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Cytokines increase engraftment of human acute myeloid leukemia cells in immunocompromised mice but not engraftment of human myelodysplastic syndrome cells

Maria Krevvata,[1,*] Xiaochuan Shan,[1,*] Chenghui Zhou,[1] Cedric Dos Santos,[1] Georges Habineza Ndikuyeze,[1] Anthony Secreto,[1] Joshua Glover,[1] Winifred Trotman,[1] Gisela Brake-Silla,[1] Selene Nunez-Cruz,[2] Gerald Wertheim,[3] Hyun-Jeong Ra,[1] Elizabeth Griffiths,[4] Charalampos Papachristou,[5] Gwenn Danet-Desnoyers,[1,#] and Martin Carroll[1,6,#]

[1]Division of Hematology and Oncology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; [2]Center for Cellular Immunotherapies, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; [3]Department of Pathology and Laboratory Medicine, Children's Hospital of Philadelphia and The University of Pennsylvania Perelman School of Medicine, Philadelphia, PA; [4]Roswell Park Cancer Institute, Buffalo, NY; [5]Department of Mathematics, Rowan University, Glassboro, NJ and [6]Veterans Administration Hospital, Philadelphia, PA, USA

MK and XS share co-authorship. #GD-D and MC share senior authorship.

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ABSTRACT

Patient-derived xenotransplantation models of human myeloid diseases including acute myeloid leukemia, myelodysplastic syndromes and myeloproliferative neoplasms are essential for studying the biology of the diseases in pre-clinical studies. However, few studies have used these models for comparative purposes. Previous work has shown that acute myeloid leukemia blasts respond to human hematopoietic cytokines whereas myelodysplastic syndrome cells do not. We compared the engraftment of acute myeloid leukemia cells and myelodysplastic syndrome cells in NSG mice to that in NSG-S mice, which have transgene expression of human cytokines. We observed that only 50% of all primary acute myeloid leukemia samples (n=77) transplanted in NSG mice provided useful levels of engraftment (>0.5% human blasts in bone marrow). In contrast, 82% of primary acute myeloid leukemia samples engrafted in NSG-S mice with higher leukemic burden and shortened survival. Additionally, all of 5 injected samples from patients with myelodysplastic syndrome showed persistent engraftment on week 6; however, engraftment was mostly low (<2%), did not increase over time, and was only transiently affected by the use of NSG-S mice. Co-injection of mesenchymal stem cells did not enhance human myelodysplastic syndrome cell engraftment. Overall, we conclude that engraftment of acute myeloid leukemia samples is more robust compared to that of myelodysplastic syndrome samples and unlike those, acute myeloid leukemia cells respond positively to human cytokines, whereas myelodysplastic syndrome cells demonstrate a general unresponsiveness to them.

Introduction

Human myeloid neoplasms represent a remarkably diverse array of blood cell diseases. Acute myeloid leukemia (AML) is a clonal hematopoietic disease characterized by an abnormal proliferation of immature leukemic blasts and by a hematopoietic differentiation block.[1] Myelodysplastic syndromes (MDS) are characterized by abnormal cell morphology and ineffective blood cell production. MDS mainly affect the elderly and their pathogenesis is not completely understood but

Correspondence:
carroll2@pennmedicine.upenn.edu

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they are thought to arise from a single transformed hematopoietic cell.[2-4] Both AML and MDS are genetically heterogeneous making functional characterization of primary human cells essential for studies of disease pathogenesis. However, primary cells from neither of these diseases survive well *in vitro*, making the use of xenotransplantation models essential for the study of primary cells. With the rapid growth in the number of immunocompromised mouse strains modified to express human proteins, studies using mice as bioreactors for human cells to test specific *in vitro* observations have become feasible. However, little work has previously been done studying how the recipient mouse affects the biology of the human disease cells. Here we compare the effect of use of NSG *versus* NSG-S mice on the relative engraftment and growth of human AML and MDS samples.

Collective studies for over three decades have described the contributions of the bone marrow microenvironment to normal hematopoiesis. Since the description of the bone marrow niche by Schofield,[5] the regulation of normal hematopoietic stem cell homeostasis by mechanisms involving non-hematopoietic cells has been extensively investigated. It is now well understood that normal stem cell self-renewal is tightly regulated, in part, by cell-extrinsic mechanisms.[6-10] Taichman and Emerson have shown that cytokines produced by osteoblasts promote proliferation of hematopoietic cells in culture[11] whereas increases in osteoblast numbers in a mouse model with constitutively active osteoblast-specific parathyroid hormone resulted in a simultaneous increase of hematopoietic stem cells.[12] As with normal hematopoiesis, several hematopoietic malignancies persist by maintaining a pool of malignant stem cells that may be partly protected by components of the microenvironment.[13,14] Conversely, leukemic stem cells induce alterations in hematopoietic regulatory functions to gain growth advantage over normal hematopoietic stem cells.[15,16] Schepers *et al.* have shown that leukemic myeloid cells secrete high levels of pro-inflammatory cytokines, creating a paracrine feedback loop that drives myeloid differentiation. At the same time, myeloid cells stimulate mesenchymal stem cells (MSC) to overproduce functionally altered osteoblastic cells with compromised ability to maintain normal hematopoietic stem cells.[17] Thus, cytokine production by the bone marrow microenvironment may modify the phenotype of malignant blood diseases.

We previously demonstrated that the NSG (NOD-Scid-IL-2Rγc^{null}) mouse is a robust recipient for human AML xenotransplantation samples, allowing a better understanding and characterization of AML biology, especially in the context of drug therapy studies.[18] However, we observed that a significant proportion of primary AML specimens showed low (0.1 to 1% human blasts in mouse bone marrow) or no (<0.1%) engraftment in NSG mice, suggesting the need for improved xenograft models.[18] Transgenic expression of human stem cell factor, granulocyte-macrophage colony-stimulating factor and interleukin-3 in NSG-S mice has been reported to enhance engraftment of primary AML samples, although only a few AML patients were compared between strains.[19] These studies did not allow for a rigorous determination of the percentage of patients' samples that engraft (an assessment for stem cell effects) or the bulk of disease burden (reflecting growth after engraftment).

A recent study attempting to develop a patient-derived xenotransplantation model for human MDS suggested that patient-derived MSC combined with the use of NSG-S immunodeficient mice, could enhance engraftment levels.[20] Indeed, the use of NSG-S mice appeared to improve engraftment levels and also maintained the malignant clone, but these studies were largely done with accompanying injection of MSC so the critical variables for engraftment of MDS samples were largely undetermined.

In this report, we compare the engraftment levels in the two above-mentioned immunodeficient mouse strains as well as the influence of MSC on relative engraftment of human AML and MDS in primary patients' samples. We describe a comprehensive paired analysis of engraftment of primary AML samples in NSG and NSG-S mice. Consistent with previous studies, the use of the NSG-S strain increased both the percentage of AML samples that engrafted and the level of engraftment. In contrast, MDS engraftment was consistently low and was not influenced significantly by the use of either mouse strain or co-injection of MSC. However, human MSC did not engraft long-term suggesting that a human microenvironment was not established. These results demonstrate that human AML cells respond positively to the three human cytokines as shown by xenografts, while these cytokines appear to be insufficient to enhance the engraftment and expansion of MDS cells. Xenotransplantation models that better mimic the human microenvironment may be necessary to establish robust MDS xenograft models.

Methods

Myelodysplastic syndrome and acute myeloid leukemia specimens

Peripheral blood, leukapheresis product, or bone marrow from AML patients were collected at the Hospital of the University of Pennsylvania after informed consent. French-American-British or World Health Organization classification and cytogenetics were determined at the Hospital of the University of Pennsylvania. For MDS samples, only bone marrow samples were used and were obtained either from the same source or from Roswell Park Cancer Institute.

Mice

Mice were used in accordance with a protocol reviewed and approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania. NSG or NSG-S mice were initially purchased from Jackson Laboratories (Bar Harbor, ME, USA) and produced at the University of Pennsylvania Stem Cell and Xenograft Core. Male and female mice 6-8 weeks of age were either sublethally irradiated (250 cGy) or chemically conditioned by intraperitoneal injection of busulfan (30 mg/kg, Otsuka America Pharmaceutical Inc.) 24 h prior to cell injections. T-cell depleted AML cells (5-10x10^6 per mouse) were transplanted *via* tail vein injection into mice.[21] Mice were euthanized no later than 16 weeks after AML injection and marrow from femora and tibiae, splenocytes and peripheral blood were harvested. Human AML engraftment was assessed by flow cytometry and defined as the percentage of human $CD45^+CD33^+$ cells in total live mononuclear cells.[22-24]

For MDS samples, intrafemoral injections with 1x10^6 human bone mononuclear cells alone or in combination with 5x10^5 *ex vivo*-expanded MSC were performed. For MDS-engrafted mice,

engraftment levels were measured in female mice by bone marrow aspiration of either the ipsilateral or contralateral femur at the time points indicated.

The methods are described in more detail in the *Online Supplementary Appendix*.

