Context and timing matters in acute myeloid leukemia: females are the superior hosts

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In this issue of *Haematologica*, Stanger *et al*. describe their evaluation of the effects of recipient sex and donor acute myeloid leukemia (AML) patients' characteristics on engraftment kinetics and survival in the commonly used, gold-standard, patient-derived xenograft (PDX) NOD.Cg-*Prkdcscid Il2rgtm1Wjl*/SzJ (NSG) immunodeficient mouse model.[1] They show that irrespective of donor sex, primary AML cells engraft better in female recipients. With extended observation times of up to a year, *in vivo* phenotypes can be obtained in female recipients which relate to clinical parameters (risk group, therapy response, cellular and clonal composition, marker profiles).[1] With this strategy, high rates of PDX engraftment were achieved even for the normally hard-to-engraft and, hence, understudied favorable-risk AML subgroup.[1] This work underscores the clinical relevance and validity of the PDX model to recapitulate aspects of AML pathogenesis and leukemia stem cell biology while highlighting experimental design caveats. The AML field relies on the NSG model to study inter- and intra-patient functional heterogeneity by assessing engraftment levels, marker profiles, clonal/genetic trajectories, drug responses, leukemia stem cell function and frequency of primary patients' samples. Therefore, methodical experimental design and detailed reporting on patient/donor as well as recipient characteristics are essential to extract meaningful, reliable, and reproducible information of clinical relevance.

Prior studies have established that engraftment efficiency upon xenotransplantation of healthy hematopoietic stem cells is greater in female than in male recipients in the NSG model and others derived from it.[2,3] This suggests that, compared to the male microenvironment, the female microenvironment is not only more leukemia-supportive but generally better in supporting human blood repopulation. Mechanistically, estrogen/estradiol was shown to stimulate murine hematopoietic stem cell cycling with retention in the supportive hematopoietic microenvironment.[4] However, such empirical investigation has primarily focused on mouse hematopoietic stem cells with limited analysis of human hematopoiesis.[4,5] In the study published in this issue, Stanger *et al*. elegantly show that AML repopulation is not significantly impacted in ovariectomized NSG mice.[1] Surprisingly, castration prior to transplantation improved AML engraftment in male recipients.[1] These data suggest that testosterone has a negative impact on AML repopulation, which could be linked to lineage skewing and niche remodeling in normal hematopoiesis.[6] While this does not preclude a role for gonad-derived sex hormones in the microenvironment prior to their removal, it implicates contrasting sex-dependent mechanisms for human normal and AML xenotransplantation. A similar study recently investigated donor-recipient sex-matching for healthy and AML donor cells and reported no significant difference in AML patient engraftment when donor cells and recipient mice were sex-matched.[3] The observed discrepancies may be attributed to a low number of paired samples (7 *vs*. 9), sample heterogeneity, mice age at transplantation, assay endpoint analysis and different cell processing and irradiation protocols.[3] Nonetheless, the poorest engraftment was observed for female AML and also female healthy donor cells transplanted into male recipients, consistent with the findings of Stanger *et al*. Of note, Stanger *et al*. used weight and, thereby, sex-adjusted irradiation dosing in contrast to the methodology reported for most PDX studies, thus minimizing potential sex biases due to irradiation insult and recovery.[1] An interesting future experimental question to address would be if their findings can be recapitulated in sex-stratified studies in PDX models (e.g., NSGW41) that do not require irradiation.[7] In the light of the PDX experiments with castrated mice, a plausible hypothesis is that androgens impact engraftment of female and male donors differently. An important caveat is that the PDX data presented following gonad removal were not stratified by donor sex.

By performing long-term engraftment assays *in vivo* for up to a year, Stanger and colleagues demonstrated the importance

