haematologica
Journal of the Ferrata Storti Foundation

# Recipient sex and donor leukemic cell characteristics determine leukemogenesis in patient-derived models

*by Anna M.P. Stanger, Marlon Arnone, Pauline Hanns, Lucca M. Kimmich, Jessica Kübler, Sarah Gekeler, Elsa S. Görsch, Lea Kramer, Marcelle Baer, Jan C. Schroeder, Taylor S. Mills, Martina Konantz, Saskia S. Rudat and Claudia Lengerke*

*Received: November 13, 2023.*
*Accepted: December 23, 2024.*

*Citation: Anna M.P. Stanger, Marlon Arnone, Pauline Hanns, Lucca M. Kimmich, Jessica Kübler, Sarah Gekeler, Elsa S. Görsch, Lea Kramer, Marcelle Baer, Jan C. Schroeder, Taylor S. Mills, Martina Konantz, Saskia S. Rudat and Claudia Lengerke. Recipient sex and donor leukemic cell characteristics determine leukemogenesis in patient-derived models.*
*Haematologica. 2025 Jan 9. doi: 10.3324/haematol.2023.284647 [Epub ahead of print]*

**Recipient sex and donor leukemic cell characteristics determine leukemogenesis in patient-derived models**

Anna M.P. Stanger[1*], Marlon Arnone[1*], Pauline Hanns[2*], Lucca M. Kimmich[1], Jessica Kübler[1], Sarah Gekeler[1], Elsa S. Görsch[1], Lea Kramer[1], Marcelle Baer[2], Jan C. Schroeder[1], Taylor S. Mills[1], Martina Konantz[2], Saskia S. Rudat[1,3], Claudia Lengerke[1,3#]

* These authors contributed equally to this work

[#] corresponding author

[1] University Clinic Tübingen, Department for Internal Medicine II, University of Tübingen, Tübingen, Germany

[2] University of Basel and University Hospital Basel, Department Biomedicine, Basel, Switzerland

[3] German Cancer Consortium (DKTK), partner site Tübingen, a partnership between DKFZ and University Hospital Tübingen, Germany

**Corresponding author:**

Claudia Lengerke, MD

Department of Internal Medicine II

Hematology, Oncology, Clinical Immunology and Rheumatology

University Hospital Tübingen

Otfried-Mueller-Str. 10

D-72076 Tübingen

Germany

Phone: +49 7071 2982777

Fax: +49 7071 293671

Email: claudia.lengerke@med.uni-tuebingen.de

**Acknowledgments**

We thank all patients for donating blood and BM. We thank the animal facility and flow cytometry department from the Department of Biomedicine in Basel and the University Hospital Tübingen.

**Funding**

This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement n° 866548, HemStem, awarded to CL), the German Cancer Consortium (DKTK) Joint Funding Program (RiskyAML, to CL), the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation, to CL, 467578951 and 539826039), the Nachwuchsgruppenprogramm of the Medical Faculty Tübingen to AMPS, and the MINT Clinician Scientist Program of the DFG to JCS.

**Author contributions**

MA, AMPS, PH and LMK designed and performed experiments analyzed data and generated tables. MA, AMPS and LMK generated figures and prepared the manuscript. JK, SG, EG, LK and MB supported *in vitro* and *in vivo* experiments, respectively and JK assisted with data analyses. TSM, SR, JCS and MK helped with proofreading, data discussion and interpretation. CL supervised the study, designed the experiments, interpreted data and supported writing the manuscript.

**Disclosures**

All of the authors declare to have no conflicts of interest to disclose.

**Data sharing**

The data that support the findings of this study are available from the corresponding author, CL, upon reasonable request.

**Abstract**

In acute myeloid leukemia (AML), leukemogenesis depends on cell-intrinsic genetic aberrations and thus, studies on AML require investigations in an *in vivo* setting as provided by patient derived xenografts (PDX) models. Here we report that, next to leukemic cell characteristics, recipient sex highly influences the outgrowth of AML cells in PDX models, with females being much better repopulated than males in primary as well as secondary transplantation assays. Testosterone may be the more important player since, strikingly, better engraftment was seen in castrated versus control male recipients, while ovariectomy did not significantly impair engraftment in females. Shorter time-to-engraftment and mouse survival were observed with adverse molecular risk, and respectively with high FLT3-ITD ratio mutated AML cells. Adverse risk AML furthermore showed higher percentages of phenotypic leukemic stem cells (LSCs), suggesting impaired differentiation capacity in these AML subtypes. Overall, we achieved successful repopulation with 14/23 (61%) favorable, 18/30 (60%) intermediate and 4/8 (50%) adverse risk AML cases in female recipient PDX models. Our data identify recipient sex as an important experimental confounder in leukemia PDX models, and the contribution of the sex hormones to leukemogenesis as an intriguing, underexplored research area.

## Introduction

Acute myeloid leukemia (AML) is known to develop from hematopoietic stem and progenitor cells (HSPCs) upon acquisition of various genetic aberrations (1). To guide therapy selection, molecular criteria that stratify AML cases in favorable, intermediate and adverse risk groups were established based on patient outcome analyses over the past decades (2).

AML cells harbor cellular subpopulations of so-called leukemic stem cells (LSCs) that are responsible for disease initiation, as well as for the fatal disease relapses commonly occurring even in patients reported to have achieved complete remission (3, 4). Similar to healthy HSPCs as their cell of origin, AML LSCs rely on microenvironmental influences that are challenging to reproduce in a cell culture dish (5-7). Patient derived xenograft (PDX) models in which human AML cells are transplanted to immune suppressed mice are instead commonly used to study human AML and LSCs *in vivo*. Given their ability to mimic disease onset, evolution, heterogeneity and the interactions between AML cells and the microenvironment, PDX models are powerful tools to investigate AML pathogenesis, drug response, and LSC biology. However, PDX models have been mainly used to study adverse molecular risk AML characterized by robust leukemic repopulation capacity, while other AML cases, e.g. of favorable risk AML, remain understudied because they hardly engraft animals in standard assays (8-10).

Here we show that, next to molecular characteristics, recipient sex highly impacts leukemogenesis in PDX models. Transplantation into female recipients promotes leukemic engraftment across various genetic subtypes, allowing robust repopulation with subtypes that were previously reported to be difficult to engraft, such as favorable risk AML. To further study the impact of sex hormones on leukemogenesis, we compared the use of castrated males and respectively ovariectomized female mice as recipients. Our results demonstrate that experiments with PDX models need to consider recipient sex to yield reliable results.

Furthermore, they indicate the impact of sex hormones and the female environment on leukemia development and therapy response as a novel intriguing area of research.

## Methods

### Patient samples

Peripheral blood (PB) samples (Supplemental table 1 and 2) and clinical data were collected following approval by the Ethics Review Board of the University Hospitals of Basel (EKNZ 2015-335) and Tübingen (953/2021B02) from patients with AML at first diagnosis. Peripheral blood mononuclear cells (PBMCs) were enriched by density gradient centrifugation (Sepmate, StemCell and Pancoll, Pan-Biotech) and viably frozen in RPMI1640 medium (Thermo Fisher) supplemented with 20% fetal bovine serum (FBS, Gibco) and 10% DMSO (AppliChem). Patient data were collected from the routine clinical database. Complete remission (CR) is defined as <5% blasts in bone marrow punctures performed in hematologic regeneration after treatment, and residual disease (RD) as ≥5% blasts in post-treatment bone marrow samples.

### Mice, xenotransplantation and homing assay

NOD.Cg-*Prkdcscid IL2rgtmWjl*/Sz (NSG) mice purchased from Jackson Laboratory (Bar Harbor, ME, USA) were bred in-house under pathogen-free conditions according to German and Swiss federal and state regulations. PBMCs were freshly thawed before each experiment. For samples with a CD33 blast count <95%, magnetic cell separation was performed to deplete CD19- and CD3-positive cells (Miltenyi Biotec). For leukemic engraftment comparisons between male and female NSG mice, $1x10^6$ cells were injected intravenously (i.v). For the subsequent kinetic engraftment observations, $0.5x10^6$ cells were

injected intra-femorally in 6-10-week-old female NSG mice. The transplantation procedure and monitoring were performed as previously described (11) and detailed in the Supplemental information. Of note, within one experiment all female and male mice displayed the same age at transplantation, but between experiments the age ranged from 6-10 weeks. All animals were pre-conditioned with sub-lethal irradiation (100 Gy/Kg) 24 hours prior to transplantation and each sample were injected into age-matched animals. Mice were considered engrafted upon detection of > 0,1% human leukemic cells among murine cells of PB, BM or other analyzed tissues. The experiment was terminated at detection of high leukemic burden (defined as > 60% of leukemic among mouse cells in the murine PB or BM), or sickness score exceeding 3, or after one-year post-transplant follow-up.

For secondary transplant assays, BM cells from mice engrafted with one primary AML were pooled, enriched for human CD33 expression as described (11) and then injected i.v. at equal numbers ($1x10^6$) as described above.

For homing experiments, $1x10^6$ primary AML cells were labeled with CFSE and injected i.v. in non-irradiated male NSG mice. 16 hours post-injection, BM and PB were sampled and analyzed for CFSE+ AML cells using flow cytometry.

**Flow cytometry analysis**

Single cell suspensions from patients or mouse BM, PB, spleen and liver were stained with fluorescently labeled antibodies against human CD33, CD34, CD117, CD45RA, CD38, CD47 (BD Biosciences), NKG2DL (R&D), CD123 (Miltenyi), GPR56, CD3 and CD19 (Biolegend). Dead cell exclusion was performed using the fixable aqua dead cell stain kit (Thermo Fisher). All marker expression values derive from live CD33 positive cells.

### Statistical analysis

Data are expressed as mean ± SD. Comparisons were performed after analysis of normal distribution using (un)paired Student t tests, Mann-Whitney-U test or one-way ANOVA. Survival analyses were performed using Kaplan-Meier. All analyses were performed with GraphPad Prism v9.4.1 and statistical significance is defined as a P value < 0.05.

## Results

### Recipient sex influences leukemogenesis in AML PDX models

To test the effect of female versus male recipient sex on leukemogenicity, we transplanted equal numbers of leukemic cells from the same patients into pre-conditioned age-matched male and female mice. Of note, donor AML cells from both female and male patients were used. Interestingly, female recipients transplanted with female donor cells showed highest leukemic infiltration (BM: 80.8 ± 28.0 %), followed by female recipients receiving male AML donor cells (BM: 54.5 ± 24.7 %). Latter were also better repopulated compared to male recipients transplanted with male AML cells (BM: 29.7 ± 31.3 %), while male recipients transplanted with female AML donor cells showed lowest repopulation rates compared to all other groups (BM: 16.0 ± 18.9 %) at all analyzed time-points and in multiple tissues (Figure 1A). Limiting dilution assays excluded a possible cell dose mediated artefact (Figure 1B). Similar results were observed in secondary transplantation assays, where leukemic cells derived from female mice successfully expanded in female recipients, but almost entirely failed to engraft in male secondary recipients (Figure 1C). Interestingly, NGS analysis of leukemic cells retrieved from mice transplanted from the same AML sample showed similar clonal compositions between female and male recipients (Figure 1D), indicating that the female environment promotes engraftment across all investigated genetic backgrounds.

Intriguingly, despite harboring lower numbers of leukemic cells, male mice showed more symptoms of disease than female recipient mice, as quantified by our “sickness score” (see Methods and Figure 1E).

Next, we asked why female mice display higher numbers of human leukemic cells when compared to experimentally matched male recipients. A potential explanation would be enhanced cell homing to the BM environment after transplantation. To test this, we injected $1x10^6$ human leukemic cells from the same donor (male and female) into age-matched male and female mice by tail vein injection and assessed the percentages of human leukemic among murine BM cells 16 hours later (Supplementary Table 2). In contrast to the clear differences observed in long-term repopulation assays, male and female recipients showed similar homing rates (Figure 1F), suggesting that the observed long-term effects are independent of this early phase of leukemic engraftment.

Another possibility would be that recipient sex biases leukemic (stem) cell expansion. In previous studies, we showed that AML cells contain LSC enriched subpopulations identified by absence of NKG2D ligand (NKG2DL) expression (12). However, female and male mice engrafted with AML cells of the same donors showed comparable percentages of NKG2DL negative AML cells, indicating that recipient sex does not specifically affect LSCs (Figure 1G).

We next sought to investigate mechanistic influences of sex hormones mediating AML repopulation in PDX, and compared AML engraftment in ovariectomized versus control female recipients, and respectively in orchiectomized (castrated) versus control male mice. Figure 2A summarizes the results obtained by transplanting n=3 different AML samples (2 male and 1 female donor) side-by-side in these recipients. Strikingly, castrated male recipients engrafted better compared to the control male recipients while the ovariectomy did not alter engraftment efficiency in female mice (Figure 2B and Supplementary Figure 1).

**Molecular AML cell characteristics impact leukemogenesis in PDX models**

Favorable risk AML cases were reported to engraft poorly in PDX models. Based on our results, we hypothesized that transplantation into female recipients may improve engraftment across all AML, including favorable risk subtypes, thus allowing a more comprehensive *in vivo* analysis of heterogeneous AML subtypes.

We transplanted 61 AML cases stratified into favorable (n=23), intermediate (n=30) or adverse (n=8) European LeukemiaNet (ELN) risk groups (13) (Supplemental table 1) into n=175 NSG female mice and followed them for up to one-year post-transplantation (11). We screened mice for leukemic repopulation by routinely performing PB analyses and BM punctures. Detectable repopulation increased over time and was documented with 36 out of the total 61 (59.02%) transplanted AML cases (with 14/23 favorable, 18/30 intermediate, and 4/8 adverse risk AML, respectively) (Table 1). Of note, animals negative in early routine BM punctures later turned positive, showing robust leukemic repopulation. This demonstrates that leukemia is initiated by rare cell populations that can remain undetectable for extended periods of time in this model – reminiscent of outcomes in patients, where relapses are often detected after several months of apparent post-therapy remission.

