulose culture and counting

Sorted human and NHP $CD34^+CD90^+$, $CD34^+CD90^-$, or NHP lineage negative cells were cultured for 2 weeks in 60 mm petri dishes containing methylcellulose media (MethoCult™ H4034 Optimum, Stemcell Technologies) in duplicate or triplicate. Each 1000 cells were mixed with 1 mL MethoCult medium without or with the addition of 0.01, 0.1, or 1 μg/mL AMG 191. The methylcellulose was subsequently vortexed to mix (10–20 seconds), and plated on cell culture dishes (35 mm × 10 mm) using a 16G blunt needle and a 1-mL syringe 20–30 minutes after vortexing. Cells were incubated in a CO_2 tissue culture for 2 weeks, and then colonies for erythroid progenitors (BFU-E), granulocyte-macrophage progenitors (CFU-GM) and multi-potential granulocyte, erythroid, macrophage and megakaryocyte progenitors (CFU-GEMM) were counted to quantify hematopoietic progenitors using an inverted microscope (Nikon Eclipse TS100).

Pharmacokinetics of anti-mouse CD117 (ACK2) antibody in mouse serum

C57BL/6(CG)-RAG2TM1.1CGN/J (B6.RAG2$^{-/-}$, CD45.2, Cat. #008449) were obtained from Jackson Laboratory. B6.RAG2$^{-/-}$ mice were pre-treated by intraperitoneal injection with (0.4 mg diphenhydramine per mouse). Following pretreatment, anti-CD117 antibody ACK2 (500 μg per mouse) was administered by retro-orbital injection. Blood (100-200 μL) was collected from tail veins of untreated and ACK2-treated RAG2$^{-/-}$ mice for 3, 6, or 9 days post-ACK2 infusion. Blood was allowed to clot by incubating at room temperature for 30 min and centrifuged for 15 min at 300 g. Serum was transferred into a new microcentrifuge tube. Using Rat IgG2b ready-SET-go ELISA kit (eBioscience), the level of ACK2 (rat anti-mouse CD117 IgG2b antibody) in serum was measured on the SpectraMax i3X (Molecular Device) according to the manufacturer's instructions.

Sorting and transplantation of mouse HSCs into ACK2 conditioned B6.RAG2$^{-/-}$ mice

Bones were harvested from limbs, pelvises, and spines of 6-8 weeks old F1 donor mice (hybrid of B6.CD45.1 × B6.CD45.2). Bone marrow mononuclear cells (BMMNC) were collected by crushing bones and subsequently lysing red blood cells (RBCs) with ACK lysis buffer. CD117^{+} cells were then enriched using lineage cell depletion kit and MACS separation columns (Miltenyi Biotec), according to the manufacturer's instructions. CD117^{+}-enriched cells were stained with PE conjugated lineage antibodies including anti-CD3 (145-2C11), anti-CD4 (GK1.5), anti-CD8a (53-6.7), anti-CD5 (53-7.3), anti-B220 (RA3-6B2), anti-Ter119 (Ter119), Mac1 (M1/70), Gr-1 (Rb6-8C5), APC-CD117 (2B8), PE-Cy7-Sca-1 (D7). All antibodies were from eBiosciences. HSCs (lineage^{-}CD117^{+}Sca^{+}) were sorted on the BD FACSAria^{TM} Cell Sorter to >90 % purity. Sorted HSC (17,500 cells per mouse) were transplanted by retro-orbital injection into unconditioned and conditioned RAG2$^{-/-}$ mice treated with ACK2 for 3, 6, or 9 days.

Engraftment analysis of transplanted B6.RAG2$^{-/-}$ mice

Spleen, blood, and limbs were harvested from transplanted mice 11 weeks post transplantation. Single cell suspension from spleen were prepared by pressing them onto 70 μm cell strainer with the plunger of a 3 ml syringe and centrifugation single cell suspension at 1,200 rpm at 4°C for 5 min. Red blood cells (RBCs) in spleen single cell suspension were lysed in 1 ml RBC lysis buffer for 5 minutes on ice. Splenocytes were then washed, counted cell numbers, and stained with the antibodies. Bone marrow mononuclear cells were collected by crushing bones from limbs and subsequently lysing RBCs. Blood (100-200 μl) obtained from the tail vein of transplanted mice was diluted with DPBS containing 10 mM EDTA to a total volume of 500 μL. An equal volume (500 μL) of 2% dextran was added to diluted blood and incubated at 37°C for 30 min. Supernatants were collected into new tubes and centrifuged at 1,200 rpm at 4°C for 5 min. RBCs were lysed in 1 ml ACK lysis buffer for 5 minutes on ice. HSC and total chimerism were assessed by staining bone marrow mononuclear cells, splenocytes, blood cells with the antibodies including FITC-anti-CD45.1 (A20), APC-anti-CD45.2 (104), APC-Cy7-CD117 (2B8), PE-Cy7-Sca-1 (D7), PE-conjugated lineage antibodies as described above. All antibodies were from eBiosciences. All stained cell samples were analyzed on LSRII flow cytometer at Stanford Shared FACS facility and data were analyzed using Flowjo software (Tristar).