Results

Increased levels of acute myeloid leukemia engraftment in NSG-S mice

We first investigated the engraftment of 77 AML samples in NSG mice, representing all French-American-British and prognostic groups (Table 1). We observed that only half of the samples (n=39, 51%) were able to engraft at a significant level (>0.5% human blasts in mouse bone marrow) (Figure 1A). In order to compare engraftment between the two mouse strains, 18 of the 39 NSG-engrafting AML samples were also injected in NSG-S mice. All 18 NSG-engrafting samples engrafted in NSG-S mice. Representative flow cytometry plots of human $CD45^+CD33^+$ cells in the bone marrow, spleen and peripheral blood from 2 AML samples that engrafted in both strains are shown in Figure 1B. Importantly, for 44% (8 out of 18) of the NSG-engrafting AML samples, the use of NSG-S mice as recipients was associated with very rapid engraftment, excessive leukemic burden, anemia, weight loss and lethargy, leading to a significantly shorter overall survival ($P \leq 0.005$) (Figure 1C). Consequently, a quantitative comparison of engraftment at the same time point for these 8 patients' samples was not feasible. The enhanced engraftment, and associated mortality, observed in 26% (8/31) of all NSG-S-engrafting samples should be taken into account when using this strain for pre-clinical studies. For 9 of the 18 patients' samples tested in both NSG and NSG-S, we were able to sacrifice the mice at the same time (Figure 1D). We observed a significantly higher leukemia burden in bone marrow and spleen from NSG-S compared to NSG mice (32±23% *vs.* 21±22% of bone marrow blasts, $P \leq 0.035$; 8±14% *vs.* 6±9% splenic blasts, $P \leq 0.0001$) (Figure 1E and F, respectively). Interestingly, NSG-S mice showed a dramatic increase in peripheral blast count (2771±7208 *vs.* 137±166 blasts/μL peripheral blood, $P \leq 0.034$) (Figure 1G). This may represent an improvement over NSG mice because it extends the usefulness of peripheral blood sampling to monitor engraftment and response to treatment in pre-clinical studies. Overall, these data demonstrate that AML engraftment in NSG-S mice is more rapid and yields a higher leukemic burden than in NSG mice.

NSG-S mice could support the engraftment of samples incapable of engrafting in NSG mice

To test whether NSG-S mice could support the engraftment of samples incapable of engrafting in NSG mice, we transplanted NSG-S mice with 21 of the 38 NSG non-engrafting samples (Figure 1A). Remarkably, 67% (14/21) of the non-engrafting samples did engraft in NSG-S mice (18±17.5% bone marrow blasts, 9.2±13.7% splenocytes, and 1799±4848 blasts/μL peripheral blood) (Figure 2A). Thus, overall our results show that 82% (32/39) of all tested AML samples engrafted in NSG-S mice, compared to 51% in NSG mice. The degree of engraftment observed in bone marrow and spleen for 11 representative patients is shown in Figure 2B. These results indicate that the presence of systemic human stem cell factor, granulocyte-macrophage colony-stimulating factor and interleukin-3 in NSG-S mice contribute to support leukemia-initiating cells for most AML samples. However, 7 out of 39 samples (18%) still failed to engraft in NSG-S mice, indicating that the bone marrow microenvironment in NSG-S mice may remain suboptimal for a minority of AML samples.

We investigated whether engraftment in NSG-S mice was correlated with surface expression of CD116 (granulocyte-macrophage colony-stimulating factor receptor), CD117 (c-kit), and CD123 (interleukin-3 receptor α–chain) on leukemic cells. As shown in Figure 2C, we found no significant difference in the density of cytokine receptor expression or cytogenetic profiles, mutations, and prognosis between NSG-S engrafting and non-engrafting samples. These results indicate that, in a small minority of AML samples, leukemia-initiating cells have requirements beyond the combination of human granulocyte-macrophage colony-stimulating factor, interleukin-3 and stem cell factor capable of supporting the vast majority of primary AML samples in mice.

Inv(16) acute myeloid leukemia shows enhanced engraftment in NSG-S mice

Core binding factor (CBF), a heterodimeric transcription factor that plays an essential role in controlling and regulating normal and leukemic differentiation, is a frequent target of gene rearrangements and mutations in AML.[25] CBF-AML patients represent 10-15% of all patients with AML and are characterized by two recurrent translocations: t(8;21)(q22;q22) and inv(16)(p13.1; q22) or t(16;16)(p13.1;q22).[26,27] These AML samples with favorable karyotypes are known to engraft poorly in NSG mice. We included 10 CBF-AML samples [2 with t(8;21) and 8 with inv(16)] in our strain comparison study, and were able to successfully engraft all 8 inv(16) AML samples in NSG-S mice (Figure 2D). Interestingly, the 2 t(8;21) samples did not show enhanced engraftment in the NSG-S mice suggesting perhaps a specific defect for the particular translocation. The presence of chromosomal abnormalities was confirmed in bone marrow blasts from engrafted NSG-S mice using a reverse transcriptase polymerase chain reaction to amplify the CBFβ-MYH11 fusion transcript and fluorescence *in situ* hybridization to detect the inv(16) breakpoint region (Figure 2E). Thus, NSG-S mice provide a permissive environment to support leukemia-initiating cells from low risk inv(16) patients. Whether this reflects a particular requirement of cytokines for inv(16) AML stem cells will require further studies.

Characterization of myelodysplastic syndrome cell engraftment in xenotransplantation models

We next turned to characterizing MDS cell engraftment in the two mouse strains. MDS engraftment in patient-derived xenotransplantation models is less well described than AML. Unlike AML, MDS should provide multi-lineage engraftment which requires further characterization. It has also been reported that MDS engraftment is enhanced by co-injection of MSC and we designed experiments to study these variables. We initially transplanted 7 MDS patient samples in NSG and/or NSG-S immunocompromised mice (Figure 3A). The patients' samples used were separately considered as high risk (MDS with excess blasts-1/2), or low risk (MDS/myeloproliferative neoplasm, unclassified low-risk MDS, therapy-related

Table 1. Clinical characteristics of the patients with acute myeloid leukemia.

