Acute myeloid leukemia including favorable-risk group samples engraft in NSG mice: just be patient

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In this issue of Haematologica, Paczulla *et al.* demonstrate that by extending the time to read out the engraftment of primary acute myeloid leukemia (AML) cells in immunodeficient IL2RG^{null} NOD/SCID (NSG) mice all types of AML samples, including favorable prognosis AML, a group that has been previously reported to be particularly difficult to engraft, could be studied.[1]

Xenotransplantation of human AML in immunocompromised animals has been critical for defining leukemic stem cells and remains the primary method for functional assessment of primary human AML biology as well as providing the best *in vivo* preclinical model. However, the use of immunodeficient mouse models to study primary AML samples has shown some limitations. Over the past decades, important advances have been made to improve patient-derived xenograft (PDX) modeling of AML through the use of mice that are more immunodeficient, such as beta 2 micro-globulin^{null} NOD/SCID mice and more recently the IL2RG knockout NOD/SCID mice.[2-4] Even with the most immunodeficient mouse model (NSG mice), only 66% of AML engraft at 10-16 weeks after transplantation.[2,3] Interestingly, the capacity to engraft has been correlated with patients' clinical outcome, with non-engrafting samples being those from the more favorable risk group.[4,5]

In this new study by Paczulla *et al.*, it appears that the dominant factor in engraftment is the speed of developing a detectable disease, as all primary AML samples, including those from the favorable-risk group, might be able to engraft in NSG mice if the duration of the experiment is prolonged for up to 1 year. The authors also found that the latency of developing a detectable level of engraftment is correlated with the frequency of leukemic stem cells (LSC) in a patient. This notion is further supported by recent data from Griessinger *et al.*, who showed *ex vivo* that non-engrafter AML samples have lower levels of leukemia long-term culture-initiating cells.[6] In addition, others have found that a high frequency of phenotypically defined CD34$^+$CD38$^-$CD123$^+$ LSC is correlated with the risk of a poor clinical outcome.[7] Lastly, using gene expression data, Dick's group identified a "leukemic stem cell gene signature" and correlated the presence of this gene signature in AML samples with the aggressiveness of the disease.[8] They subsequently refined this signature to 17 genes and confirmed their initial data in an extended cohort of patients.[9]

By investigating other components that could explain the long latency of engraftment of certain samples, the authors explored the possibility that samples from patients with a certain genetic background (favorable- or some intermediate-risk AML) might be more sensitive to microenvironmental factors for their survival and growth. Despite not detecting any significant difference in the proliferation capacity between samples from short- and long-latency "engrafter" patients, they only focused on the late time-point when a high leukemic burden was already present. Differences in the proliferative index between "non-engrafters" and "engrafters" have been reported using an *ex vivo* co-culture system[5] suggesting that the proliferative capacity of "long-latency" engrafters (originally called non-engrafters) is potentially playing a role.

Indeed, the notion that some AML cells proliferate less at an early stage in the murine environment is supported by the results obtained with the new MISTRG transgenic mouse model.[10] In this model, human cytokines are knocked into the endogenous mouse loci. In these mice, Ellegast *et al.* recently demonstrated reproducible engraftment of favorable-risk group AML samples with a shorter latency. Supporting the notion that engraftment could be impaired by cross-species differences, Majeti's group, JJ Schuringa's group and Bonnet's group reported in parallel the feasibility of engrafting previously "non-engrafter" AML samples by implanting a three-dimensional humanized bone marrow stroma scaffold/ossicles.[11-13] In this humanized microenvironment, non-engrafter samples were able to engraft with a shorter latency than that reported by Paczulla *et al.*, further suggesting that long-latency engrafters might be more sensitive to microenvironmental signals than "engrafters".

Thus, the engraftment capacity or more exactly the latency to engraft primary AML samples in immunodeficient mice is likely dependent on homing factors, survival in a foreign niche, absence of specific growth factors and supporting stroma cells as well as intrinsic differences in LSC frequency.