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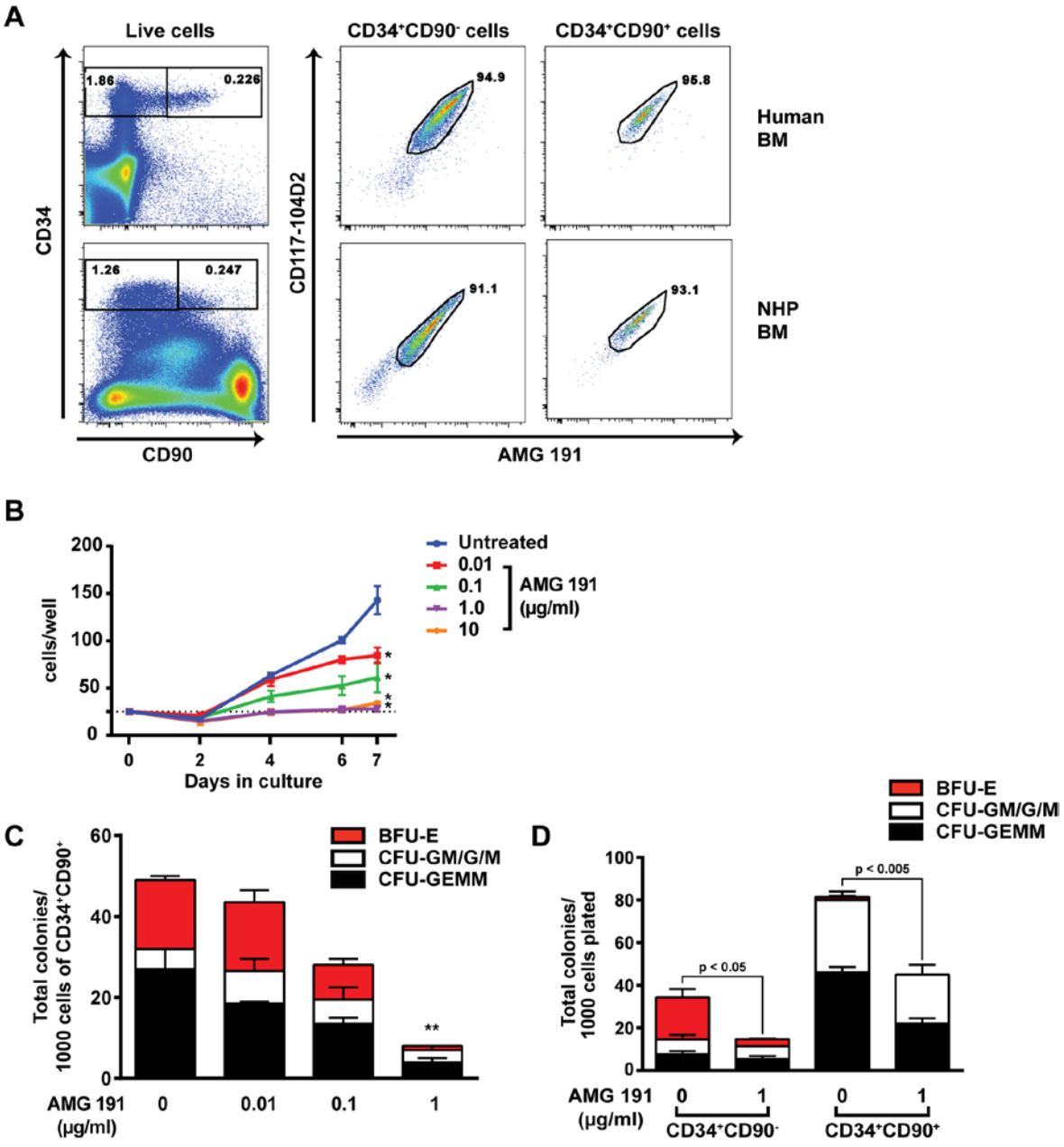
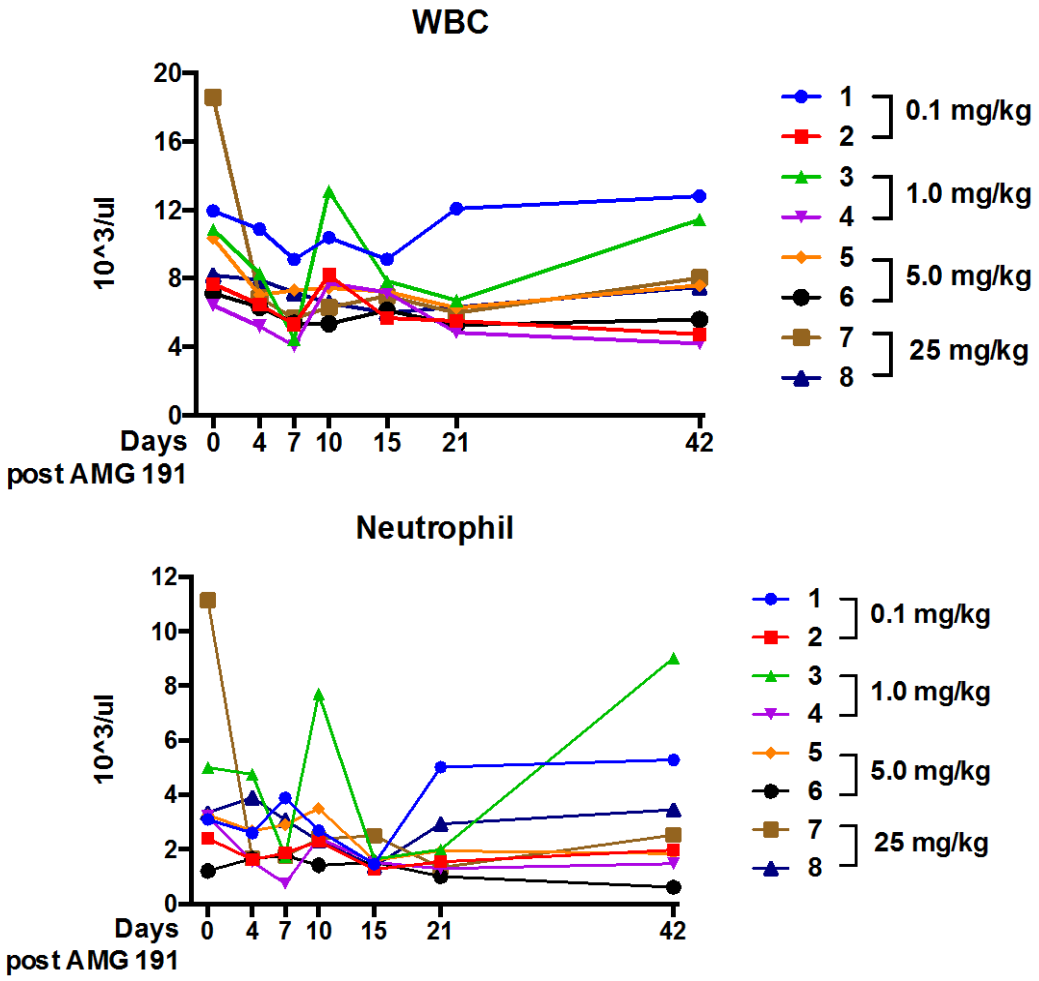