Patient ID	Age (year)	Sex	FAB classification	WBC	BM/PB	Disease status	Cytogenetics	*FLT3*	*NPM*	Blasts % (PB)	Blasts % (BM)	Engrafter in NSG	Engrafter in NSG-S
2678	32	M	AML - myeloid sarcoma	0.0723	PB	NA	Normal	NA	NA	NA	NA	MSC	ND
1532	69	M	AML with 11q23 abnormalities	0.1375	PB	Treatment related	t(11;22)(q23;q13)	WT	NA	64	NA	N	MSC
1907	82	M	AML with 11q23 abnormalities	0.115	PB	*De novo*	t(9;11), der(13;14)	NA	NA	75	NA	N	MSC
2022	63	F	AML with 11q23 abnormalities	0.0342	PB	Treatment related	t(11;16)(q23;p13.3)	WT	NA	26	NA	N	ND
3876	49	M	AML with inv16(p13;q22) or t	0.1136	PB	*De novo*	del(12), ins(14;12), inv(16),+21	D835	WT	NA	NA	N	MSC
2955	69	F	AML with inv16(p13;q22) or t	0.0797	PB	*De novo*	46,XX,inv(16)(p13.2q22)	WT	WT	72	NA	N	ND
2120	68	F	AML with inv16(p13;q22) or t	0.0145	BM	*De novo*	inv(16)(p13.2q22)	WT	NA	NA	60	N	MSC
3328	68	F	Secondary	0.0218	PB	Treatment related	inv(16)(p13.1q22)/47,idem,+8	NA	NA	50	NA	MSC	MSC
3339	29	M	AML with inv16(p13;q22) or t	0.0099	BM	*De novo*	46,XY,del(7)(q32),inv(16),(p13.3q22)/47,idem+22/46,XY	WT	WT	NA	27	N	MSC
1780	65	M	AML with inv16(p13;q22) or t	0.0269	PB	*De novo*	46,XY,inv(16)(p13.1q22)	WT	NA	11	NA	N	MSC
559	35	M	AML with inv16(p13;q22) or t	0.15	BM	*De novo*	47,XY,inv(16)(p13.3q22),+22/48,XY,idem,+9NA	NA	NA	77	N	MSC
690	46	F	AML with inv16(p13;q22) or t	0.1293	PB	*De novo*	46,XX,inv(16)(p13.3q22)/47,XX,idem+8	NA	NA	61	NA	N	MSC
1587	70	M	AML with t(8;21)(q22;q22)	0.1709	PB	Treatment related	t(8;21)(q22;q22),del(13)(q12q22)	WT	NA	91	NA	N	N
3526	34	M	AML with t(8;21)(q22;q22)	0.0315	PB	NA	t(8;21)(q22;q22)	NA	NA	67	68	N	ND
3219	55	M	AML-MLD hx prior MDS/MPN	0.14	PB	*De novo*	del(7)(p11.2)/46,XY	WT	WT	10	NA	N	ND
3198	76	F	AML-MLD hx prior MDS/MPN	0.1446	PB	*De novo*	NA	ITD	WT	21	60	MSC	MSC
3119	52	M	AML-MLD no prior MDS or MPN	0.0315	PB	*De novo*	Normal	WT	MUT	24	32	MSC	ND
2810	76	M	AML-MLD with prior MDS	0.1536	PB	Treatment related	add(20)(q11.2)	D835	WT	40	NA	N	N
2323	75	M	AML-MLD with prior MDS	0.0753	PB	*De novo*	ND	WT	NA	63	NA	N	ND
2589	65	M	AML-MLD with prior MDS	0.1164	PB	Relapsed	47,XY,+11[18]/46,XY[2]	ITD	WT	NA	NA	N	ND
3094	79	F	AML-MLD with prior MDS	0.0571	PB	Relapsed	Normal	NA	NA	4	NA	N	ND
3227	65	M	AML-MLD with prior MDS	0.0793	PB	NA	Normal	WT	NA	89	NA	MSC	ND
3229	68	F	AML-MLD with prior MDS	ND	PB	Refractory	Normal	WT	NA	NA	NA	MSC	ND
3221	65	M	AML-MLD with prior MDS	0.1616	PB	Relapsed	Normal	WT	NA	89	NA	MSC	MSC
2012	65	M	AML-MLD with prior MPN	0.1596	PB	*De novo*	Complex	WT	NA	NA	NA	MSC	ND
3406	66	M	AML-MLD with prior MPN	0.1704	PB	*De novo*	del(20)(q11.2q13.1)	WT	WT	77	70	MSC	MSC
3568	76	NA	Biphenotypic	0.2457	PB	NA	Complex	ITD	WT	NA	NA	MSC	MSC
3769	35	M	CML (myeloid blast crisis)	0.5717	PB	NA	t(9;22)(q34;q11.2)/48,idem,+8,+19	NA	NA	66	NA	N	N
1902	43	M	M0	ND	PB	Refractory	NA	NA	NA	41	90	N	MSC
2711	44	M	M1	0.326	PB	*De novo*	Normal	ITD	WT	NA	NA	N	MSC
1658	70	F	M1	0.0962	PB	*De novo*	Normal	ITD	NA	NA	NA	N	MSC
3516	25	F	M1	0.3045	PB	NA	inv(9)(p12q13)	ITD	WT	91	NA	N	ND
2141	52	M	M1	0.0454	PB	Relapsed	NA	ITD	NA	83	NA	MSC	ND
1526	78	M	M1	0.0713	PB	*De novo*	Normal	ITD	NA	78	NA	MSC	MSC
2943	65	M	M1	0.1928	PB	*De novo*	Normal	ITD	MUT	94	NA	MSC	ND
1750	63	M	M3 (APML)	0.1003	PB	NA	t(15;17)	NA	NA	NA	NA	N	MSC
1919	27	F	M4	0.1457	PB	Relapsed	NA	NA	NA	83	NA	N	MSC
3368	65	M	M4	0.2516	PB	*De novo*	NA	WT	MUT	74	90	N	MSC
3779	60	M	M4	0.1845	PB	*De novo*	NA	ITD	WT	NA	NA	N	MSC
1245	58	M	M4	0.1364	PB	*De novo*	normal	ITD	NA	NA	NA	N	ND
2017	64	M	M4	0.0766	PB	Untreated	NA	NA	NA	NA	51	N	ND
2293	52	M	M4	0.1952	PB	*De novo*	Normal	ITD	NA	87	NA	N	ND
1926	58	M	M4	0.0871	PB	Relapsed	NA	ITD	NA	89	NA	MSC	ND
1956	52	F	M4	0.1391	PB	*De novo*	47,XX,+8/46,XX	ITD	NA	51	NA	MSC	ND
2093	58	F	M4	0.079	PB	*De novo*	Normal	ITD	NA	60	NA	MSC	ND
2266	56	M	M4	0.314	PB	*De novo*	Normal	ITD	NA	90	90	MSC	ND
2522	52	M	M4	0.113	PB	Refractory	Complex	ITD	NA	NA	NA	MSC	ND
2623	58	F	M4	0.2878	PB	Relapsed	NA	ITD	NA	NA	NA	MSC	ND
3216	51	F	M4	0.1038	PB	Relapsed	t(3;3)(q21;q26),del(7)(p13),del(17)(p12),-21	WT	NA	50	NA	MSC	MSC
3254	63	M	M4	0.1595	PB	*De novo*	Complex	WT	WT	NA	NA	MSC	MSC
3965	55	M	M4	0.0577	PB	NA	Normal	ITD	WT	89	NA	MSC	MSC
3370	65	M	M4	0.2419	PB	*De novo*	Normal	WT	MUT	74	90	MSC	ND
1731	76	M	M5	0.129	PB	*De novo*	Normal	WT	NA	NA	NA	N	MSC

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3918	73	M	M5	0.1397	PB	*De novo*	Normal	WT	MUT	40	NA	N	MSC
2258	57	M	M5	0.0429	PB	*De novo*	NA	NA	NA	77	NA	N	ND
2698	43	F	M5	0.1298	PB	*De novo*	NA	ITD	MUT	87	NA	MSC	MSC
2844	73	M	M5	0.3246	PB	*De novo*	45,X,-MSC/46,XY	WT	WT	51	80	MSC	MSC
4090	68	M	NA	0.0979	PB	NA	ND	NA	NA	25	NA	N	MSC
2750	72	M	NA	0.0983	PB	*De novo*	Normal	WT	WT	73	NA	N	N
53	59	F	NA	0.1836	PB	NA	Normal	ITD	NA	NA	70	MSC	ND
3196	64	M	NA	0.0711	PB	Relapsed	47,XY,+8, t(3;17)	ITD	NA	87	NA	MSC	ND
3365	62	M	NA	0.0839	PB	*De novo*	Normal	ITD	MUT	22	80	MSC	ND
3949	59	F	NA	0.0778	PB	Refractory	Normal	ITD	NA	94	94	MSC	MSC
4133	58	M	NA	NA	PB	Relapsed	NA	ITD	NA	72	NA	MSC	MSC
2339	47	M	ND	0.349	PB	Relapsed	NA	WT	NA	80	NA	N	ND
3055	54	F	NOS	0.431	PB	*De novo*	Normal	ITD	MUT	NA	NA	MSC	ND
2748	64	F	NOS	0.1026	PB	*De novo*	Normal	NA	MUT	97	NA	N	N
1932	31	F	NOS	0.215	PB	Relapsed	Normal	NA	NA	NA	NA	N	ND
2933	56	M	NOS	0.0476	PB	*De novo*	Normal	WT	WT	76	NA	N	ND
3081	59	M	NOS	0.0224	BM	*De novo*	Normal	WT	MUT	79	90	N	ND
3033	74	M	NOS	0.038	BM	*De novo*	NA	WT	WT	NA	68	N	ND
774	37	F	NOS	0.1245	PB	*De novo*	t(7;11) (p15;p15)	ITD	NA	NA	NA	MSC	ND
2741	52	M	NOS	0.1317	PB	Relapsed	Complex	ND	NA	NA	NA	MSC	MSC
2837	43	F	NOS	0.0864	PB	*De novo*	47,XX,+8	ITD	WT	91	NA	MSC	MSC
3261	39	M	NOS	0.0955	PB	*De novo*	Normal	ITD	MUT	NA	NA	MSC	MSC
1897	64	F	Secondary	0.0348	BM	Treatment related	t(8;16) (p11.2;p13.3)	WT	NA	NA	90	N	MSC
2107	64	F	Secondary	0.0649	BM	Relapsed	t(8;16) (p11.2;p13.3)	WT	NA	94	NA	MSC	ND
2074	78	F	Secondary	0.0237	PB	Treatment related	Complex	WT	NA	90	NA	N	ND

M: male; F: female; MSC: engrafted; N: not engrafted; ND: not done; NA: not available; PB: peripheral blood; BM: bone marrow; FAB: French-American-British classification; WBC: white blood cell count (x 10^9 cells/L); ITD:internal tandem duplication; WT: wild-type; MUT: mutated.

myeloid neoplasms) (Table 2). We performed intrabone injections directly into the femoral cavity at the orthotopic site as it has been described in patient-derived xenotransplantation models for AML, that intrabone cell transplantation results in a higher probability of successful engraftment. This can be advantageous for patient-derived xenotransplantation models of MDS in particular, as the numbers of bone marrow mononuclear cells are commonly modest or low.[28] In order to address whether the presence of human stromal cells results in increased engraftment levels, MSC were *ex vivo*-expanded and co-injected along with the patients' mononuclear cells. Levels of engraftment were assessed by bone marrow aspiration at different time points throughout the experiment and are expressed as the percentage of human $CD45^+$ cells. To assess the subpopulations of cells engrafted, cells were further analyzed with the lineage-specific antibodies CD19 for B cells and CD3 for T cells as well as for the presence of stem and progenitor cells using the markers CD34 and CD38, CD123 and CD45RA, respectively (Figure 3B). In the majority of engrafted patients' samples, B and T cells were not detected or were detected at uncommonly low levels at the time points tested. In contrast, the myeloid $CD33^+$ component was present in all mice tested as well as subpopulations of $CD34^+$ and $CD38^+$ human cells. The ability to differentiate into cells of the erythroid lineage was assessed in the low and negative fractions of human $CD45^+$ cells using the erythroid differentiation markers CD71 and glycophorin A. Even though erythroid cells at the initial stages of differentiation were detected in all mice tested, cells lacked the ability to differentiate further into more mature cells (Figure 3C). These results were further confirmed by immunohistochemical analysis of decalcified bone sections, showing a broad presence of human CD33-stained cells, but absence of megakaryocytes expressing GPIIIa and erythroid cells expressing glycophorin C (Figure 3D). Overall, these results confirm that MDS cells with multi-lineage potential can potentially be transferred and maintained in immunocompromised mice.