Interestingly, mice injected with favorable-risk AML cells, despite receiving no treatment, showed a particularly late onset of leukemia (48 weeks, versus 39 or 18 weeks for intermediate or adverse risk AML cells, respectively ($p=0.0031$, and respectively $p=0.0001$) when all mice were considered and respectively 38 weeks, versus 28 for intermediate or 18 weeks for adverse risk AML cells when only engrafted mice and samples were considered (Figure 3A). Recently, Mian et al. showed that donor sex influences the engraftment of leukemic cells (14). Subdivision of the data set according to donor sex revealed decreased survival with transplanted female versus male donor cells in favorable risk AML cells (Supplementary Figure 2A), while no differences were observed with intermediate or adverse subtypes. Favorable risk AML cells also showed reduced homing rates (Supplementary Table 2) compared to intermediate ($p=0.0131$) or adverse risk samples

(p=0.0970) (Figure 3B) with more pronounced effects when female donor cells were transplanted (Supplementary Figure 2B).

FLT3 mutational status predicted time-to-leukemia and murine survival highlighting the oncogenic nature of this mutation either alone or in the setting of NPM1 mutations (Figure 3C). Interestingly, double mutated FLT3-ITD/NPM1 mutant AML showed poor survival (Figure 3C) and enhanced homing by trend (Figure 3D), when compared to FLT3 wildtype, or FLT3-ITD mutated/NPM1 wildtype cases, suggesting that the female environment has a particularly negative impact in the context of this AML subtype (Figure 3D-E). No further homing differences could be documented (Figure 3F). Of note, while NPM1 and FLT3 mutational status was assessed in all cases, next generation sequencing exploring further leukemia specific mutations was not routinely performed. The impact of further genetic aberrations on the observed results thus cannot be further explored. Next, we asked whether mouse leukemia kinetics can predict clinical outcome in patients. AML cells collected at first diagnosis from patients in whom treatment subsequently induced complete remission (CR), showed delayed leukemogenesis in xenograft models, when compared to AML cells derived from patients showing residual leukemia (RD) after treatment (Figure 4A). Lastly, we found that the FAB AML category was predictive for leukemia kinetics (Figure 4B). Together, these data indicate that our PDX model in female recipient mice reliably mimics leukemogenesis and reflects differences in AML classifications established in clinical patient cohorts (FLT3-ITD mutations, ELN, FAB). It further intriguingly suggests sex disparities generally affect AML but might have more relevant effects in certain genetic backgrounds.

In contrast, patient´s age (younger versus older than 70 years) did not significantly influence results in PDX models (Supplemental figure 2C), although as expected, patients over 70 years more frequently displayed AML with adverse molecular subtypes, when compared to patients under 70 years old (Supplemental figure 2D).

**High expression of LSC markers in adverse molecular risk AML**

The data showing lower homing capacity with favorable when compared to intermediate or adverse risk AML (Figure 3B), and *vice-versa*, enhanced homing with FLT3-ITD/NPM1 double mutated samples (Figure 3D), suggest that lower LSC content may be a determinant of better outcome in AML. We thus sought to investigate our primary and engrafted AML cells for phenotypic heterogeneity to specifically assess LSC content.

AML is known for its heterogeneity regarding cell surface marker expression (3, 15). In addition, AML cells were reported to phenotypically change during *in vitro* and *in vivo* experiments (16-18), which complicates the interpretation of experimental results. Thus, we first investigated whether propagation of patient-derived AML cells in xenograft models can maintain their phenotypic properties.

Using flow cytometry, we assessed the expression of different stem/progenitor cell surface markers on AML cells collected from female recipient xenograft animals and compared it to the originally transplanted AML cells. We observed that CD38, CD123, CD47, cKIT, and GPR56 expression did not significantly change in response to the *in vivo* transplantation experiment, while there was a decrease in CD34, and a concomitant increase in NKG2DL expression (Supplemental Figure 3A). These data highlight that PDX experiments using female mice as transplant recipients in general maintain the original phenotype of the patient's AML cells, but in some cases may increase differentiation.

Due to female recipient mice having an environment that has little influence on AML cell phenotype, we next assessed phenotypic differences in molecular subtypes of AML that have been previously understudied. We hypothesized that LSC subpopulations might be increased in adverse molecular risk AML, accounting for their higher aggressiveness in patients. Indeed, adverse risk AML samples had higher expression of the LSC marker CD34 compared to favorable or intermediate risk AML cells (Figure 5A, left panel), which was then maintained in the mouse xenografts (Figure 5A, right panel). *Vice-versa*, NKG2DL were by trend more highly expressed on favorable versus intermediate or adverse risk AML, and this

again was reflected in the phenotype of cells collected from PDX mice (Figure 5B). Furthermore, cKIT expression was higher on adverse versus favorable risk AML (Figure 6A), while GPR56, CD123, or CD47 showed similar distribution among molecular AML subtypes (Figure 6B-E). Interestingly, NKG2DL-cKIT+ subpopulations were significantly expanded in mice transplanted with intermediate and adverse risk AML compared to favorable risk AML samples (Figure 6E).

**LSC frequency among AML subtypes predicts leukemogenesis**

We next explored whether the expression of the LSC markers CD34 and cKIT, and absence for NKG2DL, correlates with leukemogenesis in our female recipient PDX model. Indeed, in line with the observation that adverse risk AML exhibits decreased expression of NKG2DL, mice transplanted with AML cells characterized by low (bottom 25%) NKG2DL expression showed increased leukemogenesis (Figure 7A). Surprisingly, while there was a significant increase of CD34+ cells in adverse versus favorable risk AML, we did not find any segregation between mice transplanted with AML containing different CD34 expression levels (Figure 7B). Lastly, we found that there was a highly significant relationship between leukemogenesis and cKIT expression, with cKIT expressing samples leading to higher leukemia burden (Figure 7C), which also correlated with increased BM homing capacity (Figure 7D). Overall, these data indicate that high LSC content promotes leukemia aggressiveness in PDX models, and supports the notion that the environment generated by transplanting AML samples into female mice allows an accurate representation of AML cell function in patients.

**Discussion**

Sex disparities are increasingly recognized as important in cancer development and therapy response (19-23). The incidence of AML and myeloid neoplasia is higher in male versus

female. In this report, we show that female recipient sex promotes leukemia induction in PDX models, enabling reconstitution across heterogeneous AML subtypes of all risk groups. Supporting the notion that the female environment promotes AML aggressiveness and/or impairs therapy response, FLT3-ITD mutated AML showed worse clinical outcome in female compared to male patients (19).

The limited cross-species reactivity hampers the growth of human leukemic cells in the mouse microenvironment, and particularly influences selected AML subtypes. “Improved” NSG strains with transgenic expression of hIL-3, hGM-CSF and hSCF, also called NGS-S were shown to improve engraftment of inv(16) favorable risk AML (10, 24-26). However, our data indicate that using female recipients and extending observation times can already enable robust repopulation with all ELN risk groups, including favorable risk AML. As expected, adverse risk AML show faster mouse repopulation and shorter survival (9, 11, 24). Of note, the revised ELN classification groups FLT3-mutated AML to intermediate disease, regardless of allelic ratio and NPM1 mutational status (13).

Phenotypic markers were largely conserved during the *in vivo* leukemic cell propagation in female PDX models. Higher LSC frequency was observed in adverse versus favorable and intermediate risk AML and correlated with enhanced long-term repopulation as well homing capacity. Consistently, LSCs from adverse risk AML showed enhanced repopulation capacity in serial re-transplantation assays (24). Overall, these data suggest that adverse risk AML contain LSC less accessible to differentiation induction which may contribute to their ability to repopulate NSG mice as well as leukemia aggressiveness in patients. However, the higher leukemic expansion in female PDX was not restricted to LSCs (27).

Our data indicate an underappreciated role of the female environment in PDX leukemogenesis and raise intriguing questions on the effects of sex disparities in patients with leukemia. A potential explanation for the sex-dependent differences could be the differences in estrogen levels. However, modulators of estrogen and its receptor were, in

contrast, described to rather induce pro-apoptotic effects in *in vitro* assays and in *in vivo* syngeneic leukemia mouse models (28, 29). In AML, as also in some other forms of hematological and solid cancers, lower incidence and better outcomes have consistently been reported for females (22, 23, 30). This is in general considered to result from a more adverse genetic landscape in male AML cases (20), and potentially associate with exposure to pro-tumorigenic environmental factors such as smoking or chemicals. Interestingly, the excess of myeloid malignancies and especially myelodysplastic neoplasms in males versus females is reversed between the onset of puberty and approximately the age of 50 years, and afterwards again shows an increase (30). Our results with castrated and respectively ovariectomized animals suggest that rather androgen deprivation than estrogen addition in fact enhances leukemogenesis in female mice. This notion is supported by a phase III trial including 330 elderly AML patients which showed improved disease-free and overall survival after the addition of androgens to post-remission maintenance therapy (31). However, since female mice show better engraftment than castrated males, estrogens or other factors in the female environment might still play a role. For example, the ratio of estrogens to androgens may be a critical force driving the sex disparities seen in AML outcomes as well as leukemic blast expansion *in vitro* and *in vivo*. As recently shown by Mian et al. (14), donor sex was also found to influence engraftment, but however in our samples to a lower extent. Future analyses will show whether leukemic cells with the same genetic background may more readily expand in female versus male patients, e.g. showing shorter time to relapse in first.

Finally, inflammatory signaling is involved in myeloid leukemogenesis and might contribute to the increased incidence of myelodysplastic neoplasms in younger women. It is recognized that female individuals have an overall higher propensity for chronic low-grade inflammation (32), but also for stronger immune responses. Hence, the female immune system might facilitate a higher rate of (pre-)leukemic clone expansion. At the same time, more efficient immune eradication of highly malignant myeloid disease clones might occur resulting in an overall lower rate of adverse risk genetics in female AML cases. Since immune responses

are blunted in PDX models, the female microenvironment factors triggering expansion might become more evident.

Intriguingly, while female PDX mice showed a higher leukemic burden, they at the same time displayed lower sickness scores when compared to male mice engrafted with cells from the same patients. Higher androgen levels, which also inhibit leukemogenesis may be responsible for the higher sickness score, as suggested by our comparison of leukemogenesis in castrated versus control male mice. Future studies are required to further analyze sex-dependent differences mediating disparities not only in leukemic cell growth but also in sickness score in females versus males, and how these findings may apply to patients with leukemia.

Together, these results reinforce that PDX models faithfully model AML but at the same time reveal recipient sex as a new essential variable that requires careful evaluation to avoid important experimental biases. Future studies will show whether they can also be used to investigate *in vivo* other yet challenging to engraft hematologic or solid cancers. Our findings highlight the impact of sex differences on AML biology and treatment outcome as an intriguing area for future research.

**References:**


1. Trumpp A, Haas S. Cancer stem cells: The adventurous journey from hematopoietic to leukemic stem cells. Cell. 2022;185(8):1266-1270.

2. Arber DA, Orazi A, Hasserjian RP, et al. International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data. Blood. 2022;140(11):1200-1228.

3. Arnone M, Konantz M, Hanns P, et al. Acute Myeloid Leukemia Stem Cells: The Challenges of Phenotypic Heterogeneity. Cancers (Basel). 2020;12(12):3742.

4. Velten L, Story BA, Hernández-Malmierca P, et al. Identification of leukemic and pre-leukemic stem cells by clonal tracking from single-cell transcriptomics. Nat Commun. 2021;12(1):1366.

5. Schuringa JJ, Schepers H. Ex vivo assays to study self-renewal and long-term expansion of genetically modified primary human acute myeloid leukemia stem cells. Methods Mol Biol. 2009;538:287-300.

6. Griessinger E, Anjos-Afonso F, Vargaftig J, et al. Frequency and Dynamics of Leukemia-Initiating Cells during Short-term Ex Vivo Culture Informs Outcomes in Acute Myeloid Leukemia Patients. Cancer Res. 2016;76(8):2082-2086.

7. Griessinger E, Vargaftig J, Horswell S, Taussig DC, Gribben J, Bonnet D. Acute myeloid leukemia xenograft success prediction: Saving time. Exp Hematol. 2018;59:66-71.e4.

8. Monaco G, Konopleva M, Munsell M, et al. Engraftment of acute myeloid leukemia in NOD/SCID mice is independent of CXCR4 and predicts poor patient survival. Stem Cells. 2004;22(2):188-201.

9. Pearce DJ, Taussig D, Zibara K, et a. AML engraftment in the NOD/SCID assay reflects the outcome of AML: implications for our understanding of the heterogeneity of AML. Blood. 2006;107(3):1166-1173.

10. 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.

11. Paczulla AM, Dirnhofer S, Konantz M, et al. Long-term observation reveals high-frequency engraftment of human acute myeloid leukemia in immunodeficient mice. Haematologica. 2017;102(5):854-864.

12. Paczulla AM, Rothfelder K, Raffel S, et al. Absence of NKG2D ligands defines leukaemia stem cells and mediates their immune evasion. Nature. 2019;572(7768):254-259.

13. Döhner H, Wei AH, Appelbaum FR, et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood. 2022;140(12):1345-1377.

14. Mian SA, Ariza-McNaughton L, Anjos-Afonso F, et al. Influence of donor-recipient sex on engraftment of normal and leukemia stem cells in xenotransplantation. Hemasphere. 2024;8(5):e80.

15. Haubner S, Perna F, Köhnke T, et al. Coexpression profile of leukemic stem cell markers for combinatorial targeted therapy in AML. Leukemia. 2019;33(1):64-74.

16. Ho TC, LaMere M, Stevens BM, et al. Evolution of acute myelogenous leukemia stem cell properties after treatment and progression. Blood. 2016;128(13):1671-1678.

17. Kageyama Y, Miwa H, Arakawa R, et al. Expression of CD25 fluctuates in the leukemia-initiating cell population of CD25-positive AML. PLoS One. 2018;13(12):e0209295.

18. Shingai Y, Yokota T, Okuzaki D, et al. Autonomous TGFβ signaling induces phenotypic variation in human acute myeloid leukemia. Stem Cells. 2021;39(6):723-736.

19. Hellesøy M, Engen C, Grob T, Löwenberg B, Valk PJM, Gjertsen BT. Sex disparity in acute myeloid leukaemia with FLT3 internal tandem duplication mutations: implications for prognosis. Mol Oncol. 2021;15(9):2285-2299.

20. De-Morgan A, Meggendorfer M, Haferlach C, Shlush L. Male predominance in AML is associated with specific preleukemic mutations. Leukemia. 2021;35(3):867-870.