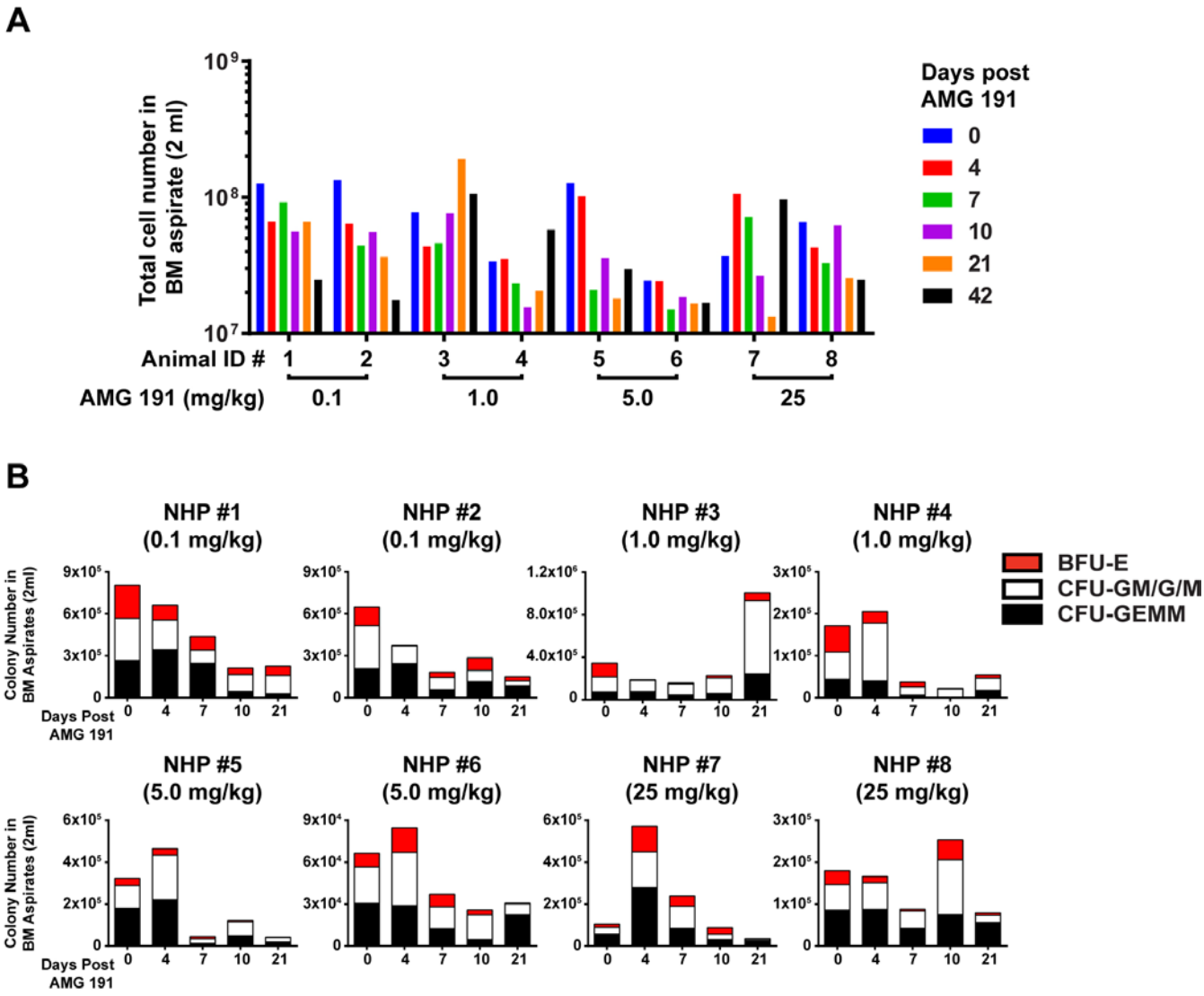
Supplemental Figure 1. AMG 191 binds to and inhibits hematopoiesis of human and NHP CD34$^+$CD90$^+$ HSCs *in vitro*.