Most myelodysplastic syndrome samples do not show sustained engraftment

The term "engraftment" used in the context of xenotransplantation studies is equated with long-term maintenance and, typically, expansion of transplanted cells. To quantitatively assess whether MDS cells engraft in NSG-S mice, we divided our samples into low- and high-risk MDS. As shown in Figure 4, engraftment of high-risk MDS was heterogeneous with 2 samples never showing robust engraftment but 2 samples showing early and sustained engraftment (Figure 4A). Only one of the 3 low-risk MDS samples demonstrated clear engraftment, whereas levels of engraftment decreased over time for the other 2 samples (Figure 4B). For comparison, a secondary AML sample shows the expected low early engraftment with increase over time (Figure 4A).

The presence of human cytokines marginally improves engraftment

Direct comparison of engraftment levels between NSG and NSG-S mice transplanted with mononuclear cells

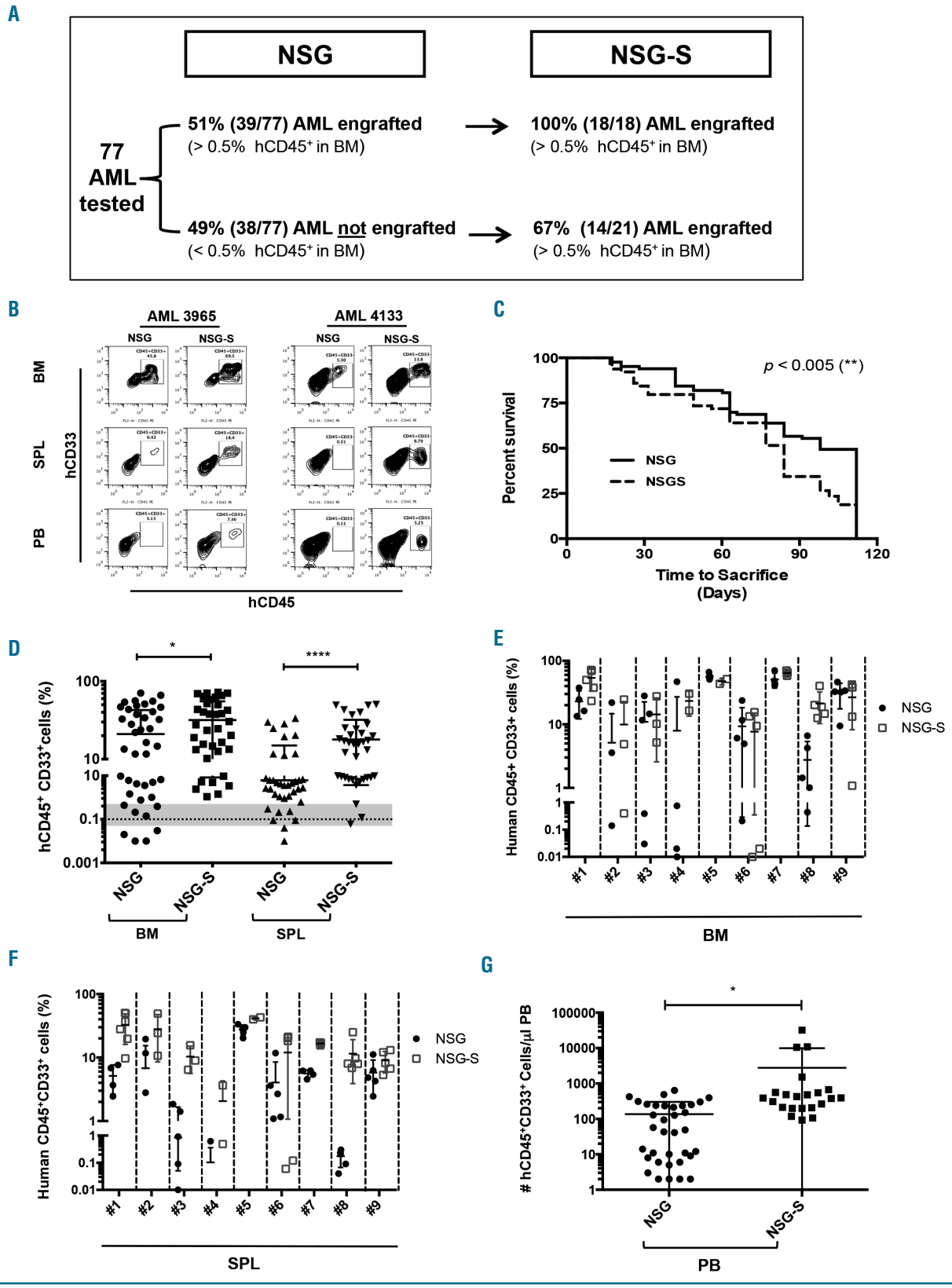

Figure 1. NSG-S mice showed higher and more rapid engraftment of primary patients' acute myeloid leukemia (AML) samples than did NSG mice. (A) Thirty-nine of the 77 AML samples screened were engrafted (hCD45+CD33+ blasts > 0.5% in mouse bone marrow) in NSG mice. All 39 AML engrafted in NSG mice also engrafted in NSG-S mice. Thirty-eight AML samples did not engraft in NSG, and approximately two-thirds of these engrafted in NSG-S mice. (B) Representative flow cytometry plots of human hCD45+CD33+ leukemia cells from bone marrow (BM), spleen (SPL) and peripheral blood (PB) samples from NSG and NSG-S mice. (C) Kaplan-Meier curves show the time to sacrifice mice for all 77 AML samples. (D) Leukemia burden of 9 AML engrafted in both NSG and NSG-S mice which were sacrificed at the same time was assessed by % of human hCD45+CD33+ cells in mouse BM and SPL. Each symbol represents one mouse. (E and F) Individual mouse BM and SPL leukemia burdens of the 9 AML engrafters are shown. Solid symbols represent data from NSG mice, and empty symbols represent data from NSG-S mice. (G) Elevated levels of leukemia burden (% hCD45+CD33+ cells) were seen in NSG-S mice.

from MDS patients revealed a small increase in the mice expressing the human cytokines at eight weeks after intrabone injections (Figure 4C). This difference was sustained at 16 weeks in all patients' samples tested but was not statistically significant at this time point (Figure 4D). Thus, in contrast to their effect on AML, human cytokines do not appear to provide a significant engraftment or growth advantage to human MDS in NSG-S mice.

Engraftment levels are independent of the presence and the origin of mesenchymal stem cells

To verify that the MSC are functional cells, we performed a phenotypic characterization and also tested the cells' ability for trilineage differentiation *in vitro* (Figure 5A-D). Overall, patient-derived and normal donor MSC appear to express all MSC markers (Figure 5A) and are able to differentiate into osteoblasts (Figure 5B), adipocytes (Figure 5C) and chondrocytes (Figure 5D), therefore having features of *bona fide* MSC.

In order to investigate whether the presence of human stromal cells might be used as a better supporting tissue for the engraftment of MDS in immunodeficient mice, *in vitro*-expanded MSC derived from patients and/or healthy donors were intrafemorally co-transplanted along with the patients' mononuclear cells. Engraftment levels were measured at eight weeks after transplantation by bone marrow

