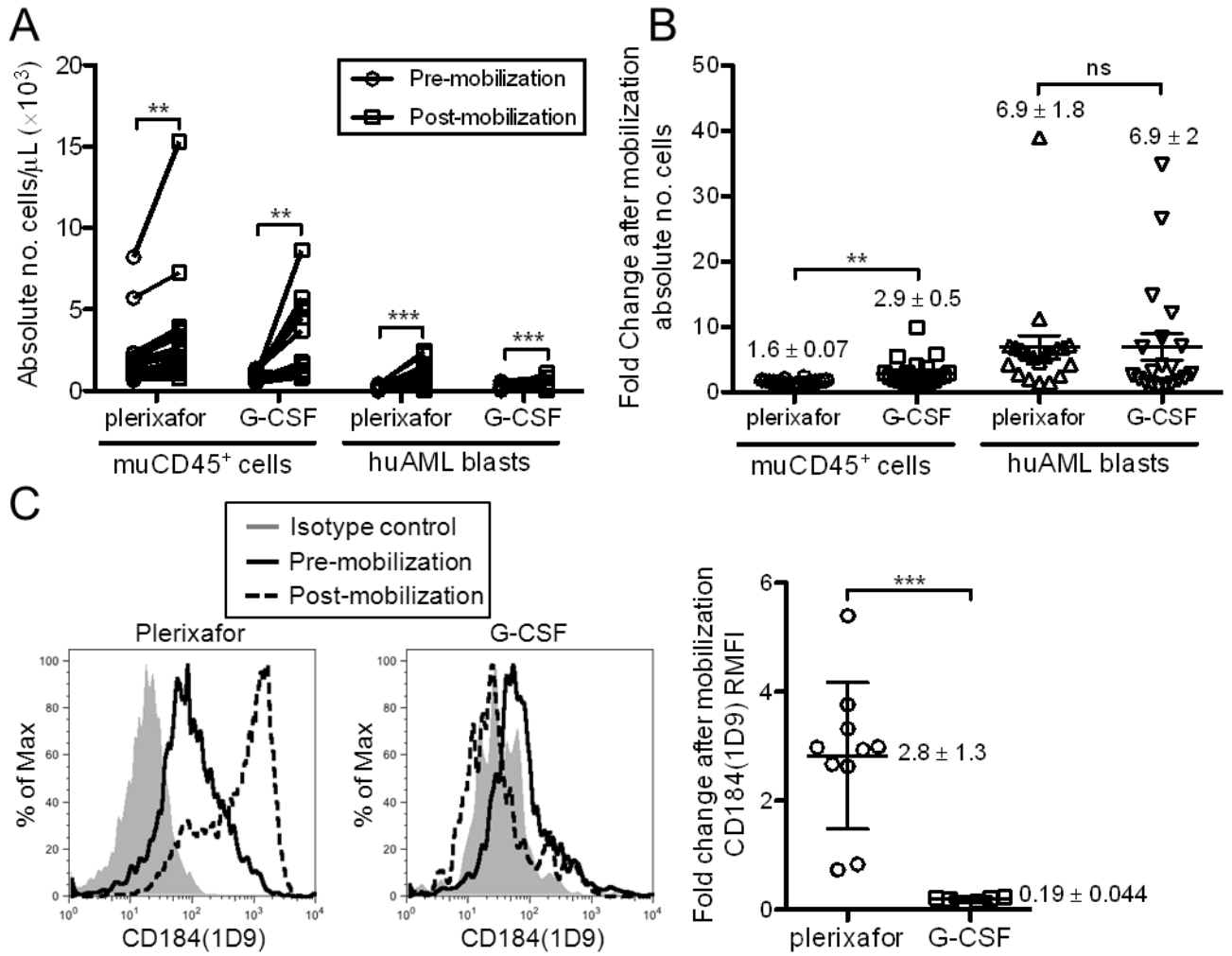


Supplemental Methods

Xenogeneic transplantation. NOD.Cg-PrkdcscidIl2rgtm1Wjl/SzJ (NOD/SCID/IL2r^{null}) mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Animals were bred and maintained according to guidelines approved by the Animal Studies Committee of Washington University School of Medicine. All experiments were performed with banked AML cells collected from a patient with French- American-British (FAB) classification system subtype of M5. Human AML cells were engrafted in NOD/SCID/IL2r^{null} mice by injecting 8×10^6 cells via tail vein injection. At 10-14 weeks post-transplantation, the recipients received G-CSF or plerixafor. Recombinant human G-CSF was administered by daily subcutaneous injection for 5 days at a dose of 250 μ g/kg. Plerixafor (Genzyme, Cambridge, MA) was supplied as a sterile isotonic aqueous solution at 20 mg/mL and was administered at a dose of 5 mg/kg as a single subcutaneous injection. Mice were bled retro-orbitally before treatment and either 2 hours after plerixafor treatment or 4 to 6 hours after the final G-CSF dose. Complete blood counts were obtained using an automated cell counter (Hemavet; CDC Technologies, Oxford, CT). Human AML engraftment and CXCR4 expression were determined by flow cytometry using anti-human CD45-FITC (clone HI30), anti-human CD184-PE (clone 1D9), anti-human CD33-APC (clone WM53), and anti-murine CD45-APC-Cy7 (clone 30F11) antibodies. The absolute number of murine CD45⁺ (muCD45⁺) or human AML (huAML) cells per μ L of blood was determined by multiplying the percent 7AAD⁻muCD45⁺huCD45⁻ (muCD45⁺) or 7AAD⁻muCD45⁻huCD45⁺huCD33⁺ (huAML) cells with the total blood cell count obtained from the automated cell counter.

Figure Legend