(A) AMG 191 binds to human and NHP HSC and HSPC. CD34$^+$ (HSPCs) or CD34$^+$CD90$^+$ (HSCs) of NHP showed a similar frequency and staining pattern compared with human BM cells. Staining of AMG 191 versus a control anti-human CD117 antibody (clone 104D2), which binds to a different epitope of CD117 and does not block binding of SCF, showed an identical staining pattern in both human and NHP cells. the majority of both human and NHP CD34$^+$CD90$^+$ and CD34$^+$CD90$^-$ cells co-stained with the anti-human CD117 reagents. Mononuclear cells were isolated from BM aspirates of human and cynomolgus macaques and stained with APC-hCD34, PE-hCD90, BV421-hCD117 (104D2), and FITC-AMG 191.

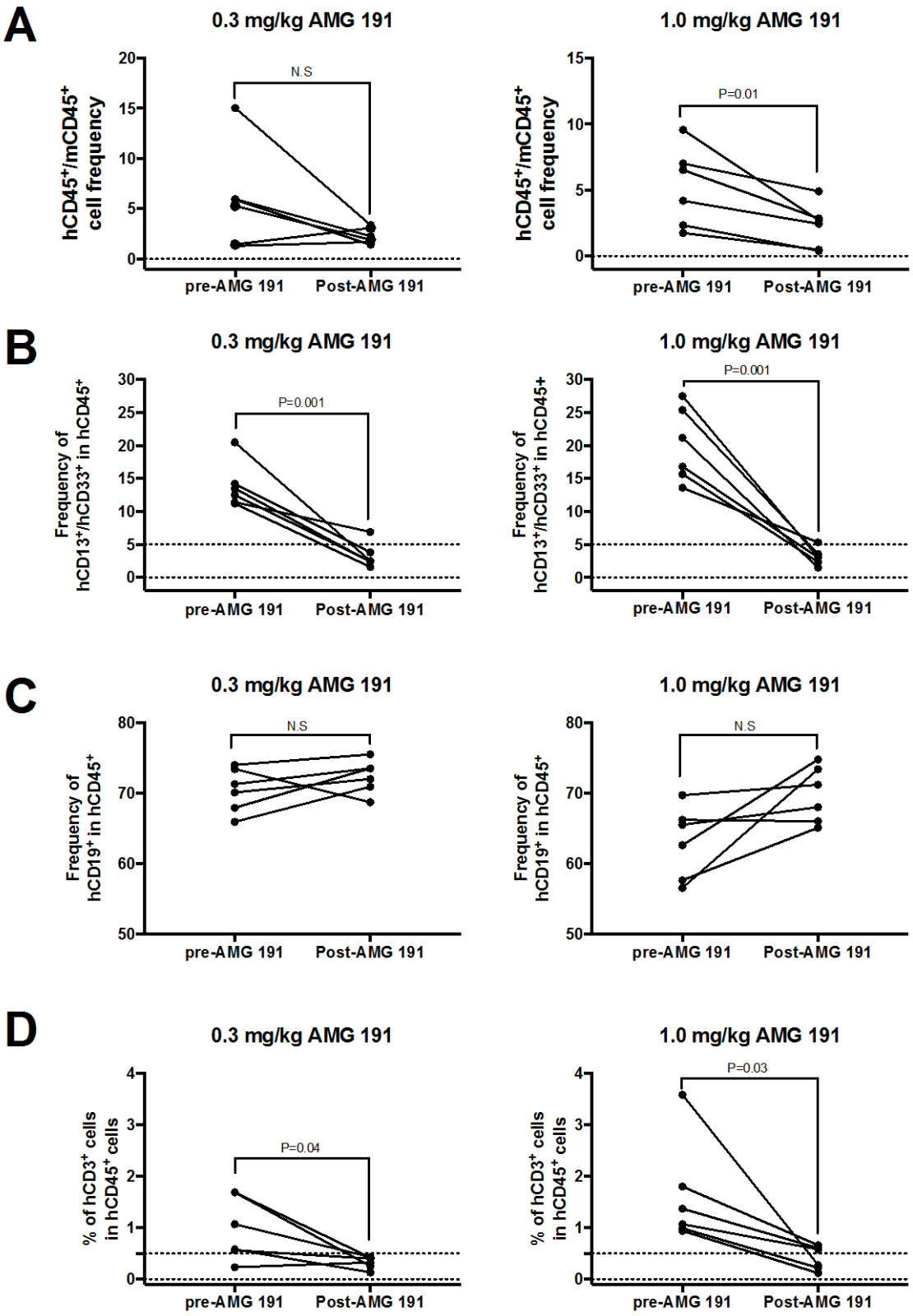
(B) AMG 191 inhibited proliferation of human BM HSCs in a dose-dependent manner in liquid culture Sorted BM human HSCs ($CD34^+CD90^+CD45RA^-CD38^-$) were plated at a density of 25 cells/well in StemSpan™ Serum-Free Medium supplemented with hSCF, hTPO, and hFlt-3L and treated with AMG 191 at 0.01−10 μg/ml or left untreated. *Untreated vs AMG 191 treated, $p<0.05$.
(C) AMG 191 inhibits hematopoiesis of human BM-derived $CD34^+CD90^+$ cells *in vitro*. AMG 191 markedly reduced methylcellulose colony formation of BFU-E and CFU-GEMM, the more primitive progenitors and mixed colonies. Lesser inhibitory effects of AMG 191 were noted on the more differentiated CFU-GM/G/M, suggesting that AMG 191's effect is more pronounced on early human hematopoietic progenitors compared to certain lineage-committed cells. The marked reduction of BFU-E colony formation by AMG 191 is consistent with the high expression pattern and function of CD117 on human erythroid progenitors.