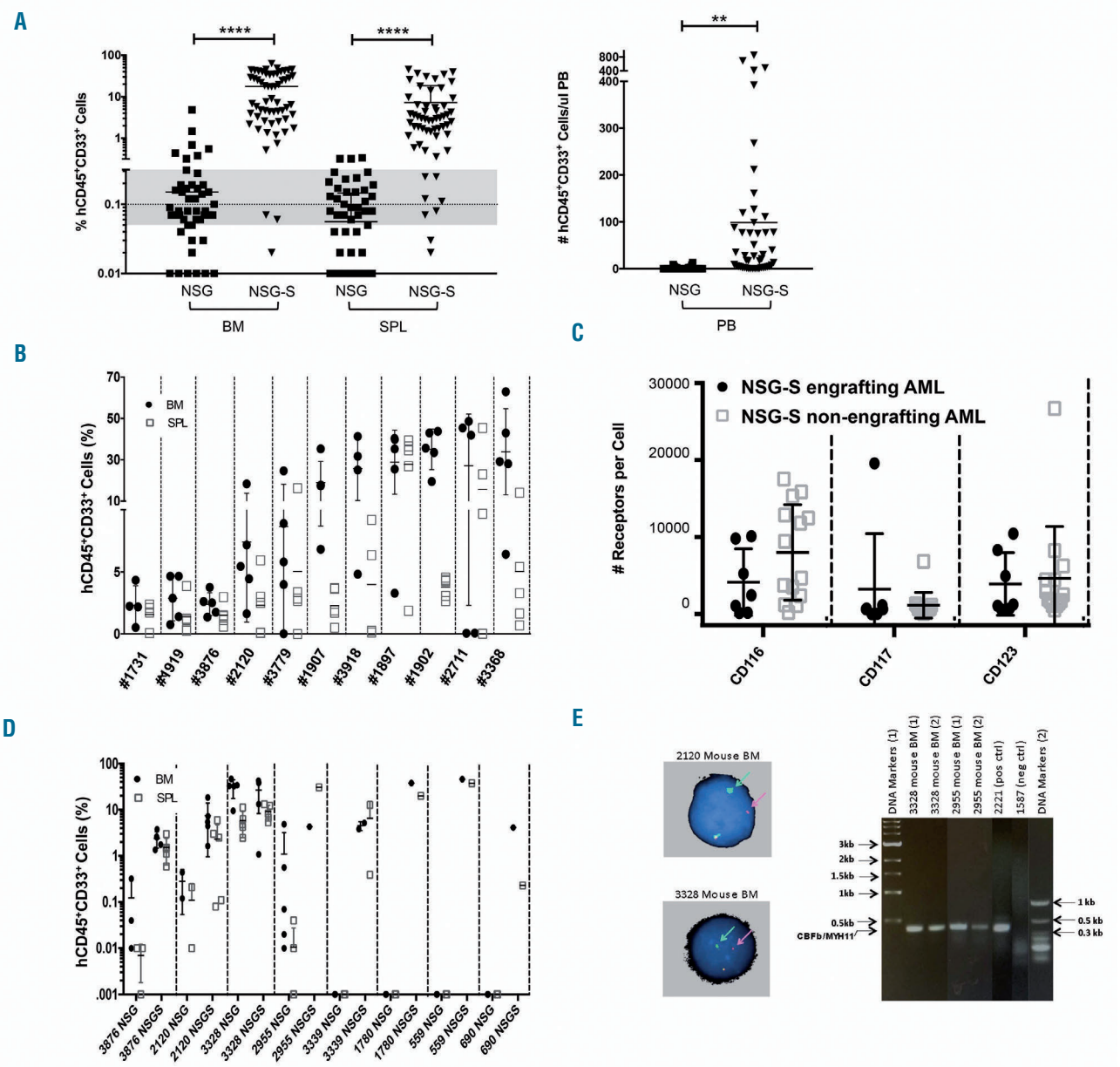

Figure 2. NSG-S mice enhanced the engraftment of human patients' acute myeloid leukemia (AML) samples more than NSG mice (non-NSG engrafted patients' samples were used). (A) Levels of hCD45$^+$CD33$^+$ leukemia blasts in bone marrow (BM), spleen (SPL) and peripheral blood (PB) of NSG or NSG-S mice (n=14) injected with the same number of AML cells. (B) Individual mouse BM and SPL leukemia burdens of 11 out of the 14 mice from (A) are shown. (C) Receptor densities (CD116, CD117 and CD123) on AML cells of the 7 non-NSG-S engrafters and 14 NSG-S engrafters were assessed as number of receptors per cell. (D) Establishment of 8 inv(16) AML patient-derived xenotransplant models in NSG-S mice. (E) The chromosomal abnormalities for inv(16) were confirmed in the BM of engrafted NSG-S mice by fluorescent *in situ* hybridization (left panels) and breakpoint reverse transcriptase polymerase chain reaction (right panel).

aspiration. No significant improvement was observed associated with the presence of human MSC in the murine environment. Interestingly, in the majority of cases in which MSC appeared to have a slightly positive effect in engraftment levels on week 8 after transplantation, the effect was lost when engraftment levels were reassessed at week 16 (*data not shown*). In a direct comparison experiment between NSG and NSG-S mice, the presence of MSC produced a mild improvement in the engraftment levels in the patients' samples tested, at eight weeks, irrespective of the mouse strain. Similarly to before, the positive effect appeared to fade at 16 weeks in both NSG and NSG-S mice (*Online Supplementary Figure S1*). Next, to assess whether MSC from various sources might contribute to MDS

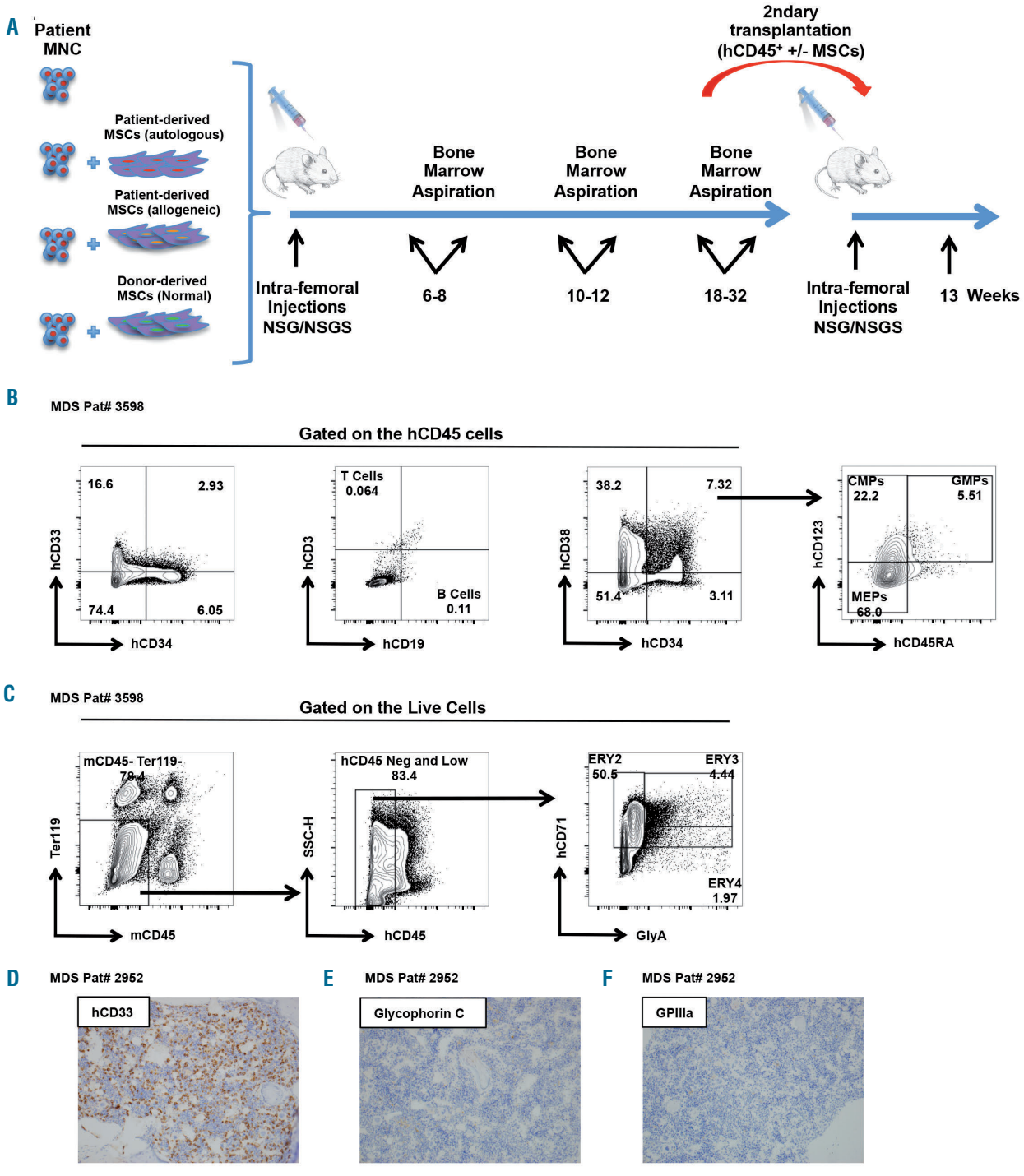

Figure 3. Myelodysplastic syndromes (MDS) cells engraft in NSG-S mice. (A) Schematic representation of experimental set up. Patient-derived bone marrow mononuclear cells (MNC) alone or in combination with mesenchymal stem cells (MSC) were intrafemorally injected in NSG-S mice. Characteristic gating strategy for analyzing the (B) myeloid (CD33), lymphoid (CD19, CD3), progenitor (CD45RA, CD123, CD38) and (C) erythroid (GlyA) subpopulations found in the bone marrow of mice engrafted with patients' bone marrow MNC samples. Immunostainings of (D) hCD33, (E) glycophorin C and (F) GPIIIa of decalcified bone marrow sections from sternums of mice engrafted with human MDS.

engraftment, we further compared engraftment of MDS mononuclear cells without MSC to healthy donor-derived MSC (normal), or allogeneic patient-derived MSC (allo) and to patient-derived autologous MSC (auto) (Figure 3A). Although some mice showed increased engraftment with different MSC samples tested, no consistent pattern of enhanced engraftment was seen that correlated with the source of the MSC (Figure 5E).

