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NSG-S mice for acute myeloid leukemia, yes. For myelodysplastic syndrome, no.

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Research on primary patient cells is a compelling challenge for scientists. Although initially limited to short experiments over hours or days, engrafting these primary human cells in immunodeficient mice today allows even more informative investigation to be carried out over weeks and months. This experiment is fascinating, probably first because it gives rise to personal and moral questions about the patient's avatar. Also, in basic research, the xenograft is the model to be used to reveal the stemness properties of a certain population of cancer cells.[1] Although today there are some *ex vivo* alternatives, the xenograft remains the gold standard technique to study cancer stem cells which are responsible for cancer initiation propagation, maintenance and evolution. Uncovering the presence of primary human leukemic cells in a sample of mouse tissue 10-16 weeks after injection, demonstrating the initial engraftment of leukemia initiating cells (LICs) causes an exhilarating sensation known to only a few lucky scientists. Absence of graft triggers the opposite sensation of complete disappointment, which has led several teams to focus their attention on this particular problem with the xenograft approach. In this issue of *Haematologica*, Krevvata *et al.* put forward fundamental new insights to help improve xenograft of acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS).[2]

Myelodysplastic syndrome and acute myeloid leukemia are myeloid neoplasms that disrupt normal hematopoiesis. This group of myeloid leukemias could be considered as a continuum consisting of a multitude of different leukemias, including all possible myeloid abnormalities. This results in a wide range of severity and patient overall survival (OS). MDS patients have globally better OS than AML patients, and some MDS evolve inevitably towards AML. Interestingly, the first attempts at AML/MDS xenograft quickly revealed, through the repartition of samples engrafting and non-engrafting the mice, that the engraftment potential was perfectly linked with the aggressiveness of the leukemia, since AML samples are usually more easy to engraft than MDSs.[3,4] Many independent studies have offered different reasons for engraftment failure, but none can satisfactorily explain it. Possible explanations are either related to the host immune environment or to the defect of the grafted cells or to the graft and host compatibility.

The innate and adaptive immune response of the host environment is an obvious and very clear obstacle for the

graft. This was the goal and the reports accross the different generation on immunodeficient recipient. Indeed previous comparisons of different models with increased immunodeficiency, NOD/SCID mice (non-obese diabetic/severe combined immunodeficiency mice), NOD/SCID beta2 (β2-microglobulin-deficient NOD-scid mice), NSG (NOD-Scid-IL-2Rγcnull) mice, showed that the degree of immunodeficiency clearly matters but is not the key to explain xenograft failure of some samples. Fewer AML cells are necessary to initiate the graft and a better engraftment is reached using more immunodeficient recipients as compared with first generation NOD/SCID mice.[5-7] However, changing the permissiveness of the recipient maintains the sample stratification in terms of engraftment potential. This means that samples with the potential to engraft better remain better engrafters and the poorer remain poorer. (In the context of this Editorial, non-engrafters remained mostly non-engrafters using different strains of immunodeficient mice.[7]) Thus, further increasing the immunodeficiency of the recipient would actually jeopardize the recipient viability without improving the overall engraftment rate.

Independently of the recipient used, it was shown that the xenograft potential of AML samples was linked to intrinsic properties of the cells injected. Engraftment failure is related to good prognosis AML and, inversely, xenograft potential is a poor prognosis marker.[8,9] Paczulla *et al.*[10] and our own team recently showed that increasing the incubation period from 10 to 30 weeks allows some successful leukemic xenografts of good prognosis-related samples incapable of engrafting NSG mice during a conventional 10-12 week period. Actually, these samples have a lower frequency of stem-progenitor cells associated with a lower expansion capacity *ex vivo* compared to poor prognosis-related samples' cells efficiently engrafting NSG mice.[11] These data suggest that the non-engrafter samples might just have a slower progression, and that the recipient residual immunity is not an insurmountable obstacle for these samples.