[3-5] One thousand sorted human BM $CD34^+CD90^+$ cells were cultured in methylcellulose medium without or with increasing concentration of AMG 191 in triplicate. BFU-E; erythroid progenitors, CFU-GEMM; multi-potential granulocyte, erythroid, macrophage and megakaryocyte progenitors, CFU-GM/G/M; granulocyte and macrophage progenitors. ** Untreated vs 1.0 μg/ml AMG 191 treated, Total colony number $p<0.05$, BFU-E $p<0.05$, CFU-GEMM $p<0.05$
(D) AMG 191 inhibits hematopoiesis of NHP $CD34^+CD90^-$ and $CD34^+CD90^+$ cells. AMG 191 impaired the formation of BFU-E and CFU-GEMM from NHP $CD34^+CD90^-$ and $CD34^+CD90^+$ cells and, similar to humans, had less effect on CFU-GM/G/M. $CD34^+CD90^+$ cells from NHP BM formed few BFU-E compared to CFU-GEMM. That BFU-E rarely arise from the more primitive NHP HSPC has also been reported in the pig-tailed macaque model.[6] One thousand of each $CD34^+CD90^+$ and $CD34^+CD90^-$ cells were sorted from NHP BM aspirates and cultured in methylcellulose medium without or with AMG 191 (1 μg/ml). Untreated vs AMG 191 treated in $CD34^+CD90^-$ cells, total colony and BFU-E, $p<0.05$, Untreated vs AMG 191 treated in $CD34^+CD90^+$ cells, total colony and CFU-GEMM, $p<0.005$. Data and error bars in (B), (C), and (D) represent the mean ± sem.
These data show that AMG 191 targets human and NHP HSCs and suppresses their hematopoietic activities in vitro.