21. Haupt S, Caramia F, Klein SL, Rubin JB, Haupt Y. Sex disparities matter in cancer development and therapy. Nat Rev Cancer. 2021;21(6):393-407.

22. Stabellini N, Tomlinson B, Cullen J, et al. Sex differences in adults with acute myeloid leukemia and the impact of sex on overall survival. Cancer Med. 2023;12(6):6711-6721.

23. Wiernik PH, Sun Z, Cripe LD, et al. Prognostic effect of gender on outcome of treatment for adults with acute myeloid leukaemia. Br J Haematol. 2021;194(2):309-318.

24. Díaz de la Guardia R, Velasco-Hernandez T, Gutiérrez-Agüera F, et al. Engraftment characterization of risk-stratified AML in NSGS mice. Blood Adv. 2021;5(23):4842-4854.

25. Krevvata M, Shan X, Zhou C, et al. Cytokines increase engraftment of human acute myeloid leukemia cells in immunocompromised mice but not engraftment of human myelodysplastic syndrome cells. Haematologica. 2018;103(6):959-971.

26. Wunderlich M, Chou FS, 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):1785-1758.

27. Notta F, Doulatov S, Dick JE. Engraftment of human hematopoietic stem cells is more efficient in female NOD/SCID/IL-2Rgc-null recipients. Blood. 2010;115(18):3704-3707.

28. Roma A, Spagnuolo PA. Estrogen Receptors Alpha and Beta in Acute Myeloid Leukemia. Cancers (Basel). 2020;12(4):907.

29. Sánchez-Aguilera A, Arranz L, Martín-Pérez D, et al. Estrogen signaling selectively induces apoptosis of hematopoietic progenitors and myeloid neoplasms without harming steady-state hematopoiesis. Cell Stem Cell. 2014;15(6):791-804.

30. Cartwright RA, Gurney KA, Moorman AV. Sex ratios and the risks of haematological malignancies. Br J Haematol. 2002;118(4):1071-1077.

31. Pigneux A, Béné MC, Guardiola P, et al. Addition of Androgens Improves Survival in Elderly Patients With Acute Myeloid Leukemia: A GOELAMS Study. J Clin Oncol. 2017;35(4):387-393.

32. Klein SL, Flanagan KL. Sex differences in immune responses. Nat Rev Immunol. 2016;16(10):626-638.

| Time points | # of engrafted samples per risk group | Total engrafted Samples (%) | # of engrafted animals per risk group | Total engrafted NSG mice (%) |
|---|---|---|---|---|
| 16 weeks | 4/23 favorable (17,39%)<br>3/30 intermediate (10,00%)<br>1/8 adverse (12,50%) | 13,11 | 8/175 favorable (2,86%)<br>6/175 intermediate (3,43%)<br>2/175 adverse (1,14%) | 9,14 |
| 26 weeks | 5/23 favorable (21,74%)<br>8/30 intermediate (26,67%)<br>2/8 adverse (25,00%) | 24,59 | 9/175 favorable (5,14%)<br>14/175 intermediate (8,00%)<br>15/175 adverse (8,57%) | 21,71 |
| 39 weeks | 11/23 favorable (47,83%)<br>12/30 intermediate (40,00%)<br>3/8 adverse (37,50%) | 42,62 | 16/175 favorable (9,14%)<br>26/175 intermediate (14,86%)<br>16/175 adverse (9,14%) | 33,14 |
| 52 weeks | 14/23 favorable (60,87%)<br>18/30 intermediate (60,00%)<br>4/8 adverse (50,00%) | 59,02 | 17/175 favorable (9,71 %)<br>37/175 intermediate (21,14%)<br>18/175 adverse (10,29%) | 41,14 |

**Table 1. Engraftment kinetic in NSG animals**

**Figure 1. Female NSG mice promote leukemic outgrowth in PDX models**

(A) Engraftment assessment using flow cytometry in bone marrow (BM; left panel) and peripheral blood (PB) and organs (right panels) at endpoint analysis. Cells isolated from the indicated tissues from both male and female NSG mice were at the same time-point screened for CD33 positive acute myeloid leukemia (AML) cells using flow cytometry (n=9 primary AML samples, n=14 female mice with female donor cells, n=28 female mice with male donor cells, n=21 male mice with female donor cells and n=19 male mice with male donor cells. (B) Limiting dilution assay. Mice were transplanted with $5x10^6$, $1x10^6$ and $0.1x10^6$ cells per mouse and at the same time-point screened for CD33 positive AML cells using flow cytometry (n=1 AML sample with n=3-4 mice per group). (C) Secondary transplantation assay; AML cells expanded in female NSG recipients were re-transplanted in female and male secondary recipients. Engraftment of CD33-positive AML cells was assessed by flow cytometry in the bone marrow, peripheral blood and spleen from male and female NSG mice at endpoint analysis (n=2 primary AML samples, 6 females and 6 males NSG recipients). (D) Next generation sequencing analysis of bulk cells retrieved from female and male recipients (n=1 AML sample). Mice (n=3 male and n=4 female) were analyzed separately. (E) Sickness score of female and male recipients transplanted with the same AML samples (n=2 AML with n=10 mice per group and sample). (F) Homing assay analyzing CFSE-positive cells in the murine BM as assessed by flow cytometry 16 hours post-transplantation (n=5 primary AML samples, 19 females and 19 males NSG animals). (G) Flow cytometric quantification of NKG2DL-negative cells in murine BM (n=3 primary AML samples, 11 females and 5 males NSG animals). D´Agostino & Pearson test was used to test for normality. For statistical significance: Student´s t test: (A) for BM, PB and spleen, (B) and (F); Mann-Whitney t-test: liver from (A); (E), and (G).

**Figure 2: Different sex hormones influence leukemic bone marrow engraftment in PDX models.** (A) Schematic overview. (B) Engraftment assessment using flow cytometry in bone marrow (BM) at endpoint analysis. Cells isolated from all groups, ovariectomized female and

orchiectomized (castrated) male next to control female and respectively control male NSG mice were collected at the same time-points and screened for CD33 positive AML cells using flow cytometry (n=3 primary AML samples, 11 orchiectomized (castrated) male, 12 ovariectomized female, 10 regular male and 12 regular female NSG recipients). D´Agostino & Pearson test was used to test for normality, Student´s t test was performed for statistical significance.

**Figure 3. Influence of leukemic characteristics in patients on leukemic cell repopulation in NSG mice.**

(A) Murine survival based on the European Leukemia Net (ELN) 2017 classification (23 favorable samples, 48 NSG mice. 30 intermediate samples, 89 NSG mice. 8 adverse samples, 38 NSG mice); (B) Homing assay analyzing CFSE-positive cells in the murine bone marrow as assessed by flow cytometry 16 hours post-transplantation (34 favorable samples, 114 NSG mice. 34 intermediate samples, 104 NSG mice. 15 adverse samples, 46 NSG mice). (C) Murine survival based on the FLT3/NPM1 mutational status (16 WT samples 34 NSG mice. 11 NPM1 single mutant samples, 43 NSG mice. 17 FLT3 single mutant, 53 NSG mice. 11 double mutant samples, 32 NSG mice); (D) Homing assay analyzing CFSE-positive cells in the murine bone marrow (BM) as assessed by flow cytometry 16 hours post-transplantation (18 single FLT3-ITD samples, 58 NSG mice. 14 double mutant samples, 46 NSG mice). (E) Murine survival based on the FLT3 mutational status and ITD/WT ratio (27 FLT3-WT samples, 77 NSG mice. 17 FLT3-ITD low samples, 48 NSG mice. 10 FLT3-ITD high samples, 36 NSG mice); (F) Homing assay analyzing CFSE-positive cells in the murine BM as assessed by flow cytometry 16 hours post-transplantation (38 WT samples, 119 NSG mice. 32 FLT3-mutated samples, 104 NSG mice. 17 FLT3-ITD low samples, 54 NSG mice. 12 FLT3-ITD high, 41 NSG mice). Statistical analysis: Log-rank test for survial analysis, Mann-Whitney-U test for Homing.

**Figure 4: Influence of therapy response and FAB classification in patients on leukemic cell repopulation in NSG mice.** Murine survival based on (A) the remission status (24 complete remission (CR) samples, 59 NSG mice. 12 resistant disease (RD) samples, 57 NSG mice) and (B) the French-American-British (FAB) classification (4 M0 samples, 11 NSG mice. 12 M1 samples, 38 NSG mice. 14 M2 samples, 39 NSG mice. 11 M4 samples, 24 NSG mice. 13 M5 samples, 33 NSG mice). Statistical analysis: Log-rank test was performed.

**Figure 5. Correlation of CD34 and NKG2DL expression with acute AML risk group.**

Samples were distributed according to their molecular characteristics into favorable, intermediate and adverse risk group samples. (A) Distribution of CD34+ cells (left) directly from patient biopsy, (right) patient-derived xenograft (PDX) cells. (B) Distribution of NKG2DL+ cells (left) directly from patient biopsy, (right) PDX cells. Acute myeloid leukemia (AML) cells were identified using CD33 positivity. n=50 primary samples (left panel A and B) n=24 primary samples in 51 NSG mice (right panel A and B) were analyzed. Statistical analysis: Kruskal-Wallis ANOVA test.

**Figure 6. Association of leukemia stem cell frequency and AML risk group**

(A-E) Distribution of marker expression between favorable, intermediate and adverse risk group samples. (A) Distribution of cKIT+ cells from PDX cells. (B) Distribution of GPR56+ cells from patient-derived xenograft (PDX) cells. (C) Distribution of CD123+ cells from PDX cells. (D) Distribution of CD47+ cells from PDX cells. (E) Distribution of NKG2DL-/cKIT+ cells from PDX cells. n=24 primary samples in 51 NSG mice were analyzed in all datasets shown. Statistical analysis: Kruskal-Wallis ANOVA test.

**Figure 7. Donor-dependent parameters influence leukemia kinetics in NSG mice**

Murine survival based on (A) the NKG2DL expression status (25 bottom 25%, 64 NSG mice. 10 top 25%, 23 NSG mice); (B) the CD34 expressing status (6 CD34-expressing samples 5-25%, 16 NSG mice. 11 CD34-expressing samples 75-100%, 35 NSG mice. 19 CD34-non-expressing samples, 47 NSG mice); (C) the cKIT expressing status (35 cKIT-expressing samples, 93 NSG mice. 7 cKIT-non-expressing samples, 19 NSG mice). (D) Homing assay analyzing CFSE-positive cells in the murine BM as assessed by flow cytometry 16 hours post-transplantation (46 cKIT-expressing samples, 144 NSG mice, 12 cKIT-non-expressing samples, 37 NSG mice). Statistical analysis: Log-Rank test for (A-C) and Mann-Whitney U-test: for (D).

Figure 1

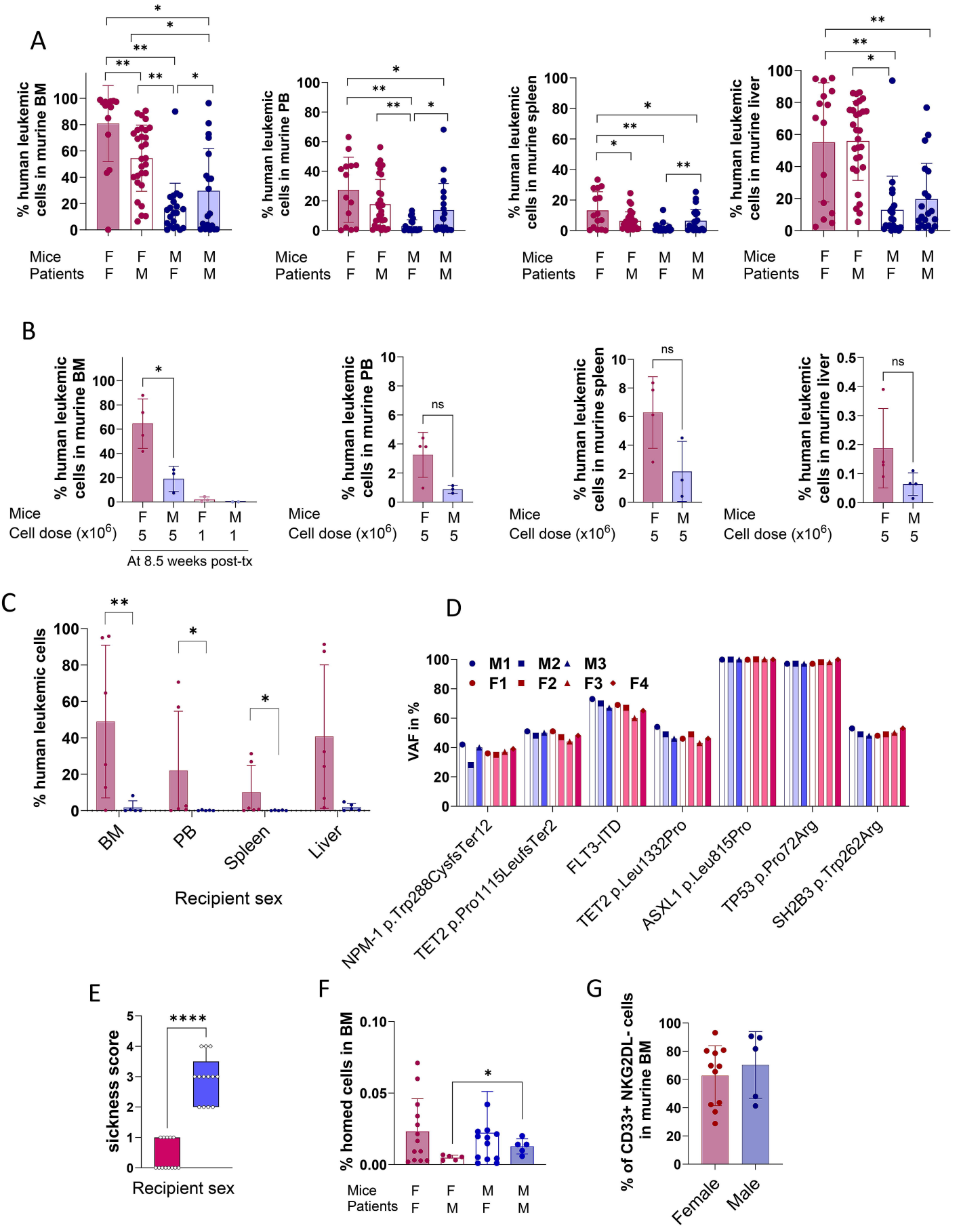
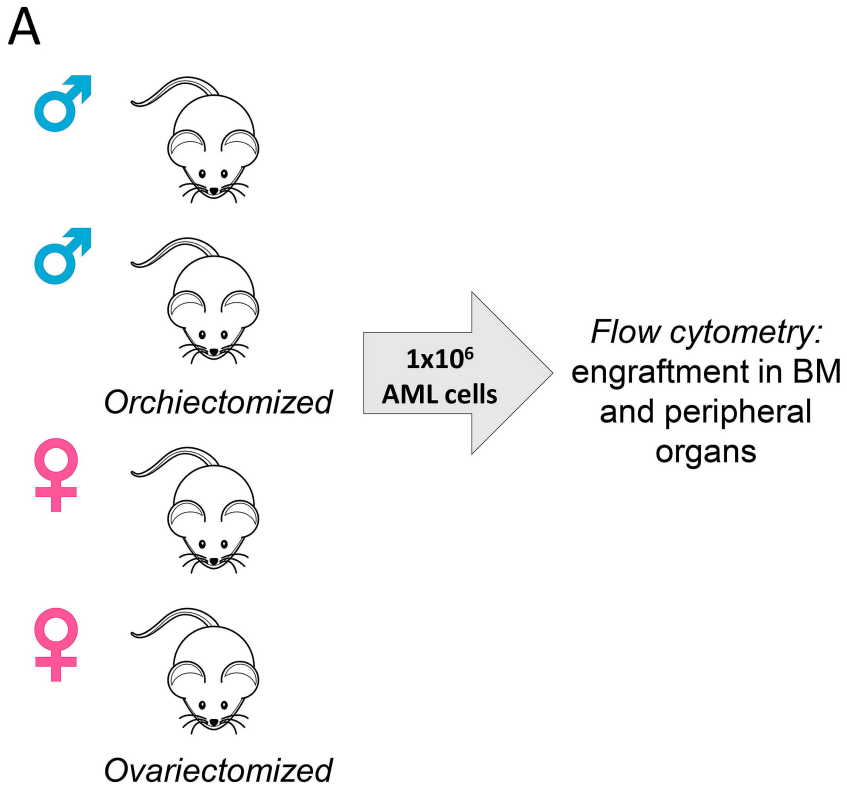

