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

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In this issue, *Arnone et al* evaluate the effects of recipient sex and donor acute myeloid leukemia (AML) patient 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 that relate to clinical parameters (risk group, therapy response, cellular and clonal composition, marker profiles) [1]. With this strategy, even high rates of PDX engraftment were achieved for the 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 (LSC) 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, LSC function and frequency of primary patient 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 (HSC) is higher in female than in male recipients in the NSG and thereof-derived models[2,3]. This suggests that the female compared to the male microenvironment is not only more leukemia-supportive but generally better in supporting human blood repopulation. Mechanistically, estrogen/estradiol was shown to stimulate murine HSC cycling with retention in the supportive hematopoietic microenvironment[4]. However, such empirical investigation has primarily been in mouse HSC with limited analysis of human hematopoiesis[4,5]. Here, *Arnone et al* elegantly show that AML repopulation is not significantly impacted in ovariectomized NSG[1]. Surprisingly, castration prior to transplantation improved AML engraftment in male recipients[1]. These data suggest 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 *Arnone et al.* Of note, *Arnone et al* used weight and thereby sex adjusted irradiation dosing in contrast to methodology reporting of most PDX studies, thereby 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 differentially impact engraftment of female vs male donors. An

important caveat is that the PDX post gonad-removal data presented was not stratified by donor sex.

By performing long-term engraftment assays *in vivo* for up to a year, Arnone and colleagues demonstrate the importance 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 show the functional heterogeneity of AML subtypes of all risk groups while simultaneously generating informative survival curves, e.g. the faster repopulation kinetics of adverse risk AML showing also shorter survival[1]. Notably, AML-related mortality in the PDX model is rarely observed within the standard experimental observation times (< 20 w) contrary to syngeneic murine AML models (e.g. MLL-AF9 model, <10 w)[9]. Hence, this data represents an important resource for preclinical PDX studies, exemplifies the vast heterogeneity of the disease, and underscores that AML is predominantly an LSC-driven disease where every sample may engraft if given enough time. Future studies are needed to address if female and male recipients differentially support long-latency AML engraftment.

Finally, a male-recipient sex bias in sickness scores was observed that 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 transplanted with male vs female donor cells from favourable risk group AML. *Arnone 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 vs. male human aged microenvironment, the female AML promoting factors at play have to be counterbalanced to result in these disease statistics. Collectively, these AML PDX studies indicate estrogen is neither a general driver nor protector from leukemia stemness as assessed by xenotransplantation.

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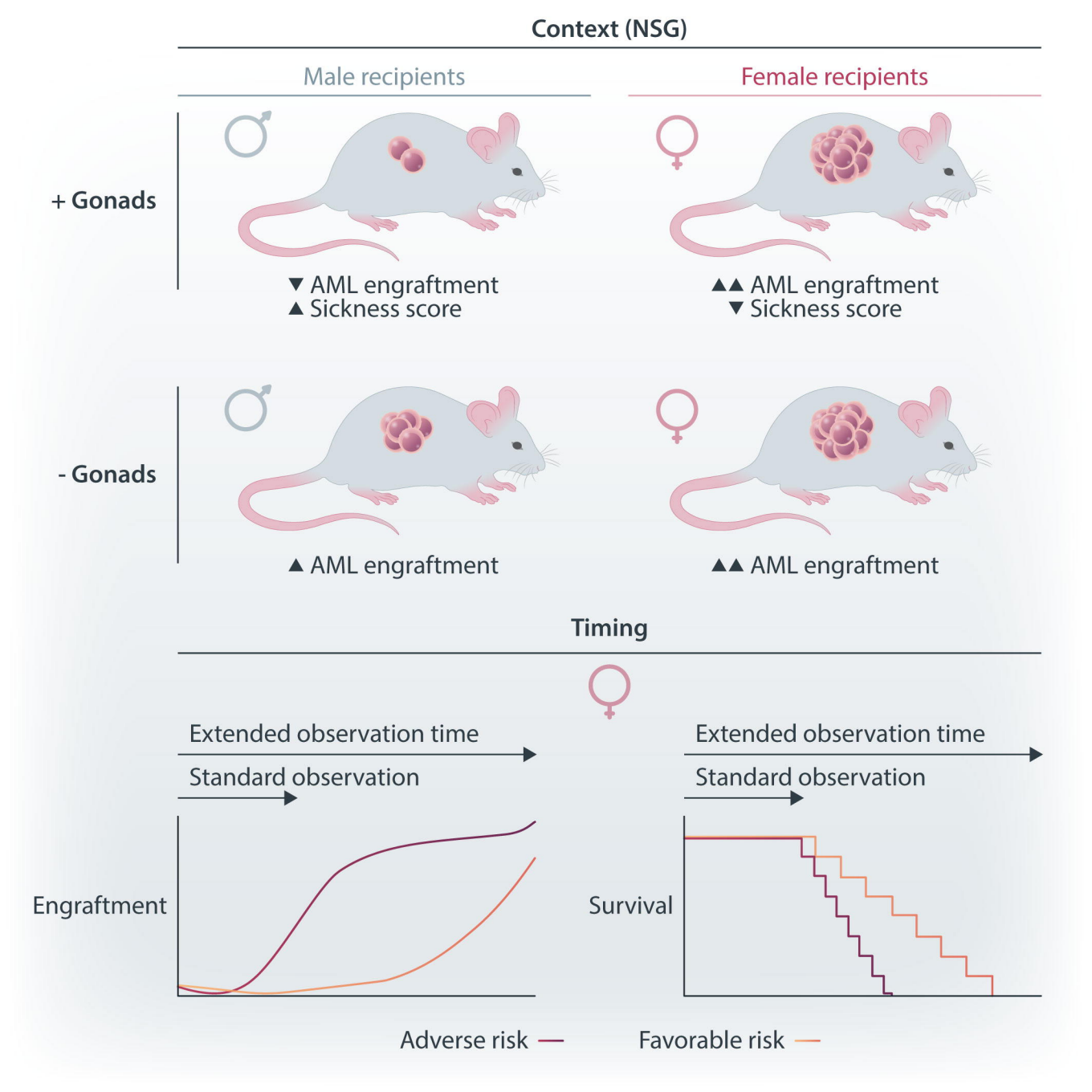
Figure 1: Human acute myeloid leukemia characteristics are impacted by recipient sex and engraftment period in the NSG immunodeficient mouse model.


Context (NSG)
Male recipients
Female recipients
+ Gonads
▼ AML engraftment
▲ Sickness score
▲▲ AML engraftment
▼ Sickness score
- Gonads
▲ AML engraftment
▲▲ AML engraftment
Timing
Extended observation time
Standard observation
Engraftment
Extended observation time
Standard observation
Survival
Adverse risk
Favorable risk