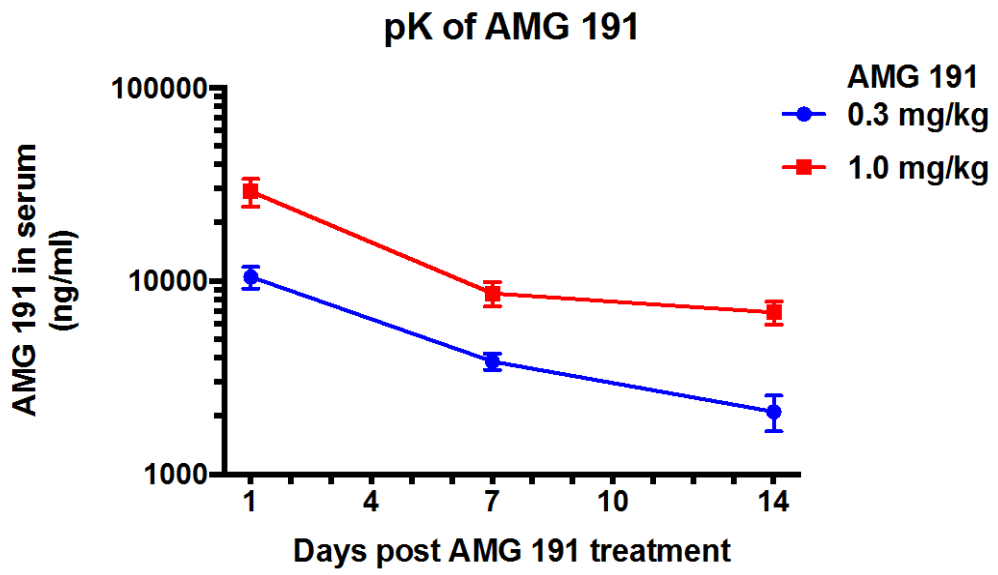
Supplemental Figure 2. White blood cell count (WBC) and absolute blood neutrophil count from individual NHP taken pre-infusion (day 0) and post-infusion of AMG 191. Colored lines correspond to individual NHP with AMG 191 doses as shown on the right. NHP #7 had elevated white cells and neutrophils and experienced emesis around the time of AMG 191 infusion indicating that it may have been ill. Although the levels of white blood cells (WBC) and neutrophils fluctuated between time points in all animals, higher doses of AMG 191 marginally decreased WBC and neutrophils but they returned to the baseline levels by day 42 in all animals.



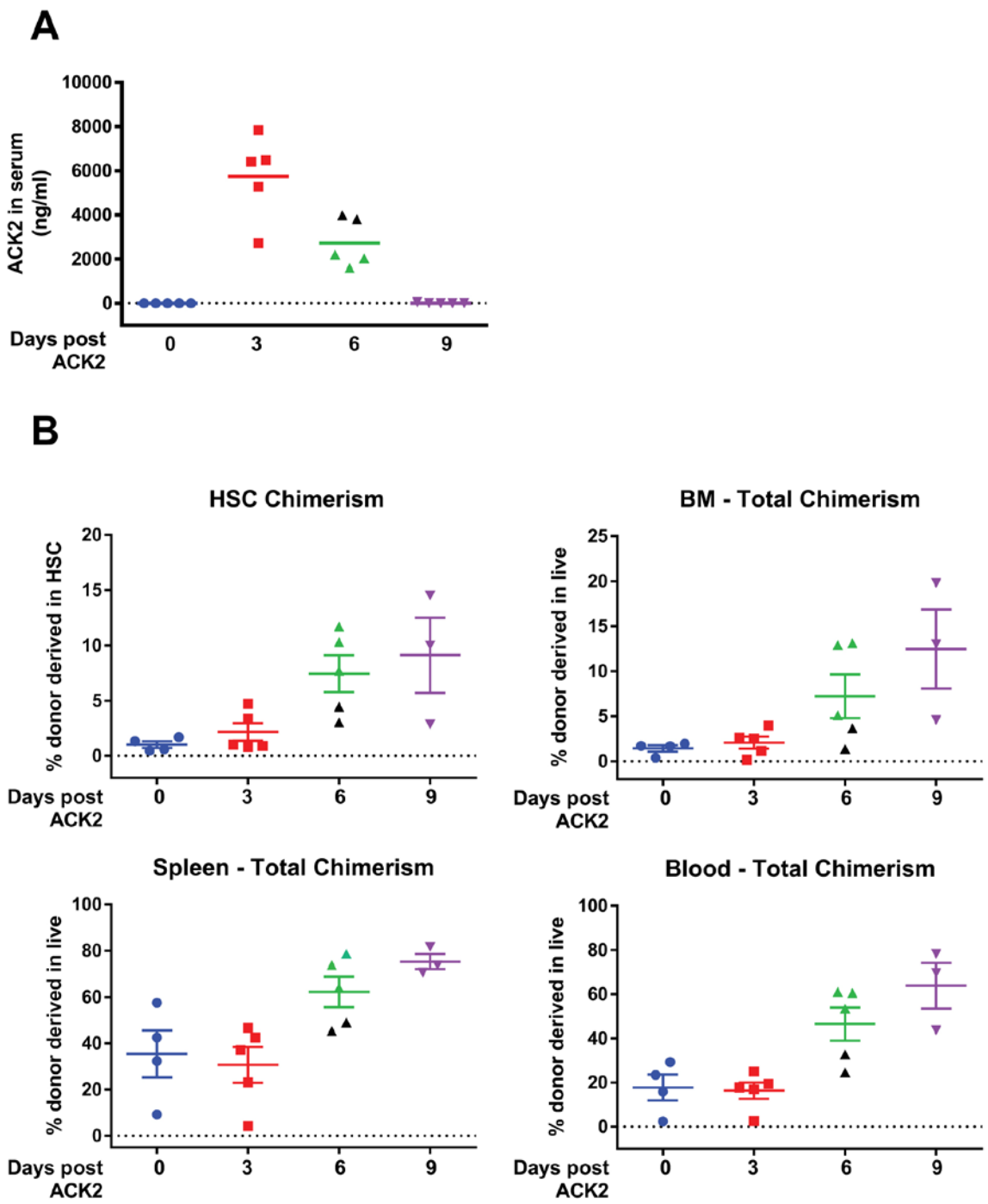
Supplemental Figure 3. Total cell numbers and progenitor colony numbers are decreased in BM aspirates by AMG 191 treatment in NHP. (A) Total cell number in 2 ml bone marrow aspirates. 2 ml of bone marrow aspirates were collected on days 0 (pre-treatment), 4, 7, 10, 21, and 42. Cell numbers in bone marrow aspirates were counted by Cellometer Auto 2000 cell counter after dilution with PBS and filtration through a 70 μm cell strainer. (B) Hematopoietic colonies from day 0, 4, 7, 10, 21 bone marrow collections. Normalized total colony numbers of progenitors cultured in methylcellulose media. Lineage negative cells (Lin^-) containing HSCs and progenitor populations were sorted on the FACSAria™ to >95% purity. 20,000 sorted Lin^- cells were cultured in methylcellulose media for 2 weeks in duplicate. Total colony numbers were calculated based on colony numbers obtained from 20,000 of lineage negative cells and total cell numbers obtained from 2 ml of BM aspirates. The average numbers of colonies are presented.