A
% human leukemic cells in murine BM
% human leukemic cells in murine PB
% human leukemic cells in murine spleen
% human leukemic cells in murine liver
Mice F F M M
Patients F M F M
B
% human leukemic cells in murine BM
% human leukemic cells in murine PB
% human leukemic cells in murine spleen
% human leukemic cells in murine liver
Mice F M F M
Cell dose (x10^6) 5 5 1 1
At 8.5 weeks post-tx
Mice F M
Cell dose (x10^6) 5 5
ns
C
% human leukemic cells
BM
PB
Spleen
Liver
Recipient sex
D
VAF in %
M1 M2 M3
F1 F2 F3 F4
NPM-1 p.Trp288CysfsTer12
TET2 p.Pro1115LeufsTer2
FLT3-ITD
TET2 p.Leu1332Pro
ASXL1 p.Leu815Pro
TP53 p.Pro72Arg
SH2B3 p.Trp262Arg
E
sickness score
Recipient sex
F
% homed cells in BM
Mice F F M M
Patients F M F M
G
% of CD33+ NKG2DL- cells in murine BM
Female
Male

Figure 2


A
Orchiectomized
1x10⁶
AML cells
Flow cytometry:
engraftment in BM
and peripheral
organs
Ovariectomized

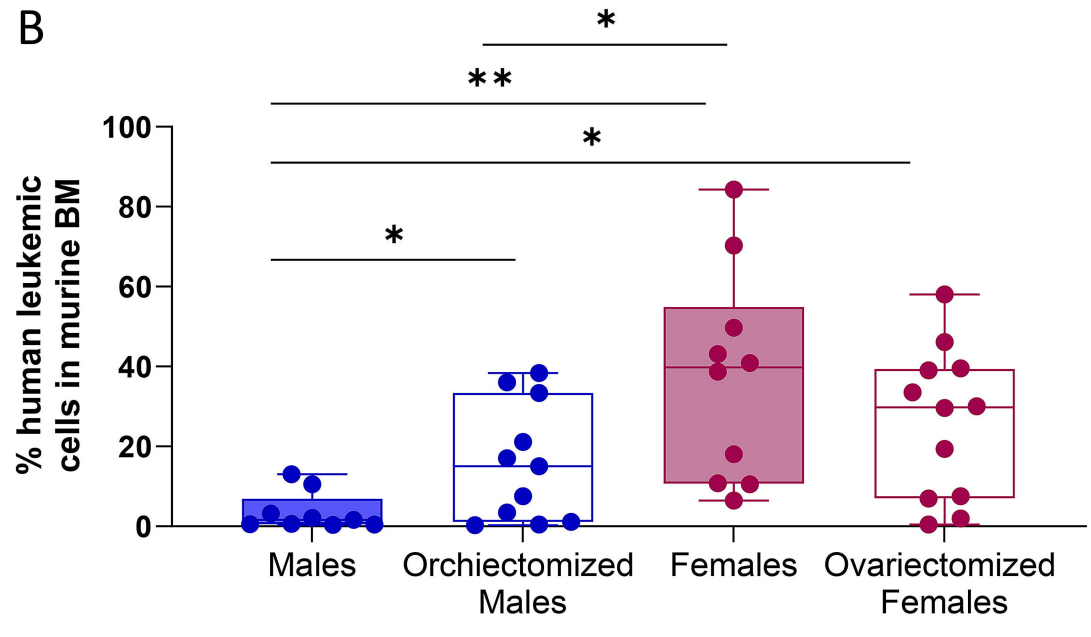

B
% human leukemic
cells in murine BM
100
80
60
40
20
0
*
**
*
*
Males
Orchiectomized
Males
Females
Ovariectomized
Females

Figure 3

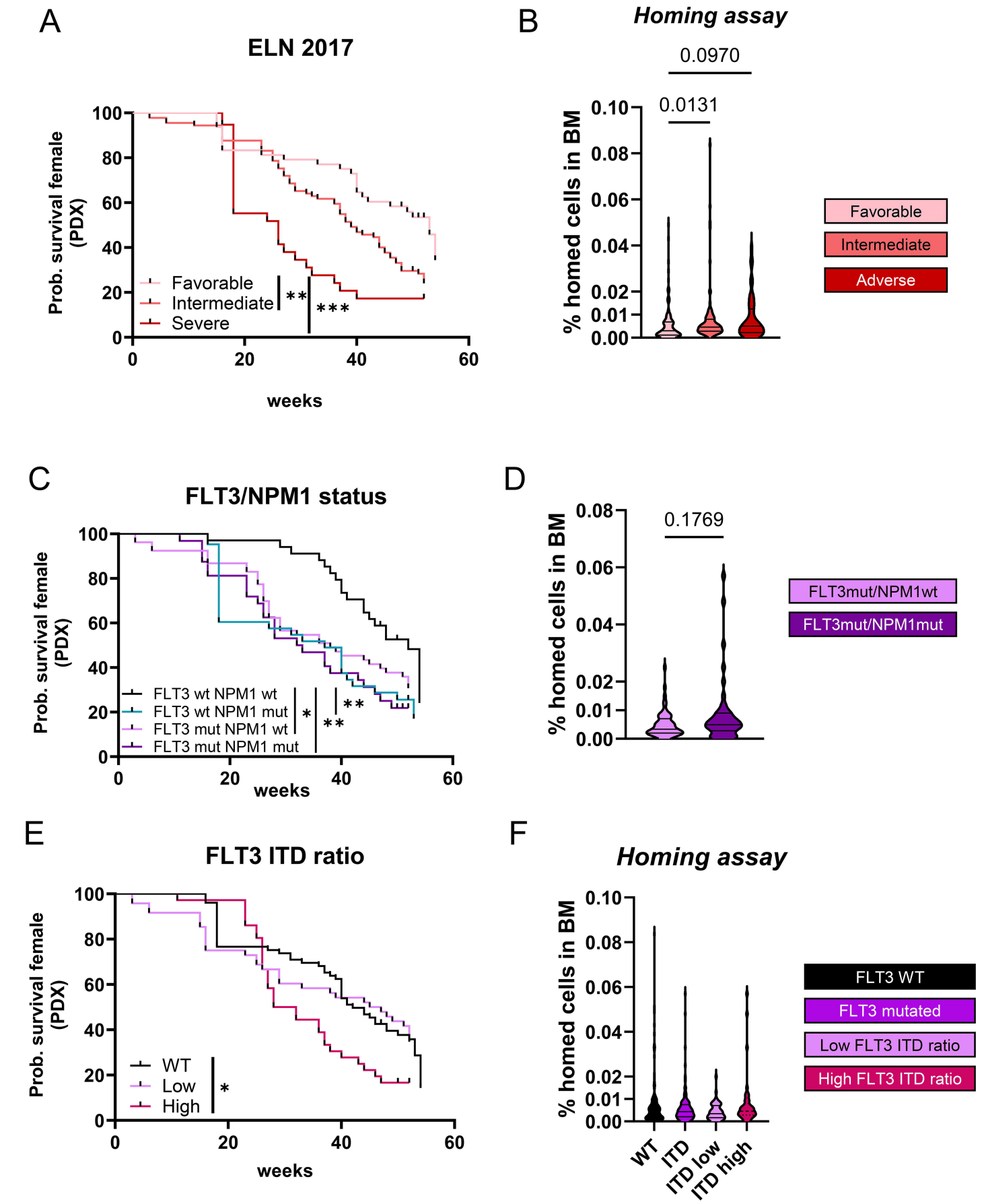

A
ELN 2017
Prob. survival female (PDX)
100
80
60
40
20
0
0
20
40
60
weeks
Favorable
Intermediate
Severe
**
***
B
Homing assay
0.0970
0.0131
% homed cells in BM
0.10
0.08
0.06
0.04
0.02
0.01
0.00
Favorable
Intermediate
Adverse
C
FLT3/NPM1 status
Prob. survival female (PDX)
100
80
60
40
20
0
0
20
40
60
weeks
FLT3 wt NPM1 wt
FLT3 wt NPM1 mut
FLT3 mut NPM1 wt
FLT3 mut NPM1 mut
*
**
**
D
0.1769
% homed cells in BM
0.08
0.06
0.04
0.02
0.01
0.00
FLT3mut/NPM1wt
FLT3mut/NPM1mut
E
FLT3 ITD ratio
Prob. survival female (PDX)
100
80
60
40
20
0
0
20
40
60
weeks
WT
Low
High
*
F
Homing assay
% homed cells in BM
0.10
0.08
0.06
0.04
0.02
0.01
0.00
WT
ITD
ITD low
ITD high
FLT3 WT
FLT3 mutated
Low FLT3 ITD ratio
High FLT3 ITD ratio

Figure 4

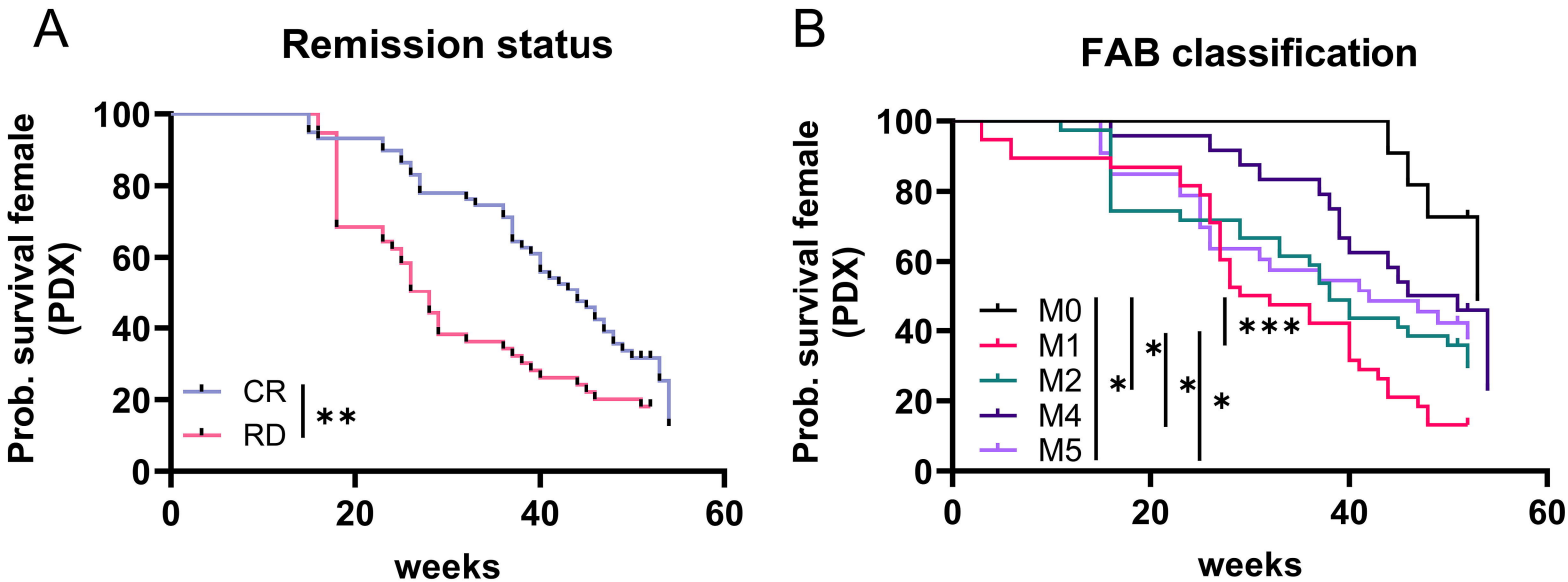

A
Remission status
Prob. survival female (PDX)
100
80
60
40
20
0
CR
RD
**
0
20
40
60
weeks
B
FAB classification
Prob. survival female (PDX)
100
80
60
40
20
0
M0
M1
M2
M4
M5
*
*
*
*
***
0
20
40
60
weeks