To better understand the transient effect of the presence of MSC on the levels of engraftment observed for some of the patients' samples tested, *in vitro*-expanded MSC were labeled using a lentiviral plasmid expressing green fluorescent protein and luciferase. Those cells were further transplanted *via* intrabone injections in NSG mice and monitored over time. *In vivo* imaging revealed gradually decreasing levels of luminescence (Figure 5F). Luminescence was solely detected in the area of the injected femur and its levels were completely diminished by week 4 after transplantation.

Long-term engraftment of myelodysplastic syndrome cells in NSG-S mice

To evaluate whether long-term engraftment of MDS cells can be achieved in NSG-S mice, human CD45 cells, isolated from the bone marrow of well-engrafted primary recipients, were selected and intrafemorally transplanted into secondary recipients with or without MSC according to the experimental plan (Figure 6A). Thirteen weeks after transplantation the mice were sacrificed. Analysis of the different subpopulations identified the presence of myeloid $CD33^+$ cells, as well as $CD34^+CD38^-$ cells. As in the primary recipients, no B or T cells were detected (Figure 6B). Small erythroid populations in secondary recipients were also unable to differentiate further to more mature erythroid cells (Figure 6C). All mice showed increased levels of engraftment compared to the engraftment levels of the primary recipients at the same time

Table 2. Clinical samples used for the MDS engraftment studies.

Patient ID#	Diagnosis	Risk level
2970	MDS/MPN	Low
2381	Therapy related myeloid neoplasm	Low
108	Unclassified MDS	Low
3598	MDS-EB-1	High
2952	MDS-EB-1	High
4712	MDS-EB-1	High
3282	MDS-EB-2	High

MDS: myelodysplastic syndrome; MPN: myeloproliferative neoplasm; EB: excess blasts.

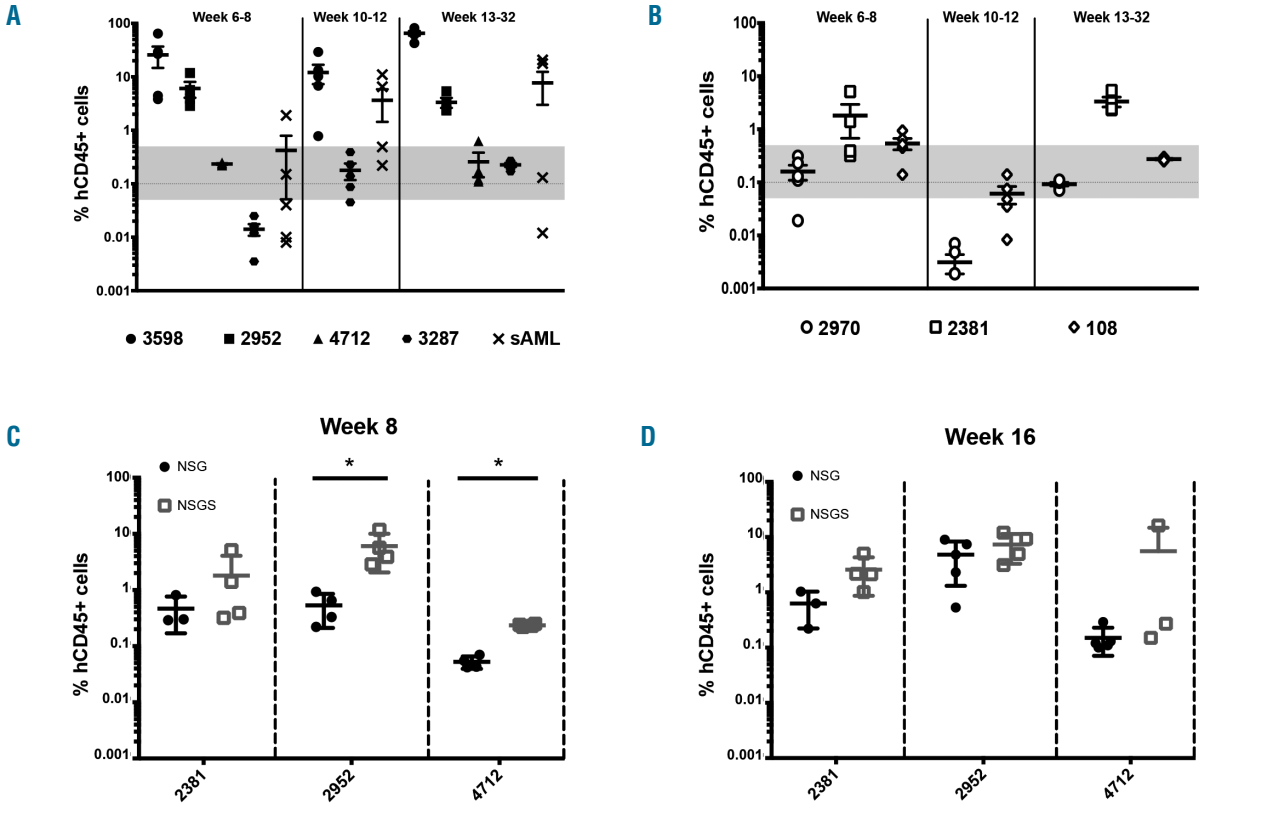

Figure 4. Most myeloproliferative syndrome (MDS) samples do not show sustained engraftment and human cytokines moderately enhance engraftment. Time course representation of the percentages of $hCD45^+$ cells in the bone marrow of xenografted NSG-S mice injected with (A) high risk and (B) low risk human MDS patients' mononuclear cells. The gray line corresponds to the 0.1% threshold used in the study and the gray zone reflects a broader area of uncertainty of engraftment. Bone marrow mononuclear cells from patients diagnosed with MDS were intrafemorally injected into NSG and NSG-S mice in a strain comparison experiment. Percentage of $hCD45^+$ cells found in the murine bone marrow was evaluated at (C) eight and (D) 16 weeks after transplantation.

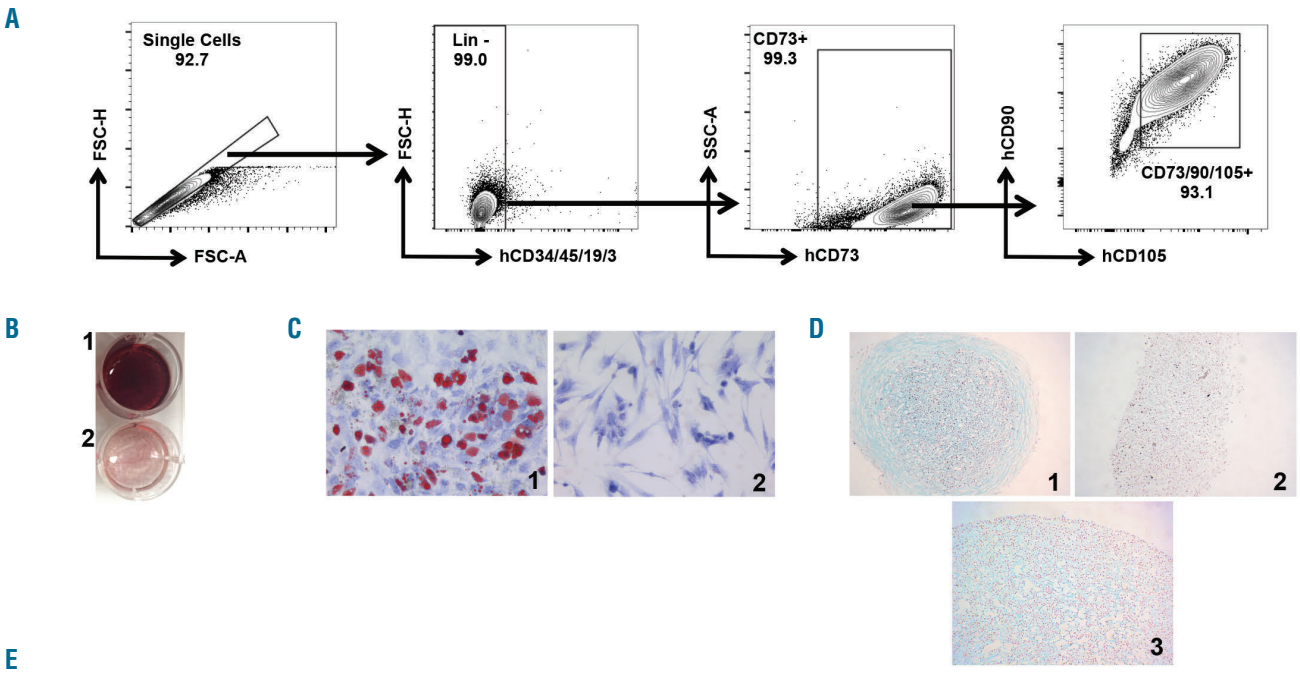

Figure 5. Myelodysplastic syndrome (MDS) cell engraftment is not affected by mesenchymal stem cells (MSC). (A) Representative phenotypical characterization of the *ex vivo*-expanded human MSC used in the transplantation experiments. Trilineage *in vitro* differentiation of *ex vivo* expanded MDS patient MSC into (B) osteoblasts, (C) adipocytes, and (D) chondrocytes. [(1) *Differentiated*, (2) *Undifferentiated*, (3) *Positive Control*] (E) Bone marrow human mononuclear cells were intrafemorally transplanted into NSG-S mice alone or in combination with *ex vivo*-expanded MSC isolated from various origins. Percentages of hCD45$^+$ cells present in the murine bone marrow at 6-8 weeks post transplantation. Red line at 0.1% represents the positive engraftment threshold level. Comparisons showed no significant statistical differences between the four groups. (F) NSG mice intrafemorally transplanted with labeled MSC-green fluorescent protein-luciferase. Bioluminescence levels were measured *in vivo* at the timepoints indicated.