A
0.3 mg/kg AMG 191
1.0 mg/kg AMG 191
hCD45+/mCD45+ cell frequency
N.S
P=0.01
pre-AMG 191
Post-AMG 191
B
0.3 mg/kg AMG 191
1.0 mg/kg AMG 191
Frequency of hCD13+/hCD33+ in hCD45+
P=0.001
P=0.001
pre-AMG 191
Post-AMG 191
C
0.3 mg/kg AMG 191
1.0 mg/kg AMG 191
Frequency of hCD19+ in hCD45+
N.S
N.S
pre-AMG 191
Post-AMG 191
D
0.3 mg/kg AMG 191
1.0 mg/kg AMG 191
% of hCD3+ cells in hCD45+ cells
P=0.04
P=0.03
pre-AMG 191
Post-AMG 191

Supplemental Figure 4. (A) Effects of AMG 191 on engrafted human cells in BM of NSG mice two-weeks post injection. (A) Ratios of total human-$CD45^+$/mouse-$CD45^+$ ($hCD45^+$/$mCD45^+$) cell, (B) myeloid cells, (C) B cells, and (D) T cells frequencies in BM of humanized NSG mice without (pre-AMG 191) and with AMG 191 (0.3 mg/kg and 1.0 mg/kg). Ratio of $hCD45^+$/$mCD45^+$ cell frequency in BM was decreased by AMG 191 treatment in most mice (4 of 6) that receive 0.3 mg/kg and all mice (6 of 6) that received 1.0 mg/kg. Amongst $hCD45^+$ cells, myeloid cells demonstrated the highest level of depletion by AMG 191 treatment as assessed by the frequency of $hCD13^+hCD33^+$ cells.
The % decrease of myeloid cell frequency in $hCD45^+$ cells by AMG 191 in mice treated with 0.3 mg/kg and 1.0 mg/kg AMG 191 was 75 ± 17 and 83 ± 10 (avg ± std), respectively. The relative frequency of B cells ($hCD19^+$) amongst $hCD45^+$ appeared to increase. AMG 191 caused a modest decrease of T cell ($hCD3^+$) frequency within $hCD45^+$ cells. The % decrease of T cell frequency in $hCD45^+$ cells by AMG 191 in mice treated with 0.3 mg/kg and 1.0 mg/kg AMG 191 was 48 ± 43 and 70 ± 17 (avg ± std), respectively. BM aspirates were collected from femurs of humanized NSG mice before (pre-AMG 191) and 2 weeks after AMG 191 treatment (post-AMG 191). Mononuclear cells were prepared from BM aspirates and stained with antibodies. Cell frequency was analyzed by FACS. P values were obtained using paired student *t*-test.



Supplemental Figure 5. Pharmacokinetics of AMG 191 in serum of humanized NSG mice treated with 0.3 mg/kg and 1.0 mg/kg AMG 191. At day14 post-AMG 191 injection, 1700 – 2700 ng/ml and 6000 – 7800 ng/ml of AMG 191 was detected in sera from mice injected with 0.3 mg/kg and 1.0 mg/kg of AMG 191, respectively. This data indicates that time to clearance of AMG 191 in NSG mice is dose dependent. AMG 191 was administered at indicated doses by a single intravenous injection. Serum was prepared from peripheral blood collected from treated mice by tail venipuncture (n=3 mice at each time point). The residual AMG 191 in serum was measured by electrochemiluminescent method (See materials and methods for details). Data and error bars represent the mean ± sem.