Figure 5

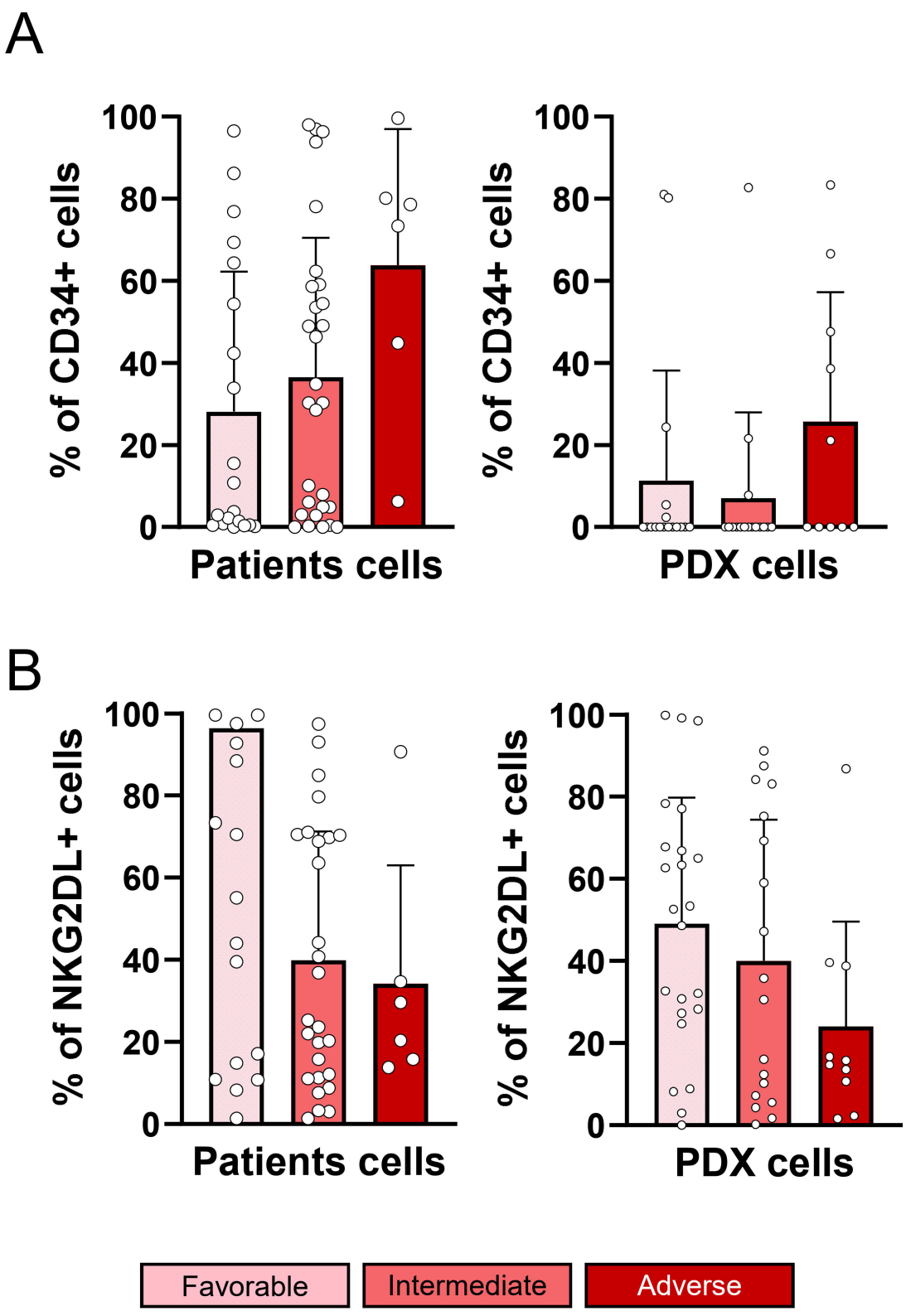

A
% of CD34+ cells
100
80
60
40
20
0
Patients cells
% of CD34+ cells
100
80
60
40
20
0
PDX cells
B
% of NKG2DL+ cells
100
80
60
40
20
0
Patients cells
% of NKG2DL+ cells
100
80
60
40
20
0
PDX cells
Favorable
Intermediate
Adverse

Figure 6

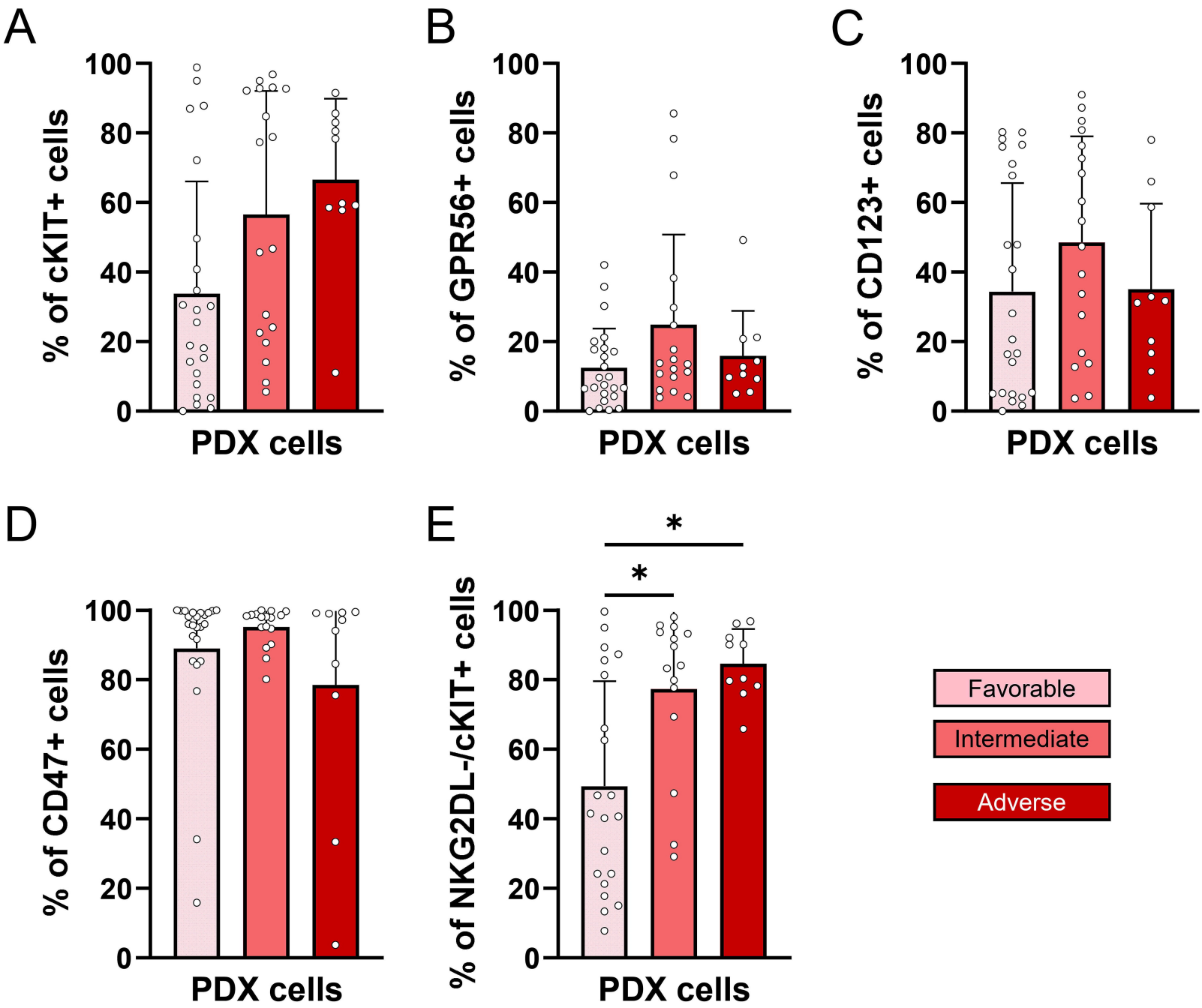
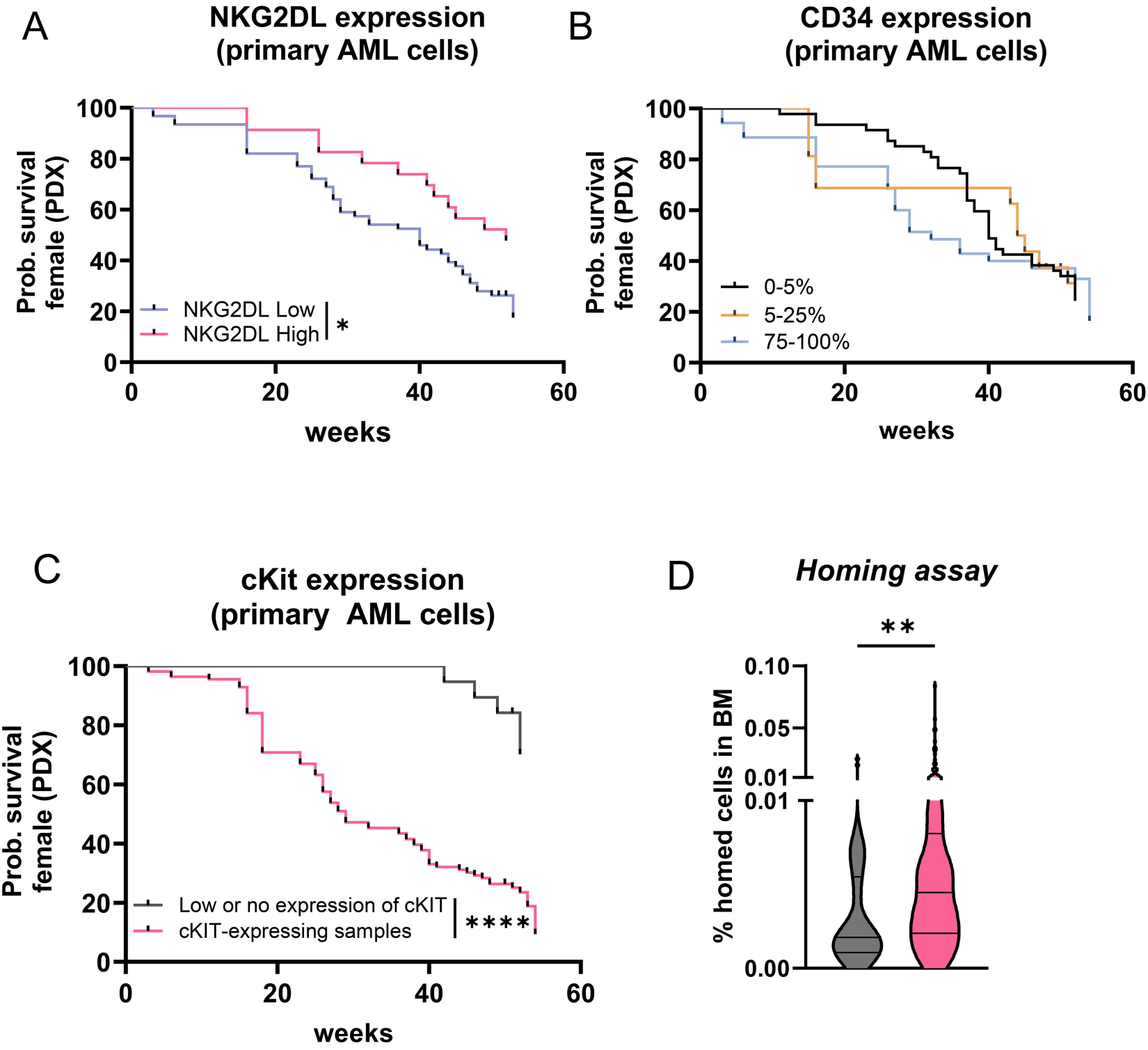
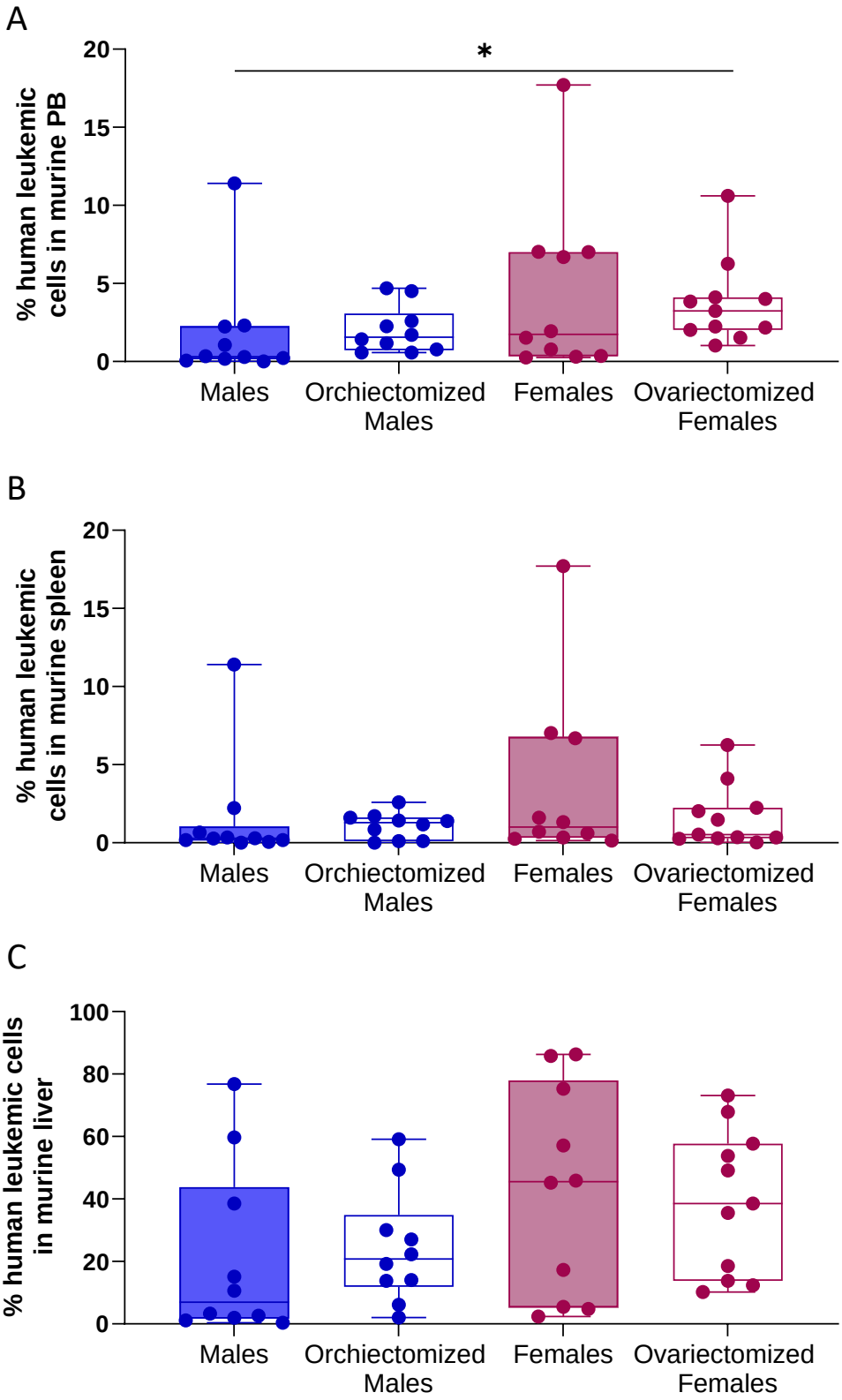
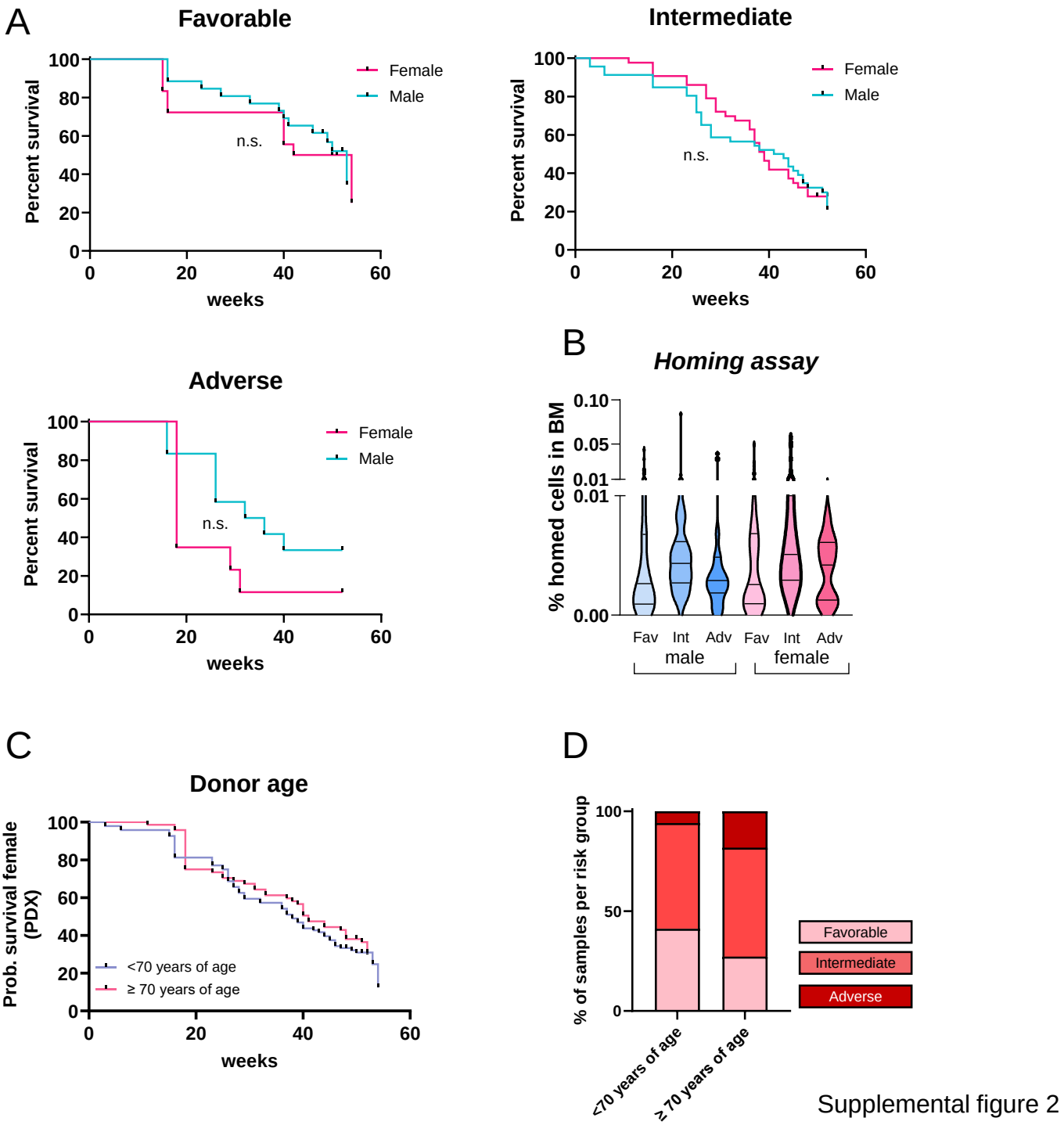