Eventually, beyond the recipient immunity or the grafted cells defect, the last explanation for xenograft failure is the lack of a human specific microenvironment support for some categories of leukemia samples. In the last decade, one strategy approaching the issue from two different directions was adopted to try to improve the compatibility of the animal for human cells. The approach had been to humanize the murine recipient either genetically (by forcing the expression of human cytokines) or by injecting the mice with cellular components of the human bone marrow microenvironment (BMME). Different immunodeficient mouse strains expressing various human cytokines have been generated over the last decade and are reviewed by Theocharides *et al.*[12] Among them, the NSG-S mice used by Krevvata *et al.*[2] is an engineered strain, with knock-in for human stem cell factor (SCF), granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-3 in the background of the NSG recipient.[13] Alternatively, humanizing recipient BMME is achieved by injection of stromal cells of the human BMME, such as mesenchymal stem cells (MSC), or endothelial cells, or osteoblast progenitors.[14-16] For different reasons, intravenous (IV) or intra-bone marrow bolus of stromal cells is less and less used for the benefit of humanized ectopic ossicle approaches, subcutaneously implanted by surgery with either matrigel scaffold, sponges, or ceramic seeded with human stromal cells. These methods have certainly improved the situation since they recently resulted in successful engraftment of samples previously defined as non-engrafters, including good pronosis AML.[15,16] However two disadvantages were reported for these models. First, these ectopic leukemic grafts of good prognosis-related samples were reported to not invade recipient bone marrows, thus actually limiting the size of the human leukemic population in this particular case.[15,16] Secondly, some protocols are quite demanding to handle in routine lab practice, such as the pre-treatment of the ectopic niche with parathyroid hormone to favor the osteoblastic differentiation of the MSC prior to the introduction of the leukemic cells.[15] Thus, direct IV injection will probably remain the most common *in vivo* protocol to explore the LIC compartment, and increasing the chances of successful engraftment in this setting is of particular interest.

Krevvata *et al.* show on a large cohort (n=77) of AML patients that 82% of AML samples engraft NSG-S recipient *versus* 50% in the NSG strain.[2] Sixty-seven percent of non-engrafter AML in the NSG strain become engrafters in the NSG-S strain during a conventional incubation period. This was also true for good prognosis inv16 AML, which are core binding factor (CBF) mutated AML known to repeatedly fail xenograft procedure. NSG-S also presents the advantages of faster engraftment and a leukemic burden present in the peripheral blood similar to that of patients, allowing simple blood sampling for longitudinal monitoring. However, the downside of the NSG-S model is the management of the leukemia progression that reduces viability of the cohorts. Xenografted with the same sample in NSG-S mice die faster than in NSG. Although the swiftness and quantity of engraftment is clearly shown in this model, further comparative tests should investigate the quality of the graft, for example, to exclude LIC alteration and exhaustion. The Authors eventually found that 18% of the samples remain non-engrafters in NSG-S, providing opportunities for further investigation into graft failures. Engrafting good prognosis AML samples, including CBF-AML, is a big step forward, opening up opportunities for previously impossible investigation, such as identifying the phenotype of their LICs, or analyzing their *in situ* behavior in the endosteum by intravital microscopy, or the possibility of comparison of clonal architecture and clonal evolution *in vivo* with poor prognosis AML samples. This model could also allow the *in vivo* comparison of drug resistance mechanisms of these two groups of patients on the condition of first determining whether NSG-S mice can support an induction regimen, as Farge *et al.* have recently shown for NSG mice.[17]

The second part of the study of Krevvata *et al.* reports negative results but is nonetheless equally important. The Authors have performed a deep analysis of low- and high-risk MDS sample engraftment in NSG-S mice with or without MSCs co-injection. MSCs from different origins were tested: healthy donor-derived MSC (normal), allogeneic patient-derived MSC (allo), or patient-derived

autologous MSC (auto). These results are very interesting because previous publications from two other groups arrived at opposite conclusions.[18-20] Krevvata *et al.* confirm the observation of Rouault-Pierre *et al.* and demonstrate that MDS only offer transient benefit from the cytokine stimulation in the NSG-S model, and actually tend to exhaust their engraftment level over time.[2] It is also the first study presenting a comprehensive paired analysis of engraftment that clearly establishes that MDS engraftment is not enhanced by co-injection of MSC, in contrast to previous reports. Overall, this work suggests that improving the MDS xenograft model remains a key challenge. Further testing should be performed using other newly developed immunodeficient mouse models, such as the four genes encoding human cytokines MISTRG (M-CSFh/h IL-3/GM-CSFh/h hSIRPAtg TPOh/h Rag2-/-Il2rg-/-) strain or NBSGW mice (mouse stem cell factor receptor mutated in the background of NSG), to eventually develop better MDS xenografts. In the perspective of this study, future investigations could explore and try to understand how and why an IL3, SCF and GM-SCF cytokine cocktail can be beneficial for supporting LICs of good prognosis AML but not of MDS, and how their distinct epigenetic regulators and DNA methylation patterns might be involved in this differential response.

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