A
ACK2 in serum (ng/ml)
10000
8000
6000
4000
2000
0
Days post ACK2
0
3
6
9
B
HSC Chimerism
% donor derived in HSC
20
15
10
5
0
Days post ACK2
0
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6
9
BM - Total Chimerism
% donor derived in live
25
20
15
10
5
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Days post ACK2
0
3
6
9
Spleen - Total Chimerism
% donor derived in live
100
80
60
40
20
0
Days post ACK2
0
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Blood - Total Chimerism
% donor derived in live
100
80
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Days post ACK2
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Supplemental Figure 6. Serum levels of anti-mouse CD117 antibody (ACK2) are inversely correlated with the degree of donor chimerism. (A) PK analysis of ACK2 in B6.RAG2$^{-/-}$ (CD45.2) mice administered a single IV injection of ACK2 (500 μg/mouse). The levels of ACK2 in serum were measured at 3, 6, and 9 days post-infusion using an anti-rat IgG2b ELISA kit and quantified with the SoftMax program (n=5 mice/group). ACK2 remained in the serum at levels of 2700 - 6500 ng/ml on Day 3, at 1600 - 4000 ng/ml on Day 6, and was undetectable on Day 9 post injection. Colored symbols correspond to individual mice. Black triangles in the Day 6 group highlight mice that had relatively high serum levels of ACK2 on that day (3800 and 4000 ng/ml) (B) HSC and total blood chimerism of RAG2$^{-/-}$ mice conditioned with ACK2 as in (A) and transplanted with congenic immune competent [F1] CD45.1/CD45.2 donor HSC (17,500 cells/mouse). Chimerism at 11 weeks post-transplantation is shown. HSC chimerism was assessed as percentages of CD45.1/CD45.2 cells in HSC (lineages^{-}CD117^{+}Sca^{+}) in BM (top left panel). Total chimerism was assessed as percentages of CD45.1/CD45.2 cells in live cells from BM, spleen, and blood. Level of chimerism was inversely correlated with serum level of ACK2 at the time of donor HSC infusion. This inverse relationship is particularly notable in the Day 6 group; recipient mice marked by the black triangles had serum levels of $\geq$3800 ng/ml whereas mice marked by the green triangles had residual ACK2 levels of $\leq$2200 ng/ml on that day. Mice transplanted on Day 3 when the serum levels were >3000 ng/ml had uniformly poor engraftment as reflected by the low chimerism levels. These data suggest that the threshold level of serum ACK2 above which ACK2 prevents donor HSCs from engrafting is roughly 2200 ng/ml.

Frequency of hCD45$^+$ cells in live BM cells (%)

Mouse	Pre-AMG 191	2 weeks Post-AMG 191	6 weeks Post-HCT
Unconditioned #1	89.7	95.3	89.2
Unconditioned #2	91.7	90.9	91.5
Unconditioned #3	59.6	95.6	94.7
Unconditioned #4	57.1	90.8	86.8
Unconditioned #4	67.4	93.5	52.7
Unconditioned #5	35.0	91.3	80.6
Unconditioned #6	94.9	91.6	91.4
Unconditioned #7	86.5	62.2	89.4
Unconditioned #8	83.6	88.8	92.3
AMG 191 (0.3 mg/kg) #1	92.5	76.6	1.71
AMG 191 (0.3 mg/kg) #2	85.4	68.8	1.99
AMG 191 (0.3 mg/kg) #3	56.2	62.5	2.16
AMG 191 (0.3 mg/kg) #4	83.7	58.3	0.47
AMG 191 (0.3 mg/kg) #5	83.0	65.2	4.97
AMG 191 (0.3 mg/kg) #6	58.7	74.5	32.4
AMG 191 (1.0 mg/kg) #1	85.6	82.1	2.93
AMG 191 (1.0 mg/kg) #2	62.3	32.2	2.90
AMG 191 (1.0 mg/kg) #3	86.7	72.3	2.34

Supplemental Table 1. Frequency changes of hCD45$^+$ cells in BM of humanized NSG mice conditioned with AMG 191 and subsequently transplanted with a second CD34$^+$ HSPC graft at 6 weeks post transplantation. At the time of injection, mice treated with AMG 191 had high percentages of human cells ranging from 56–93% of all live cells in the BM. The frequency of hCD45$^+$ cells in BM of unconditioned mice that received the mCitrine-marked second graft remained in the range of 35–95% (n=9 mice). In contrast, AMG 191 treated and transplanted mice had frequencies of hCD45$^+$ cells amongst live cells that range from 0.5–32.4% at 6 weeks post-HCT (0.3 mg/kg AMG 191, n=6 mice and 1.0 mg/kg AMG 191, n=3 mice). These data suggest that AMG 191 effectively depleted the engrafted human cells. The second human donor cell graft could engraft in the AMG 191 treated but not unconditioned mice (Fig. 3). However, this second human donor cell graft did not robustly expand in BM of these mice, probably due to the unfavorable mouse BM microenvironment and competition from the endogenous mouse HSPC which likely expanded into the vacated niches while the anti-human CD117 directed AMG 191 was present. Pooled cord blood CD34$^+$ cells transduced with mCitrine expressing lentivirus were transplanted into humanized NSG mice with or without AMG 191 treatment (0.3 and 1.0 mg/kg). Six weeks after transplantation, cell frequencies were assessed in BM aspirates of transplanted humanized NSG mice. Cell frequency was analyzed by FACS.