A
% of cKIT+ cells
100
80
60
40
20
0
PDX cells
B
% of GPR56+ cells
100
80
60
40
20
0
PDX cells
C
% of CD123+ cells
100
80
60
40
20
0
PDX cells
D
% of CD47+ cells
100
80
60
40
20
0
PDX cells
E
*
*
% of NKG2DL-/cKIT+ cells
100
80
60
40
20
0
PDX cells
Favorable
Intermediate
Adverse

Figure 7


A
NKG2DL expression
(primary AML cells)
Prob. survival
female (PDX)
100
80
60
40
20
0
0
20
40
60
weeks
NKG2DL Low
NKG2DL High
*
B
CD34 expression
(primary AML cells)
Prob. survival
female (PDX)
100
80
60
40
20
0
0
20
40
60
weeks
0-5%
5-25%
75-100%
C
cKit expression
(primary AML cells)
Prob. survival
female (PDX)
100
80
60
40
20
0
0
20
40
60
weeks
Low or no expression of cKIT
cKIT-expressing samples
****
D
Homing assay
**
% homed cells in BM
0.10
0.05
0.01
0.01
0.00

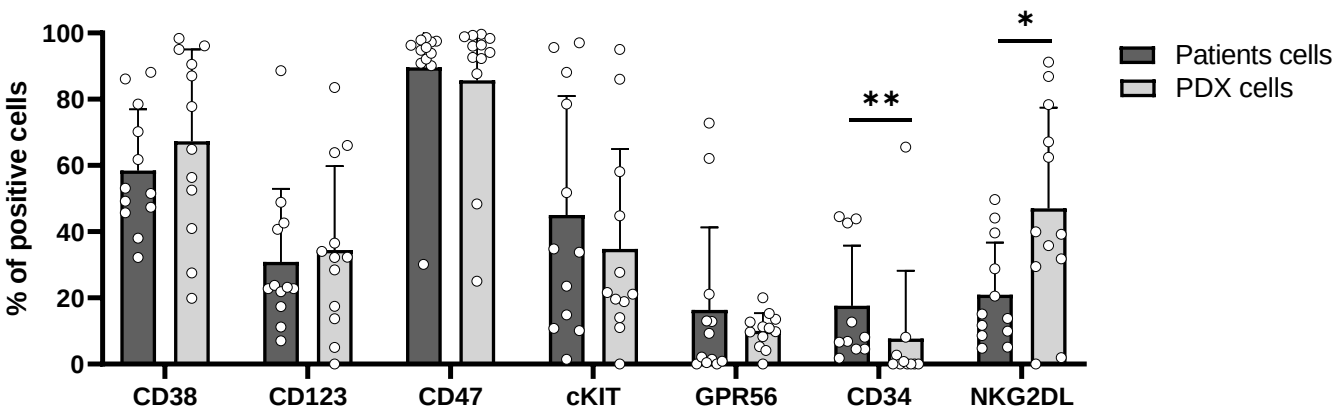



Suppl. Figure 1




Supplemental figure 2

A




Supplemental figure 3

**Supplementary information – Methods.**

**Next Generation sequencing**

DNA and RNA of collected bone marrow samples were extracted on the QIAcube instrument (Qiagen) using QIAamp DNA Blood Mini QIAcube Kit (Qiagen) and RNeasy Mini QIAcube Kit (Qiagen). DNA and RNA concentrations were measured with Qubit HS dsDNA Assay Kit Qubit HS RNA Assay Kit (Thermo Fisher Scientific). Automated library preparation with Oncomine Myeloid Research Assay (Thermo Fisher Scientific) with the Ion Chef instrument according manufacturer's instructions. DNA and RNA libraries were sequenced with Ion GeneStudioTM S5 Plus System (Thermo Fisher Scientific) using a 530 chip. Sequence alignment (hg19) and base calling was performed with Torrent Suite software version 5.12.3 (Thermo Fisher Scientific). Variant calling as well as annotation was done with Ion Reporter software version 5.20 (Thermo Fisher Scientific).

**Xenotransplantation and homing**

For the transplantation procedure, NSG mice were anesthetized by intraperitoneal injection of a mixture of ketamin (65 mg/kg, Streulipharma, Uznach, Switzerland) and xylazin (13 mg/kg, Streulipharma). Animals were assessed for human leukemic cells using PB bleeds and intrafemoral bone marrow (BM) puncture (1) performed routinely at 16, 26, 39 weeks post-transplantation, or at signs of disease. If high burden of leukemic cells was detected during these investigations in one or more mice of an experimental group, final analysis of the entire group was performed.

Mice were additionally followed bi-weekly for weight loss, grin scale, and fur appearance to determine their sickness score. All mice underwent final analysis of leukemic cells in PB, BM, liver and spleen. BM samples from engrafted animals were collected and viably frozen using standard procedures for further analysis.

For homing experiments, $1x10^6$ primary AML cells were labeled with CFSE and injected i.v. in male NSG mice without prior irradiation. 16 hours after injection, BM and PB were sampled for subsequent flow cytometric analyses of CFSE+ AML cells.

**Supplemental Figure legends.**

**Supplemental Figure 1. Modulation of sex hormones influence leukemic outgrowth in patient-derived xenograft models.** Engraftment assessment using flow cytometry in peripheral blood (PB; A), spleen (B) and liver (C) at endpoint analysis. Cells isolated from all groups, ovariectomized female and orchiectomized male as well as regular female and regular male NSG mice from the indicated tissues were screened at the same time-point for CD33 positive acute myeloid leukemia (AML) cells using flow cytometry (n=3 primary AML samples, 11 orchiectomized male, 12 ovariectomized female, 10 regular male and 12 regular female NSG recipients).

**Supplemental Figure 2. Donor-dependent parameters influence leukemia kinetics in NSG mice**. (A) Murine survival based on the European Leukemia Net (ELN) 2017 classification (20 favorable samples (n=12 male with n=26 NSG mice and n=8 female donors with n=18 mice); 30 intermediate samples (n=16 male with n=46 NSG mice and n=14 female donors with n=43 mice); 6 adverse samples (n=3 male donors with n=12 NSG mice and n=3 female donors with n=23 NSG mice). (B) Homing assay analyzing CFSE-positive cells in the murine bone marrow as assessed by flow cytometry 16 hours post-transplantation (14 favorable male samples in 49 NSG mice and 14 favorable female samples in 43 NSG mice; 19 intermediate male samples in 59 NSG mice and 14 intermediate female samples in 42 NSG mice; 6 adverse male samples in 20 NSG mice and 4 adverse female samples in 12 NSG mice). (C) Murine survival based on the patient´s age (34 patients below 70-year-old, 96 NSG mice. 22 patients above 70-year-old, 72 NSG mice). (D) Quantification of samples based on age and risk group association (below 70-year old: 14 favorable acute myeloid leukemia (AML), 18 intermediate

AML and 2 adverse AML. Above 70-year old: 6 favorable AML, 12 intermediate and 4 adverse AML).