Supplemental Figure 1. Human AML engraftment and mobilization in NOD/SCID/IL2r^{null} mice. NOD/SCID/IL2r^{null} mice were injected retro-orbitally with 8×10^6 human AML (huAML) blast cells. At 10-14 weeks post-transplantation, recipients were treated with G-CSF 250 μ g/kg per day subcutaneously for 5 days (n = 20) or a single injection of plerixafor at 5 mg/kg (n = 20) subcutaneously. Peripheral blood samples collected before or after mobilization were analyzed on an automated cell counter. Human AML engraftment and mobilization was determined by flow cytometry using monoclonal antibodies specific for human CD45, CD33, CD184 and murine CD45. (A) Absolute number of murine CD45⁺ (muCD45⁺) and huAML blasts before and after mobilization. The absolute number of muCD45⁺ or huAML blast cells per μ L of blood was determined by multiplying the percent 7AAD⁻muCD45⁺huCD45⁻ (muCD45⁺) or 7AAD⁻muCD45⁻huCD45⁺huCD33⁺ (huAML) cells with the total blood cell count. (B) Change in muCD45⁺ and huAML blasts in the peripheral blood expressed as fold-change relative to baseline at 2 hours after administration of plerixafor or 4 to 6 hours after the final G-CSF dose. (C) Relative changes in the amount of cell surface-expressed CXCR4. The flow cytometric histograms show overlays of CXCR4 surface expression on huAML blasts before and after mobilization with plerixafor or G-CSF. Relative mean fluorescence intensity ratios (RMFI) were calculated by dividing the mean fluorescence intensity of CD184 by the mean fluorescence intensity of a rat IgG2a isotype control. Change in surface CD184 expression on huAML blasts is expressed as the fold-change in CD184 RMFI relative to baseline at 2 hours after administration of plerixafor or 4 to 6 hours after the final G-CSF dose. The data (mean \pm SEM) are pooled from two (G-CSF mobilization) or three (plerixafor mobilization) independent experiments. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.



Supplemental Figure 1.