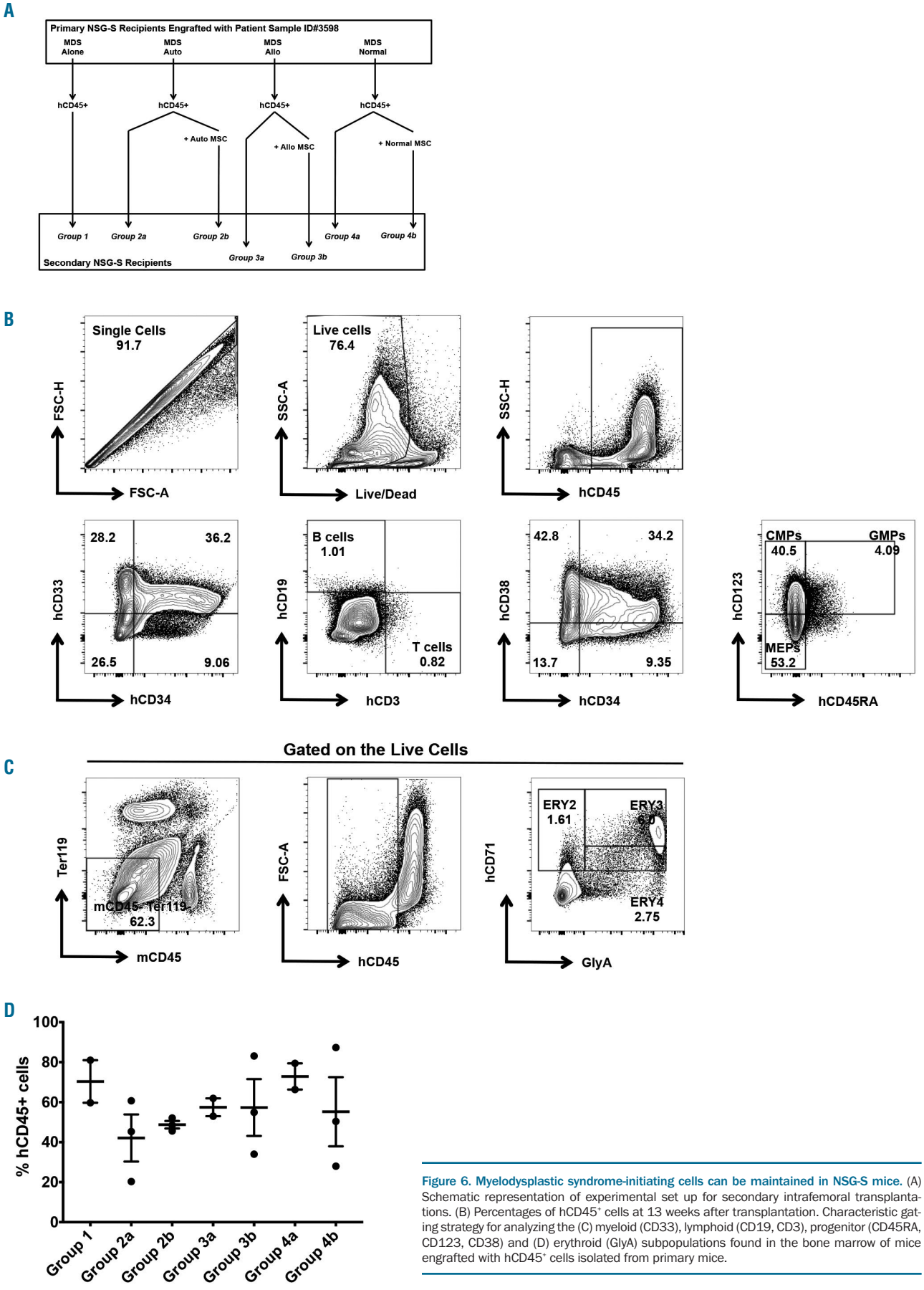

Figure 6. Myelodysplastic syndrome-initiating cells can be maintained in NSG-S mice. (A) Schematic representation of experimental set up for secondary intrafemoral transplantations. (B) Percentages of hCD45$^+$ cells at 13 weeks after transplantation. Characteristic gating strategy for analyzing the (C) myeloid (CD33), lymphoid (CD19, CD3), progenitor (CD45RA, CD123, CD38) and (D) erythroid (GlyA) subpopulations found in the bone marrow of mice engrafted with hCD45$^+$ cells isolated from primary mice.

point (Figure 6D), whereas the presence of MSC did not appear to improve engraftment regardless of the origin of the MSC (Figure 6D).

Discussion

Ongoing development of so-called "humanized" mouse strains that combine immunodeficiency with expression of human proteins or cells permits increasingly sophisticated modeling of human diseases in xenotransplantation models. Here we have used the NSG and NSG-S strains to study the effects of human cytokines on engraftment and growth of human myeloid diseases, AML and MDS. Our results show that NSG-S mice represent a significantly improved patient-derived xenotransplantation model to accelerate and enhance leukemic engraftment compared to NSG mice, and to support engraftment for the vast majority of all primary AML samples, making this model particularly useful for pre-clinical studies. In contrast, engraftment of human MDS in NSG or NSG-S mice is possible but not robust and in several cases not sustained. High-risk MDS is more likely to achieve long-term engraftment compared to low-risk MDS. The expression of human cytokines in NSG-S mice marginally improves engraftment levels. In developing this model we also note that human MSC do not provide long-term MSC engraftment in NSG-S mice and do not contribute to MDS cell engraftment or expansion. Overall, these results are consistent with *in vitro* studies suggesting that AML cells are actually more responsive to bone marrow-derived cytokines than MDS cells, at least as measured by engraftment and growth in immunocompromised mouse strains.[29-32]

Engraftment of AML samples in NSG mice has been the standard measure of assessment of leukemic stem cells for the last ten years. However, our results with AML overall, and particularly with inv(16) AML, demonstrate that severe combined immunodeficiency leukemia engrafting ability, the functional definition of leukemic stem cells, can be strongly affected by the recipient mouse strain and the extent to which the recipient environment contains human cytokines or cytokines that cross-react with human cytokine receptors. More formal exploration of stem cells comparing NSG and NSG-S mice will further define the dependency of severe combined immunodeficiency leukemia-initiating cells on cytokines. Furthermore, these studies raise significant questions about AML biology that should be addressable using the NSG-S model. Previous authors have suggested that AML cells require pathological activation of signaling pathways for full transformation.[33] However, our data demonstrate that the majority of AML samples may remain responsive to cytokines produced by the bone marrow as was also shown by Ellegast *et al.*[34] Given the recent results that effective 3rd-generation FLT3 inhibitors are clinically active but not curable it may be of therapeutic value to consider whether cytokines can regulate cell survival allowing AML cells to escape FLT3 inhibition.[35]

In contrast to AML, human MDS xenotransplantation has not been thoroughly studied. Although we studied a modest number of MDS samples, our results comparing MDS injections into NSG *versus* NSG-S mice with or without MSC co-injection are similar to other recently described results.[36] A critical question is the formal definition of an MDS-engrafting cell, the functional equivalent of the severe combined immunodeficiency leukemia-initiating cell as initially defined by Bonnet and Dick.[22] AML stem cells typically traffic to the marrow after xenotransplantation, remain quiescent for some weeks and then initiate expansion. In contrast, MDS cells in our study were injected into the marrow where they appear to remain but do not expand for several months. Some samples, from patients with high-risk MDS, demonstrated expansion and our results show that MDS initiating cells can be maintained in NSG-S mice. More specifically, secondary recipient NSG-S mice injected with human CD45 cells isolated from primary recipient mice developed abnormal hematopoiesis identical to that of the primary recipients, accompanied by a significant expansion of the phenotypic stem/progenitor cell compartment, which is typical in cases of AML passaged into secondary xenotransplanted animal models. Whereas genetic analysis showed the presence of identical genetic lesions between the patients' samples and the primary recipient animals (*data not shown*), the selection of an unidentified minor AML clone in the secondary recipient animals is a possibility that requires further exploration. Examination of the human cell populations present in the murine bone marrow revealed signs of abnormal hematopoiesis reflected by the presence of stem/progenitor cells, the prevalence of the myeloid component and, in the majority of cases, the complete absence of lymphoid cells. Erythropoiesis was compromised and even though cells at the initial stages of human erythroid differentiation (ERY1, ERY2) were present in both the bone marrow and the spleen (*data not shown*), these cells were not capable of further differentiation, suggesting a blockage in the erythroid differentiation process, as has been previously described.[37] Our findings suggest that the development of a patient-derived xenotransplantation model for MDS is possible, but not yet robust, and that the definition of MDS stem cells may include long-term engraftment but not expansion. The development of an ectopic human niche might provide a solution since promising results have been reported in several types of leukemia.[38,39]

The use of MSC to humanize the murine bone marrow niche has been proposed as a method to enhance MDS engraftment.[20] However, we demonstrated that human MSC engrafted into murine bone marrow do not establish long-term engraftment. It is theoretically possible that short-term survival of human MSC may promote engraftment of MDS cells but this was not seen in our studies. The reason for this discrepancy from other studies is not clear, but it is notable that AML engraftment is known to be highly variable among animal colonies.