**Supplemental Figure 3. Phenotypic analyses of patient- vs. mouse-derived AML cells**. (A) Flow cytometric quantification of positive cells for leukemic stem cell (LSC) markers from matched samples pre- and post-transplantation (n= 16 primary AML samples). Paired t-test: (CD38 and NKG2DL). Wilcoxon t-test: (CD123, CD47, cKIT, GPR56 and CD34)

**Supplemental Table 1. Patient informations, including the fraction of leukemic cells in the BM of each animal in longterm analyses.**

| Patient # | ELN | % leukemic cells in murine BM | Age/ Sex | FAB | Remis-sion | CD 34 | CD 33 | CD 117 | CD56 | ITD ratio | FLT3 | NPM1 | CD 133 | HLA-DR | MPO | NKG2 DL | CD14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | Intermediate | 85,9\|88,9 | 50 F | M1 | CR | pos | pos | pos | neg | high | ITD | MUT | neg | neg | pos | 3,05 | neg |
| **4** | Intermediate | 84,5 | 70 M | M5 | CR | neg | pos | pos | pos | WT | WT | WT | neg | pos | low | 79,67 | pos |
| **5** | Adverse | 2,85\|0,016\| 2,16\| 0,00397\|59,0\| 89,1\|71,5 | 40 M | M1 | RD | pos | pos | pos | neg | high | ITD | WT | pos | pos | pos | 34,7 | neg |
| **7** | Favorable | 0,00758\|0,76\|0, 21 | 70 M | M5 | n.a | neg | pos | neg | pos | WT | WT | MUT | pos | pos | neg | 99,63 | pos |
| **8** | Intermediate | 0 | 70 M | M4 | RD | pos | pos | pos | neg | low | ITD | WT | pos | pos | pos | n.a | pos |
| **10** | Intermediate | 0,011\|0 | 30 M | M2 | CR | pos | pos | pos | neg | low | ITD | WT | pos | pos | pos | 22 | neg |
| **13** | Favorable | 0,016\|0,08\|0 | 55 M | M2 | CR | neg | pos | n.a | n.a | WT | WT | MUT | n.a | n.a | n.a | 10,82 | n.a |
| **14** | Favorable | 0,34 | n.a | n.a | n.a | n.a | n.a | n.a | n.a | WT | WT | MUT | n.a | n.a | n.a | n.a | n.a |
| **15** | Favorable | 84,5 | 26 F | M5 | CR | neg | pos | low | pos | WT | WT | MUT | neg | pos | pos | 92,8 | pos |
| **16** | Favorable | 0\|0\|0 | 52 F | M5 | CR | neg | pos | neg | neg | low | ITD | MUT | neg | pos | pos | 62,19 | pos |
| **17** | Adverse | 0\|0 | n.a | n.a | RD | n.a | n.a | n.a | n.a | n.a. | n.a | n.a | n.a | n.a | n.a | n.a | n.a |
| **18** | Favorable | 0,25\|82,9 | 61 F | M4 | CR | pos | pos | pos | neg | WT | WT | WT | pos | pos | pos | n.a | neg |
| **22** | Intermediate | 0,019\|2,99\|60,4 | 73 F | M2 | CR | n.a | n.a | n.a | n.a | WT | WT | MUT | n.a | n.a | n.a | 19,91 | n.a |
| **23** | Adverse | 97,0 | n.a | n.a | n.a | pos | pos | pos | neg | n.a. | ITD | WT | neg | pos | low | 13,8 | neg |
| **25** | Intermediate | 0,023\|2,14 | 71 F | M5 | n.a | pos | neg | n.a | n.a | WT | WT | MUT | n.a | n.a | n.a | 12,1 | pos |
| **29** | Favorable | 1,12\|0,91 | n.a | n.a | n.a | n.a | n.a | n.a | n.a | n.a. | n.a | n.a | n.a | n.a | n.a | n.a | n.a |
| **32** | Intermediate | 68,6\|66,1\|59,1 | 64 M | M2 | n.a | pos | pos | n.a | n.a | n.a. | n.a | n.a | n.a | n.a | n.a | 69,8 | n.a |
| **34** | Intermediate | 0\|0,3 | 83 M | M0 | n.a | n.a | pos | n.a | n.a | low | ITD | WT | n.a | n.a | n.a | 25,3 | n.a |
| **38** | Intermediate | 0,11\|0,081\|0,01 1 | 58 F | M2 | RD | pos | pos | pos | pos | low | ITD | WT | n.a | pos | n.a | 3,33 | neg |
| **40** | Intermediate | 0,59\|1,08\|0,3\|2, 37 | 35 F | M4 | RD | n.a | pos | n.a | n.a | WT | WT | WT | n.a | n.a | n.a | 93 | n.a |
| **41** | Intermediate | 10,2\|72,0\|73,5 | 45 F | M4 | RD | pos | pos | pos | pos | low | ITD | WT | pos | pos | neg | 70,5 | pos |
| **42** | Favorable | 74,0 | 62 M | M4 | CR | pos | pos | pos | neg | WT | WT | WT | pos | pos | pos | 55,1 | pos |
| **43** | Favorable | 0\|0\|0 | 73 F | M4 | n.a | pos | pos | pos | neg | low | ITD | WT | pos | pos | pos | 72,5 | pos |
| **44** | Intermediate | 0,19\|29,7 | 71 M | M5 | n.a | neg | pos | pos | low | WT | WT | WT | low | pos | pos | 97,45 | low |
| **49** | Adverse | 0,15\|0,23\|0,14 | 74 M | M5 | n.a | pos | pos | neg | pos | high | ITD | WT | neg | pos | low | 90,7 | pos |
| **51** | Favorable | 0,73\|14,7\|0,24 | 46 M | M0 | CR | pos | pos | pos | neg | WT | WT | MUT | neg | pos | pos | 1,39 | neg |
| **52** | Intermediate | 0,04\|39,9\|0,069 \|50,6 | 68 F | M2 | RD | pos | pos | pos | neg | low | ITD | WT | pos | pos | pos | 23,6 | neg |
| **53** | Favorable | 83,8\|86,8\|71,1 | 75 F | M1 | n.a | neg | pos | pos | neg | WT | WT | MUT | pos | pos | pos | 14,84 | neg |
| **55** | Favorable | 90,3 | 33 M | M4 | CR | pos | pos | pos | neg | WT | WT | WT | pos | pos | pos | 70,5 | pos |
| **56** | Intermediate | 0,02\|0\|0 | 71 F | M2 | n.a | neg | pos | pos | pos | high | ITD | MUT | neg | pos | pos | 40,8 | neg |
| **57** | Intermediate | 0,1\|92,5\|0,0069 2 | 36 M | M4 | RD | pos | pos | pos | low | low | ITD | WT | low | pos | pos | 70,4 | pos |
| **58** | Intermediate | 9,58\|0,018\|33,8 | 71 F | M1 | CR | pos | pos | pos | low | WT | WT | WT | pos | pos | pos | 20,3 | low |
| **59** | Favorable | 72,3\|86,9 | 50 F | M2 | n.a | pos | pos | pos | neg | low | ITD | MUT | pos | pos | pos | 17,1 | neg |
| **62** | Favorable | 81,3 | 50 M | M1 | CR | pos | pos | pos | n.a | WT | WT | WT | pos | pos | n.a | 8,3 | neg |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **63** | Adverse | 83,8\|65,0 | 58 M | M2 | n.a | pos | pos | pos | pos | n.a. | n.a | n.a | pos | pos | low | 20,4 | neg |
| **64** | Intermediate | 86,2\|18,8\|83,9\|97,3 | 62 M | M1 | RD | pos | pos | pos | neg | high | ITD | MUT | pos | pos | low | 15,8 | neg |
| **65** | Favorable | 93,1\|93,4 | 81 M | M5 | n.a | neg | pos | pos | pos | WT | WT | MUT | neg | pos | neg | 99,61 | pos |
| **66** | Favorable | 82,7 | n.a | n.a | n.a | n.a | n.a | n.a | n.a | n.a. | n.a | n.a | n.a | n.a | n.a | n.a | n.a |
| **69** | Intermediate | 0\|0\|0,98\|0,99 | 62 M | M1 | n.a | pos | pos | pos | pos | low | ITD | WT | pos | pos | low | 11 | neg |
| **70** | Intermediate | 65,4\|91,8\|85,6 | 23 M | M5 | RD | pos | pos | pos | neg | high | ITD | WT | pos | pos | pos | 36,9 | low |
| **72** | Favorable | 82,1\|0,77 | 72 M | M1 | n.a | n.a | n.a | n.a | n.a | WT | WT | MUT | n.a | n.a | n.a | 10,9 | n.a |
| **73** | Intermediate | 0,25\|0,74\|8,97\|0,02 | 68 M | M5 | CR | neg | pos | pos | | high | ITD | MUT | low | pos | neg | 84,96 | pos |
| **74** | Favorable | 94,6\|98,9\|88,1 | 58 F | M5 | CR | pos | pos | pos | pos | low | ITD | MUT | neg | post | neg | 73,4 | pos |
| **75** | Intermediate | 0,5\|0\|0,031\|0,039 | 23 F | M2 | CR | n.a | n.a | n.a | n.a | WT | WT | WT | n.a | n.a | n.a | 44,2 | n.a |
| **76** | Favorable | 0,036 | 61 F | M2 | CR | pos | pos | pos | n.a | WT | WT | WT | pos | pos | pos | 12,0 | neg |
| **77** | Adverse | 0\|0,077 | 73 F | M1 | n.a | pos | pos | pos | neg | WT | WT | WT | pos | pos | pos | 15,8 | neg |
| **78** | Adverse | 0 | 70 F | M4 | n.a | n.a | n.a | n.a | n.a | WT | WT | WT | n.a | n.a | n.a | 29,6 | n.a |
| **80** | Intermediate | 0,074\|0,08 | 79 F | M2 | n.a | n.a | n.a | n.a | n.a | low | ITD | WT | n.a | n.a | n.a | 11,3 | n.a |
| **82** | Favorable | 0\|0 | 45 M | M4 | CR | pos | pos | n.a | n.a | WT | WT | WT | n.a | n.a | n.a | 97,5 | n.a |
| **85** | Intermediate | 0,02\|0,053\|0,72 | 52 M | M1 | CR | pos | pos | n.a | n.a | high | ITD | MUT | n.a | n.a | n.a | 1,39 | n.a |
| **89** | Intermediate | 0,11\|92,9\|38,5\|35,3 | 21 F | M1 | CR | pos | pos | pos | pos | high | ITD | WT | pos | pos | pos | 63,6 | neg |
| **94** | Favorable | 0,04\|0,018\|1,1 | 82 M | M2 | n.a | n.a | n.a | n.a | n.a | low | ITD | Mut | n.a | n.a | n.a | 8;16 | neg |
| **95** | Intermediate | 0,042\|0,12\|0,031 | 53 M | M0 | n.a | pos | pos | pos | pos | WT | WT | WT | pos | low | neg | n.a | neg |
| **96** | Favorable | 0,00204\|0,014\|0,021 | 34 M | M1 | CR | pos | pos | pos | neg | WT | WT | WT | pos | pos | pos | 39,5 | neg |
| **100** | Intermediate | 0,023\|0,018\|0,032 | 49 M | M0 | RD | pos | pos | neg | neg | WT | WT | WT | pos | pos | neg | 8,75 | neg |
| **101** | Favorable | 0\|0,2 | 50 M | M5 | CR | neg | pos | low | neg | low | ITD | Mut | neg | pod | pod | 88,42 | pos |
| **122** | Intermediate | 13,2\|0,15\|68,9\|0,27 | 72 M | M5 | CR | pos | pos | pos | n.a | low | ITD | WT | n.a | pos | n.a | 7,59 | neg |
| **123** | Intermediate | 0,12\|83,6\|91,3\|2,94 | 72 M | M2 | n.a | pos | pos | low | n.a | low | ITD | WT | pos | pos | n.a | 68,9 | pos |
| **124** | Intermediate | 0,025\|1,45\|0,15 | 54 F | M4 | CR | neg | pos | pos | neg | high | ITD | MUT | pos | pos | low | 71,14 | pos |
| **125** | Intermediate | 0,15\|0,034\|0,1 | 83 F | n.a | n.a | n.a | n.a | n.a | n.a | n.a. | n.a | n.a | n.a | n.a | n.a | n.A | n.a |
| **131** | Adverse | 3,59\|8,33\|0,39\|3,17\|10,3\|38.4\|0,24\|2,93\|0\|16,1\|2,21\|5,97\|7,43\|1,35\|0,015\|2,77\|1,38\|0\|62,0\|1,75 | 74 F | n.a | RD | neg | pos | pos | pos | n.a. | WT | MUT | n.a | n.a | pos | n.a | neg |

**Supplemental Table 2. Patient informations, including the fraction of CFSE-positive cells indicating homing to the BM per animal in Homing analyses.**