Supplemental Table 1. Adverse Events by Grade and Frequency

	AE GRADE				
	1	2	3	4	5
Allergy					
Allergic Reaction/Hypersensitivity	2	1			1
Allergic Rhinitis					
Cardiovascular					
Atrial Fibrillation	1				
Atrial Flutter		1	1		
Bradycardia	9	1			
Bruising	1	1			
Cardiac Ischemia/Infarct					
Edema	29	10			
Hematoma	3	1			
Hemorrhage	6	2	1		
Hypertension	5	5			
Hypotension	6	8	3		
Palpitations	1				
Tachycardia	35	1			
Thrombosis/Embolism		1			
Vasovagal Episode		1			
Constitutional					
Blurred Vision					
Decreased Appetite	1				
Diaphoresis	2				
Fatigue	11	6	2		
Fever	1				
Fever-No Infection	4	1	50	1	
Infection	3	2	8		2
Weakness	16	18	2		
Weight Gain					
Weight Loss	1				
Dermatologic					
Alopecia	1				
Bumps		3			
Cellulitis	13	7	1		
Hand-Foot Reaction					
Pruritus	8	2			
Rash	10	11			
Ulceration	1		1		
Endocrine					
Hot Flashes	1				
Night Sweats					
Gastrointestinal					
Anorexia	16	13	4		
Ascites					
Colitis					
Constipation	14	1			
Dehydration					
Diarrhea	32	9	2		
Dyspepsia/Heartburn	3	2			
Dysphagia	1	2			
Flatulence					
Hemorrhoids	2	1			
Ileus					
Indigestion	1				
Mucositis	21	12	2		

Nausea	36	12	2	
Stomatitis	1	3		
Vomiting	12	11		
Hematological				
Hemoglobin		18	12	
Leukocytes (Wbc)	1	2	6	46
Lymphopenia				
Neutrophils (Anc)			2	25
Platelets	2	3	10	28
Hepatic				
Alt	7	7	3	1
Ast	9	2	1	1
Bilirubin	7	2	3	
Hepatomegaly	1			
Jaundice	1	1		
Metabolic/Laboratory				
Alkaline Phosphatase	16	4		
Chloride	1			
Hyperalbumin	1			
Hypercalcemia	1			
Hypercholesterolemia				
Hyperkalemia	1	1		
Hyponatremia	1	1		
Hyperphosphatemia	2			
Hypertriglyceridemia				
Hypoalbumin	20	9		
Hypocalcemia	30	7		
Hypokalemia	13	2	5	
Hyponatremia	15		1	
Hypophosphatemia	5	1	2	
Magnesium	1			
Phosphate		1		
Neurological				
Confusion	4		3	
Dizziness	5			
Headache	18	5	2	
Insomnia	10	7		
Mood Alteration	1	1		
Mood Alteration - Anxiety	9	7		
Mood Alteration - Depression	2	1		
Mood Alteration - Lethargy	1		1	
Neuropathy (Type _____)	5	1		
Numbness	3	1		
Rigors	5	4		
Seizure				
Singultus	2			
Somnolence			1	
Syncope				
Taste Alteration	1	1		
Tingling	3	1		
Tremor		1	1	
Pulmonary				
Atelectasis		1		
Cough	8	6		
Dyspnea (Sob)	7	7		
Hypoxia			1	
Pneumonia			1	
Pneumonitis/Pulmonary Infiltrates				

Pneumothorax				
Tachypnea	3		1	
Renal/Genito-Urinary				
Creatinine				
Dysuria				
Eye Redness	2			
Hematuria				
Incontinence	3	2	1	
Renal Failure		1	1	
Urinary Frequency/Urgency	4	1	1	1
Urinary Retention	3			
Urine Color Change				
Uti	2			
Vision Change	3			
Vision/Occular				
Vison Blur	3	4		
Pain				
Pain	78	49	2	

Supplemental Table 2. Expression of surface markers in peripheral blood and bone marrow blasts by response to treatment.

Antigen	n	RMFI Bone Marrow Blasts			n	RMFI Peripheral Blood Blasts		
		CR/CRi	Persistent Disease	P		CR/CRi	Persistent Disease	P
CD123	28	3529	4044	0.57	36	289	2317	0.34
CD184 (12G5)	28	3347	4455	0.50	37	326	2307	0.29
CD184 (1D9)	25	3879	4321	0.94	35	332	1771	0.38
CD31	15	2151	6380	0.32	21	129	1714	0.30
CD44	12	2057	5216	0.42	19	121	1719	0.17
CD49D	27	4111	4232	0.68	36	320	2364	0.36
CD58	12	9799	7576	0.87	16	132	1876	0.52
CD62L	15	2067	6462	0.32	21	148	1783	0.30
CXCR7	14	2250	4896	0.25	22	135	2281	0.28