In summary, AML cells demonstrate enhanced engraftment in NSG-S mice compared to NSG mice. MDS cells do not demonstrate a similar response to either the human cytokines produced by the NSG-S strain or by co-injection of human MSC. The findings of these studies are consistent with those of previous *in vitro* studies of AML and MDS and may suggest that MDS cells are not capable of responding to human cytokines with an increase in survival or growth. Further work may determine whether this is a fundamental aspect of the ineffective hematopoiesis that defines MDS.

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References

1. Tallman MS, Gilliland DG, Rowe JM. Drug therapy for acute myeloid leukemia. Blood. 2005;106(4):1154–1163.
2. Walter MJ, Shen D, Ding L, et al. Clonal architecture of secondary acute myeloid leukemia. N Engl J Med. 2012;366(12):1090–1098.
3. Will B, Zhou L, Vogler TO, et al. Stem and progenitor cells in myelodysplastic syndromes show aberrant stage-specific expansion and harbor genetic and epigenetic alterations. Blood. 2012;120(10):2076–2086.
4. Woll PS, Kjällquist U, Chowdhury O, et al. Myelodysplastic syndromes are propagated by rare and distinct human cancer stem cells in vivo. Cancer Cell. 2014;25(6):794–808.
5. Schofield R. The relationship between the spleen colony-forming cell and the haemopoietic stem cell. Blood Cells. 1978;4(1-2):7–25.
6. Lane SW, Scadden DT, Gilliland DG. The leukemic stem cell niche: current concepts and therapeutic opportunities. Blood. 2009;114(6):1150–1157.
7. Suda T, Arai F, Hirao A. Hematopoietic stem cells and their niche. Trends Immunol. 2005;26(8):426–433.
8. Wilson A, Trumpp A. Bone-marrow haematopoietic-stem-cell niches. 2006;6(2):93–106.
9. Méndez-Ferrer S, Lucas D, Battista M, Frenette PS. Haematopoietic stem cell release is regulated by circadian oscillations. Nature. 2008;452(7186):442–447.
10. Yamazaki S, Ema H, Karlsson G, et al. Nonmyelinating Schwann cells maintain hematopoietic stem cell hibernation in the bone marrow niche. Cell. 2011;147(5):1146–1158.
11. Taichman RS, Emerson SG. The role of osteoblasts in the hematopoietic microenvironment. Stem Cells. 1998;16(1):7–15.
12. Calvi LM, Adams GB, Weibrecht KW, et al. Osteoblastic cells regulate the haematopoietic stem cell niche. Nature. 2003;425(6960):841–846.
13. Kremer KN, Dudakovic A, McGee-Lawrence ME, et al. Osteoblasts protect AML cells from SDF-1-induced apoptosis. J Cell Biochem. 2014;115(6):1128–1137.
14. Tavor S, Petit I, Porozov S, et al. CXCR4 regulates migration and development of human acute myelogenous leukemia stem cells in transplanted NOD/SCID mice. Cancer Res. 2004;64(8):2817–2824.
15. Colmone A, Amorim M, Pontier AL, Wang S, Jablonski E, Sipkins DA. Leukemic cells create bone marrow niches that disrupt the behavior of normal hematopoietic progenitor cells. Science. 2008;322(5909):1861–1865.
16. Zhang Y, Xie R-L, Croce CM, et al. A program of microRNAs controls osteogenic lineage progression by targeting transcription factor Runx2. Proc Natl Acad Sci USA. 2011;108(24):9863–9868.
17. Schepers K, Pietras EM, Reynaud D, et al. Myeloproliferative neoplasia remodels the endosteal bone marrow niche into a self-reinforcing leukemic niche. Stem Cell. 2013;13(3):285–299.
18. Sanchez PV, Perry RL, Sarry JE, et al. A robust xenotransplantation model for acute myeloid leukemia. Leukemia. 2009;23(11):2109–2117.
19. Wunderlich M, Chou F-S, Link KA, et al. AML xenograft efficiency is significantly improved in NOD/SCID-IL2RG mice constitutively expressing human SCF, GM-CSF and IL-3. Leukemia. 2010;24(10):1785–1788.
20. Medyouf H, Mossner M, Jann J-C, et al. Myelodysplastic cells in patients reprogram mesenchymal stromal cells to establish a transplantable stem cell niche disease unit. Cell Stem Cell. 2014;14(6):824–837.
21. Wunderlich M, Brooks RA, Panchal R, Rhyasen GW, Danet-Desnoyers G, Mulloy JC. OKT3 prevents xenogeneic GVHD and allows reliable xenograft initiation from unfractionated human hematopoietic tissues. Blood. 2014;123(24):e134–144.
22. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med. 1997;3(7):730–737.
23. Ishikawa F, Yoshida S, Saito Y, et al. Chemotherapy-resistant human AML stem cells home to and engraft within the bone-marrow endosteal region. Nat Biotechnol. 2007;25(11):1315–1321.
24. Sarry J-E, Murphy K, Perry R, et al. Human acute myelogenous leukemia stem cells are rare and heterogeneous when assayed in NOD/SCID/IL2Rγc-deficient mice. J Clin Invest. 2011;121(1):384–395.
25. Speck NA, Gilliland DG. Core-binding factors in haematopoiesis and leukaemia. Nat Rev Cancer. 2002;2(7):502–513.
26. Mrózek K, Bloomfield CD. Chromosome aberrations, gene mutations and expression changes, and prognosis in adult acute myeloid leukemia. Hematology Am Soc Hematol Educ Program. 2006;2006(1):169–177.
27. Mrózek K, Marcucci G, Paschka P, Whitman SP, Bloomfield CD. Clinical relevance of mutations and gene-expression changes in adult acute myeloid leukemia with normal cytogenetics: are we ready for a prognostically prioritized molecular classification? Blood. 2007;109(2):431–448.
28. Kushida T, Inaba M, Hisha H, et al. Intrabone marrow injection of allogeneic bone marrow cells: a powerful new strategy for treatment of intractable autoimmune diseases in MRL/lpr mice. Blood. 2001;97(10):3292–3299.
29. Miyauchi J, Kelleher CA, Yang YC, et al. The effects of three recombinant growth factors, IL-3, GM-CSF, and G-CSF, on the blast cells of acute myeloblastic leukemia maintained in short-term suspension culture. Blood. 1987;70(3):657–663.
30. Hoang T, Haman A, Goncalves O, Wong GG, Clark SC. Interleukin-6 enhances growth factor-dependent proliferation of the blast cells of acute myeloblastic-leukemia. Blood. 1988;72(2):823–826.
31. Vellenga E, Ostapovicz D, Orourke B, Griffin JD. Effects of recombinant Il-3, Gm-Csf, and G-Csf on proliferation of leukemic clonogenic cells in short-term and long-term cultures. Leukemia. 1987;1(8):584–589.
32. Miyauchi J, Kelleher CA, Wong GG, et al. The effects of combinations of the recombinant growth-factors Gm-Csf, G-Csf, Il-3, and Csf-1 on leukemic blast cells in suspension-culture. Leukemia. 1988;2(6):382–387.
33. Kelly LM, Gilliland DG. Genetics of myeloid leukemias. Annu Rev Genomics Hum Genet. 2002;3:179–198.
34. Ellegast JM, Rauch PJ, Kovtonyuk LV, et al. inv(16) and NPM1mut AMLs engraft human cytokine knock-in mice. Blood. 2016;128(17):2130–2134.
35. Perl AE, Altman JK, Cortes J, et al. Selective inhibition of FLT3 by gilteritinib in relapsed or refractory acute myeloid leukaemia: a multicentre, first-in-human, open-label, phase 1-2 study. Lancet Oncol. 2017;18(8):1061–1075.
36. Rouault-Pierre K, Mian SA, Goulard M, et al. Preclinical modeling of myelodysplastic syndromes. Leukemia. 2017;31(12):2702-2708.
37. Shi H, Yamamoto S, Sheng M, , et al. ASXL1 plays an important role in erythropoiesis. Sci Rep. 2016;6:28789.
38. Reinisch A, Thomas D, Corces MR, et al. A humanized bone marrow ossicle xenotransplantation model enables improved engraftment of healthy and leukemic human hematopoietic cells. Nat Med. 2016;22(7):812–821.
39. Abarrategi A, Foster K, Hamilton A, et al. Versatile humanized niche model enables study of normal and malignant human hematopoiesis. J Clin Invest. 2017;127(2):543–548.