| Patient # | ELN | % CFSE pos cells in murine BM | Age/ Sex | FAB | Remis-sion | CD 34 | CD 33 | CD 117 | CD 56 | ITD ratio | FLT3 | NPM1 | CD 133 | HLA-DR | MPO | NKG2 DL | CD14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | Intermediate | 0,048\|0,033\|0,057 | 50 F | M1 | CR | pos | pos | pos | neg | high | ITD | MUT | neg | neg | pos | 3,05 | neg |
| **3** | Adverse | 0,00125\|0,00416\|0,0082 | 70 F | M2 | RD | pos | pos | pos | neg | WT | WT | WT | pos | pos | low | 52,7 | pos |
| **4** | Intermediate | 0,0837\|0,00604\|0,011 | 70 M | M5 | CR | neg | pos | pos | pos | WT | WT | WT | neg | pos | low | 79,67 | pos |
| **5** | Adverse | 0,0032\|0,00223\|0,00318\|0,00341\|0,00262\|0,0024 | 40 M | M1 | RD | pos | pos | pos | neg | high | ITD | WT | pos | pos | pos | 34,7 | neg |
| **6** | Adverse | 0,032\|0,039\|0,034 | 66 M | M1 | RD | neg | pos | pos | neg | WT | WT | WT | pos | pos | low | 13,1 | neg |
| **7** | Favorable | 0,00186\|0,00208\|0,00166 | 70 M | M5 | n.a | neg | pos | neg | pos | WT | WT | MUT | pos | pos | neg | 99,63 | pos |
| **8** | Intermediate | 0,00799\|0,00748\|0,00794 | 70 M | M4 | RD | pos | pos | pos | neg | low | ITD | WT | pos | pos | pos | n.a | pos |
| **10** | Intermediate | 0,00509\|0,0084\|0,00478 | 30 M | M2 | CR | pos | pos | pos | neg | low | ITD | WT | pos | pos | pos | 22 | neg |
| **12** | Adverse | 0,00174\|0,00243 | 83 M | n.a | n.a | pos | pos | pos | n.a | WT | WT | WT | pos | pos | pos | 16,3 | pos |
| **13** | Favorable | 0,0013\|0,00201\|0,00302 | 55 M | M2 | CR | neg | pos | n.a | n.a | WT | WT | MUT | n.a | n.a | n.a | 10,82 | n.a |
| **14** | Favorable | 0,00884\|0,0066\|0,00564 | n.a | n.a | n.a | n.a | n.a | n.a | n.a | WT | WT | MUT | n.a | n.a | n.a | n.a | n.a |
| **15** | Favorable | 0,00111\|0,000645\|0,000935 | 26 F | M5 | CR | neg | pos | low | pos | WT | WT | MUT | neg | pos | pos | 92,8 | pos |
| **16** | Favorable | 0,000937\|0,00786 | 52 F | M5 | CR | neg | pos | neg | neg | low | ITD | MUT | neg | pos | pos | 62,19 | pos |
| **17** | Adverse | 0,014\|0,017\|0,025 | n.a | n.a | RD | n.a | n.a | n.a | n.a | n.a. | n.a | n.a | n.a | n.a | n.a | n.a | n.a |
| **18** | Favorable | 0,00767\|0,00627\|0,00977\|0,00596 | 61 F | M4 | CR | pos | pos | pos | neg | WT | WT | WT | pos | pos | pos | n.a | neg |
| **19** | Adverse | 0,000656\|0,00176\|0,000657 | 76 F | M4 | CR | neg | pos | neg | neg | WT | WT | WT | neg | pos | pos | 82,2 | pos |
| **22** | Intermediate | 0,00248\|0,0045\|0,00419 | 73 F | M2 | CR | n.a | n.a | n.a | n.a | WT | WT | MUT | n.a | n.a | n.a | 19,91 | n.a |
| **23** | Adverse | 0,00571\|0,00563\|0,00905 | n.a | n.a | n.a | pos | pos | pos | neg | n.a. | ITD | WT | neg | pos | low | 13,8 | neg |
| **24** | Favorable | 0,00242\|0,008\|0,00774\|0,00964 | n.a | M4 | PR | pos | pos | n.a | n.a | WT | WT | WT | n.a | n.a | n.a | 44 | n.a |
| **25** | Intermediate | 0,00897\|0,00586\|0,0036 | 71 F | M5 | n.a | pos | neg | n.a | n.a | WT | WT | MUT | n.a | n.a | n.a | 12,1 | pos |
| **28** | Adverse | 0,011\|0,024\|0,012 | n.a | n.a | n.a | n.a | n.a | pos | n.a | n.a. | n.a | n.a | n.a | n.a | n.a | n.a | n.a |
| **29** | Favorable | 0,00488\|0,00345\|0,00644\|0,00179\|0,011\|0,00635 | n.a | n.a | n.a | n.a | n.a | n.a | n.a | n.a. | n.a | n.a | n.a | n.a | n.a | n.a | n.a |
| **30** | Favorable | 0,00482\|0,00683\|0,00424\|0,00515 | 43 F | M4 | CR | neg | pos | neg | neg | WT | WT | MUT | neg | pos | pos | 96,28 | pos |
| **32** | Intermediate | 0,00344\|0,00425\|0,00536 | 64 M | M2 | n.a | pos | pos | n.a | n.a | n.a. | n.a | n.a | n.a | n.a | n.a | 69,8 | n.a |
| **34** | Intermediate | 0,00432\|0,00519\|0,00257 | 83 M | M0 | n.a | n.a | pos | n.a | n.a | low | ITD | WT | n.a | n.a | n.a | 25,3 | n.a |
| **36** | Favorable | 0,043\|0,033\|0,00101 | 63 M | M2 | n.a | neg | pos | n.a | n.a | WT | WT | MUT | n.a | n.a | n.a | 25,62 | n.a |
| **37** | Favorable | 0,00683\|0,00579\|0,00628 | 81 F | M4 | RD | neg | pos | pos | neg | WT | WT | MUT | pos | pos | pos | 57,19 | neg |
| **38** | Intermediate | 0,00166\|0,00325\|0,00205 | 58 F | M2 | RD | pos | pos | pos | pos | low | ITD | WT | n.a | pos | n.a | 3,33 | neg |
| **39** | Intermediate | 0,00347\|0,00321\|0,00383 | 63 M | M5 | n.a | pos | pos | pos | neg | n.a. | n.a | n.a | pos | pos | pos | n.a | neg |
| **40** | Intermediate | 0,051\|0,015\|0,019 | 35 F | M4 | RD | n.a | pos | n.a | n.a | WT | WT | WT | n.a | n.a | n.a | 93 | n.a |
| **41** | Intermediate | 0,00451\|0,00199\|0,00224 | 45 F | M4 | RD | pos | pos | pos | pos | low | ITD | WT | pos | pos | neg | 70,5 | pos |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **42** | Favorable | 0,00376\|0,00345\|0,00277 | 62 M | M4 | CR | pos | pos | pos | neg | WT | WT | WT | pos | pos | pos | 55,1 | pos |
| **43** | Favorable | 0,00256\|0,000381\|0,00026\|0,00147 | 73 F | M4 | n.a | pos | pos | pos | neg | low | ITD | WT | pos | pos | pos | 72,5 | pos |
| **44** | Intermediate | 0,01\|0,00805\|0,00562 | 71 M | M5 | n.a | neg | pos | pos | low | WT | WT | WT | low | pos | pos | 97,45 | low |
| **45** | Intermediate | 0,00318\|0,00597\|0,00919 | 68 M | M1 | RD | pos | pos | pos | low | high | ITD | MUT | pos | pos | pos | 22,1 | low |
| **46** | Adverse | 0,00661\|0,00129\|0,00537 | 70 F | M1 | RD | pos | pos | pos | neg | WT | WT | WT | pos | low | pos | 6,69 | neg |
| **49** | Adverse | 0,00229\|0,00684\|0,00357 | 74 M | M5 | n.a | pos | pos | neg | pos | high | ITD | WT | neg | pos | low | 90,7 | pos |
| **50** | Adverse | 0,00475\|0,00488\|0,00347 | 76 M | M5 | n.a | n.a | n.a | n.a | n.a | WT | WT | WT | n.a | n.a | n.a | 99,83 | n.a |
| **51** | Favorable | 0,016\|0,017\|0,021 | 46 M | M0 | CR | pos | pos | pos | neg | WT | WT | MUT | neg | pos | pos | 1,39 | neg |
| **52** | Intermediate | 0,01\|0,00377\|0,00164 | 68 F | M2 | RD | pos | pos | pos | neg | low | ITD | WT | pos | pos | pos | 23,6 | neg |
| **53** | Favorable | 0,029\|0,049\|0,00145 | 75 F | M1 | n.a | neg | pos | pos | neg | WT | WT | MUT | pos | pos | pos | 14,84 | neg |
| **54** | Favorable | 0,0017\|0,00091\|0,00204 | 41 M | M4 | CR | neg | pos | low | neg | WT | WT | MUT | neg | neg | pos | 87,16 | pos |
| **55** | Favorable | 0,012\|0,00654\|0,00979 | 33 M | M4 | CR | pos | pos | pos | neg | WT | WT | WT | pos | pos | pos | 70,5 | pos |
| **56** | Intermediate | 0,00495\|0,00445\|0,00693 | 71 F | M2 | n.a | neg | pos | pos | pos | high | ITD | MUT | neg | pos | pos | 40,8 | neg |
| **57** | Intermediate | 0,00871\|0,00503\|0,00434 | 36 M | M4 | RD | pos | pos | pos | low | low | ITD | WT | low | pos | pos | 70,4 | pos |
| **58** | Intermediate | 0,011\|0,01\|0,003 | 71 F | M1 | CR | pos | pos | pos | low | WT | WT | WT | pos | pos | pos | 20,3 | low |
| **59** | Favorable | 0,0035\|0,00908 | 50 F | M2 | n.a | pos | pos | pos | neg | low | ITD | MUT | pos | pos | pos | 17,1 | neg |
| **62** | Favorable | 0,00264\|0,0016\|0,00456 | 50 M | M1 | CR | pos | pos | pos | n.a | WT | WT | WT | pos | pos | n.a | 8,3 | neg |
| **64** | Intermediate | 0,00632\|0,00454\|0,00616 | 62 M | M1 | RD | pos | pos | pos | neg | high | ITD | MUT | pos | pos | low | 15,8 | neg |
| **65** | Favorable | 0,000663\|0,000116\|0,000353\|0,00111\|0,000792\|0,00268 | 81 M | M5 | n.a | neg | pos | pos | pos | WT | WT | MUT | neg | pos | neg | 99,61 | pos |
| **66** | Favorable | 0,00435\|0,00232\|0,00318 | n.a | n.a | n.a | n.a | n.a | n.a | n.a | n.a. | n.a | n.a | n.a | n.a | n.a | n.a | n.a |
| **69** | Intermediate | 0,00156\|0,00207\|0,00027 | 62 M | M1 | n.a | pos | pos | pos | pos | low | ITD | WT | pos | pos | low | 11 | neg |
| **70** | Intermediate | 0,00201\|0,00129\|0,00078\|1 | 23 M | M5 | RD | pos | pos | pos | neg | high | ITD | WT | pos | pos | pos | 36,9 | low |
| **71** | Favorable | 0,00347\|0,00178\|0,00484 | 76 F | M1 | RD | neg | pos | pos | n.a | WT | WT | MUT | n.a | low | pos | 25,54 | neg |
| **72** | Favorable | 0,0093\|0,01\|0,0065 | 72 M | M1 | n.a | n.a | n.a | n.a | n.a | WT | WT | MUT | n.a | n.a | n.a | 10,9 | n.a |
| **73** | Intermediate | 0\|0,00335, 0,00493\|0,015\|0,00897 | 68 M | M5 | CR | neg | pos | pos | | high | ITD | MUT | low | pos | neg | 84,96 | pos |
| **74** | Favorable | 0,00045\|0,00158\|0,00022 6 | 58 F | M5 | CR | pos | pos | pos | pos | low | ITD | MUT | neg | post | neg | 73,4 | pos |
| **75** | Intermediate | 0,00231\|0,00522\|0,00728 | 23 F | M2 | CR | n.a | n.a | n.a | n.a | WT | WT | WT | n.a | n.a | n.a | 44,2 | n.a |
| **76** | Favorable | 0,00163, 0,000954\|0,00115 | 61 F | M2 | CR | pos | pos | pos | n.a | WT | WT | WT | pos | pos | pos | 12,0 | neg |
| **77** | Adverse | 0,00627\|0,00559\|0,00424 | 73 F | M1 | n.a | pos | pos | pos | neg | WT | WT | WT | pos | pos | pos | 15,8 | neg |
| **79** | Intermediate | 0,00213\|0,00118\|0,00213 | 67 M | M4 | n.a | pos | pos | pos | neg | n.a. | n.a | n.a | neg | pos | pos | n.a | neg |
| **80** | Intermediate | 0,0083\|0,00756\|0,013 | 79 F | M2 | n.a | n.a | n.a | n.a | n.a | low | ITD | WT | n.a | n.a | n.a | 11,3 | n.a |
| **82** | Favorable | 0,000832\|0,000619\|0,000951\|0,00289\|0,00155 | 45 M | M4 | CR | pos | pos | n.a | n.a | WT | WT | WT | n.a | n.a | n.a | 97,5 | n.a |
| **83** | Adverse | 0,014\|0,018 | n.a | n.a | n.a | n.a | n.a | n.a | n.a | n.a. | n.a | n.a | n.a | n.a | n.a | n.a | n.a |
| **84** | Intermediate | 0,012\|0,00924\|0,011 | | M2 | | | | | | high | ITD | Mut | | | | | |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **85** | Intermediate | 0,00301\|0,00414\|0,00434 | 52 M | M1 | CR | pos | pos | n.a | n.a | high | ITD | MUT | n.a | n.a | n.a | 1,39 | n.a |
| **89** | Intermediate | 0,00619\|0,012\|0,000294 | 21 F | M1 | CR | pos | pos | pos | pos | high | ITD | WT | pos | pos | pos | 63,6 | neg |
| **90** | Adverse | 0,000152\|0,00108\|0,000565 | 77 M | M5 | RD | neg | pos | pos | pos | WT | WT | WT | neg | pos | pos | 97,85 | pos |
| **91** | Favorable | 0,016\|0,017\|0,021 | 64 F | M1 | n.a | neg | pos | pos | neg | n.a. | n.a | n.a | neg | neg | pos | n.a | neg |
| **93** | Favorable | 0,00184\|0,000813\|0,000687 | 46 F | M5 | n.a | neg | pos | neg | n.a | n.a. | n.a | n.a | neg | pos | pos | n.a | pos |
| **94** | Favorable | 0,0045\|0,000362\|0,00466\|0\|0,000619\|0,000483 | 82 M | M2 | n.a | n.a | n.a | n.a | n.a | low | ITD | Mut | n.a | n.a | n.a | 8;16 | neg |
| **95** | Intermediate | 0,00509\|0,00519\|0,00673 | 53 M | M0 | n.a | pos | pos | pos | pos | WT | WT | WT | pos | low | neg | n.a | neg |
| **96** | Favorable | 0 | 34 M | M1 | CR | pos | pos | pos | neg | WT | WT | WT | pos | pos | pos | 39,5 | neg |
| **97** | Adverse | 0,015\|0,00525\|0,018 | n.a | M2 | n.a | n.a | n.a | n.a | n.a | n.a. | ITD | WT | n.a | n.a | n.a | n.a | n.a |
| **98** | Favorable | 0,00204\|0,00108 | n.a | n.a. | n.a | n.a | n.a | n.a | n.a | n.a. | n.a | n.a | n.a | n.a | n.a | n.a | n.a |
| **100** | Intermediate | 0,00243\|0,00361 0,00165 | 49 M | M0 | RD | pos | pos | neg | neg | WT | WT | WT | pos | pos | neg | 8,75 | neg |
| **101** | Favorable | 0,02\|0,0069\|0,00664\|0,0076 | 50 M | M5 | CR | neg | pos | low | neg | low | ITD | Mut | neg | pod | pod | 88,42 | pos |
| **122** | Intermediate | 0,00326\|0,00279\|0,00341 | 72 M | M5 | CR | pos | pos | pos | n.a | low | ITD | WT | n.a | pos | n.a | 7,59 | neg |
| **123** | Intermediate | 0,00137\|0,0018\|0,00575 | 72 M | M2 | n.a | pos | pos | low | n.a | low | ITD | WT | pos | pos | n.a | 68,9 | pos |
| **124** | Intermediate | 0,00349\|0,00598\|0,00554 | 54 F | M4 | CR | neg | pos | pos | neg | high | ITD | MUT | pos | pos | low | 71,14 | pos |
| **125** | Intermediate | 0,00271\|0,00309\|0,00273 | 83 F | n.a | n.a | n.a | n.a | n.a | n.a | n.a. | n.a | n.a | n.a | n.a | n.a | n.A | n.a |
| **126** | Favorable | 0,00294\|0,00337\|0,00233\|0,00675 | n.a | M2 | n.a | n.a | n.a | n.a | n.a | n.a. | n.a | n.a | n.a | n.a | n.a | n.a | neg |
| **127** | Favorable | 0,00107\|0,025\|0,00231 | 58 F | M3 | n.a | neg | pos | neg | pos | n.a. | ITD | MUT | neg | neg | n.a | n.a | pos |

**Reference:**

1. Paczulla AM, Dirnhofer S, Konantz M, et al. Long-term observation reveals high-frequency engraftment of human acute myeloid leukemia in immunodeficient mice. Haematologica. 2017 May;102(5):854-864.