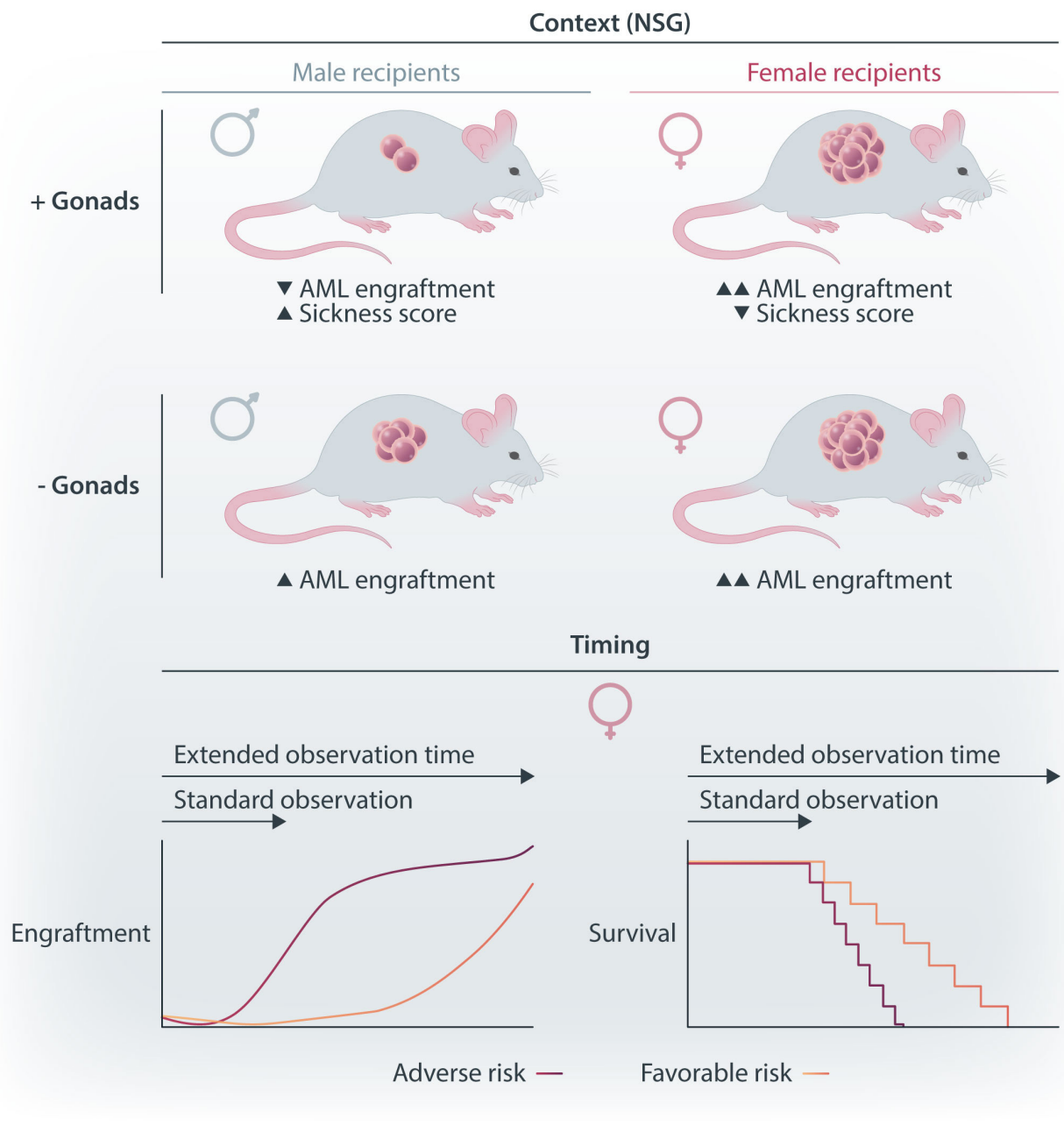

Figure 1. Human acute myeloid leukemia characteristics are impacted by recipient sex and engraftment period in the NSG immunodeficient mouse model. AML: acute myeloid leukemia.

of long transplantation timelines to capture long-latency AML.[1] Standard AML xenograft studies are observed for up to 12-16 weeks.[8] Although the assays were performed only in female recipients, the observations effectively showed the functional heterogeneity of AML subtypes of all risk groups while simultaneously generating informative survival curves, such as the faster repopulation kinetics of adverse-risk AML also showing shorter survival.[1] Notably, AML-related mortality in the PDX model is rarely observed within the standard experimental observation times (<20 weeks) unlike in syngeneic murine AML models (e.g., the MLL-AF9 model, <10 weeks).[9] Hence, these data represent an important resource for preclinical PDX studies, exemplify the vast heterogeneity of the disease, and underscore that AML is predominantly a disease driven by leukemia stem cells in which every sample may engraft if given enough time. Future studies are needed to address whether female and male recipients support long-latency AML engraftment differently.

Finally, a male-recipient sex bias in sickness scores was observed which appeared to be uncoupled from donor engraftment level, suggesting that the microenvironment may determine frailty.[1] Sickness scores were observed to be higher in male recipients despite higher disease burden/engraftment in female mice, and female recipients survived better when transplanted with male rather than female donor cells from favorable-risk group AML. Stanger *et al*. showcased the validity of using female recipients exclusively to obtain clinically relevant phenotypes while also lowering costs by faster detectable engraftment and reducing the risk of male mice dropping out due to sickness.[1] We are not aware of any cohort study correlating the blast percentage and sex-specific survival outcomes in AML patients. A conclusive long-term engraftment and survival study would entail transplanting every AML pairwise into recipients of both sexes and measuring sickness scores. Ultimately, considerations must be made to interpret AML engraftment observations in young female recipient mice in light of a human disease that has an all-age 1.5-fold leukemia risk incidence and mortality in males *versus* females.[10] Assuming the same mechanisms are at play in the female *versus* male human aged microen-

vironment, the female AML-promoting factors at play have to be counterbalanced to result in these disease statistics. Collectively, these AML PDX studies indicate that estrogen is neither a general driver nor a protector from leukemia stemness as assessed by xenotransplantation.

Disclosures

No conflicts of interest to disclose.

Contributions

KBK and SZX wrote the manuscript and conceived the figure.

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