Humanizing the microenvironment via the use of new transgenic mice and/or via the use of three-dimensional scaffolds should clearly help to shorten the latency of disease development, extending the use of the PDX model to all AML samples. Shortening the latency to detect engraftment will not only reduce the cost of maintaining mice but will also provide a more useful PDX model for predicting patients' responses to potential therapies.

As new strains of mice become available and novel and more complex three-dimensional humanized scaffolds are developed, it will be necessary to examine in more detail the value of the different PDX models for predicting patients' responses. Indeed, as indicated by assessment of the subclonal architecture of individual PDX, some reports suggest highly selective engraftment of specific subclones in PDX mice.[14,15] Paczulla *et al.* report that, despite the long latency to develop leukemia, the phenotypic and genetic features in mouse-derived *versus* pre-transplanted AML cells are conserved. Since the subclonal composition of the AML after PDX will influence responses to therapies, reproducing similar clonal architecture in PDX as in pre-transplant samples is clearly an important aspect in the development of valuable preclinical PDX models.

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Better acute graft-*versus*-host disease outcomes for allogeneic transplant recipients in the modern era: a tacrolimus effect?

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Acute graft-*versus*-host disease (GvHD) continues to be an important complication following allogeneic hematopoietic stem-cell transplantation (HSCT) in the modern era. With matched related and unrelated donors, the cumulative incidence of acute GvHD remains approximately 40-60%, respectively.[1] Survival outcomes for patients undergoing HSCT have however improved over the last few decades because of improvements in non-relapse mortality rather than relapse incidence.[2,3] It is an interesting conundrum that improvement in non-relapse mortality and survival has occurred despite a lack of sentinel advancements in acute GvHD prophylaxis or treatment. Calcineurin inhibitors are the cornerstone of prophylaxis, while steroids remain the mainstay of treatment.[4] The question arises whether improvements in non-relapse mortality and survival are due: (i) solely to improved management of acute GvHD complications (infections and organ toxicity); (ii) to better rates of acute GvHD response to steroid-based therapy; or (iii) to a secular shift in the nature and severity of acute GvHD over time.

Khoury *et al.* now offer some insights into these important questions in this issue of Haematologica.[5] In a large registry analysis (n=2905) from the Center for International Blood and Marrow Transplant Research (CIBMTR), they evaluate the incidence and outcomes of grade II-IV acute GvHD developing within 100 days after myeloablative, HLA-matched HSCT over three successive time periods [1999-2001 (n=497), 2002-2005 (n=962), 2006-2012 (n=1446)]. These periods overlap with important advances in supportive care (e.g., azoles for fungal infections, valacyclovir for cytomegalovirus).[6,7] The predominant GvHD prophylaxis regimens were tacrolimus-based (n=1767; 60.7%) or cyclosporine (CsA)-based (n=1077; 37.1%). Patients in the tacrolimus and CsA groups were well-balanced with regard to baseline characteristics (except for more matched unrelated donor and peripheral blood stem-cell grafts in the tacrolimus cohort). The authors then compared the outcomes of patients in each time period stratified by GvHD prophylaxis (CsA-based *versus* tacrolimus-based) and grade of acute GvHD (grade II *versus* grades III-IV).

Several interesting observations resulted. Firstly, the severity of acute GvHD appears to have decreased over time. The proportion of patients with grades III-IV severe acute GvHD in the most recent time period (2006-2012) has decreased by 20% compared to that in the earliest time period (1999-2001). This could be due to a true decrease in acute GvHD severity or a drift within acute GvHD categories, with more grade II patients being identified and reported to the CIBMTR. Simultaneously, there are fewer patients with concurrent three-organ (gut/skin/liver) involvement in recent years compared to previous years, while the proportion of patients with gut acute GvHD with or without skin involvement has increased significantly. Secondly, on multivariate analysis, in the subgroup of HSCT recipients with grades II-IV acute GvHD who received tacrolimus prophylaxis, overall survival (Figure 1, from the original article) and non-relapse mortality have improved in the modern era. The improvement appears to be due to fewer deaths from organ toxicity and infection. Interestingly, this improvement is not seen in HSCT recipi-