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Response to NK cell content does not seem to influence engraftment in *ex vivo* T cell depleted haploidentical stem cell transplantation

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In our recent publication in *Stem Cell Reports* (Lobo de Figueiredo-Pontes et al., 2021), we assessed the role of NK cells on hematopoietic stem cell (HSC) fate during HSC transplantation and concluded that NK cells, possibly via secretion of cytokines (and in particular IFNγ), negatively affect HSC frequency and function. In their letter in this issue of *Stem Cell Reports*, Merli and colleagues report carrying out an interesting attempt to translate our observations to clinics (Merli et al., 2022). They performed a retrospective analysis of 2 cohorts of pediatric patients transplanted after *ex vivo* T cell depletion (Locatelli et al., 2017; Merli et al., 2021; Lang et al., 2014) and concluded that the amount of NK cells in the graft does not have an impact in the transplantation outcome. While we acknowledge the value of their analysis, we believe that there are critical factors between both studies that prevent Merli et al. from reaching our same conclusions.

First, the main aim of our study was to deepen our understanding of the mechanisms that control HSC fate in the context of transplantation. Thus, we employed simplified and reductionist pre-clinical models that do not resemble the complexity of clinical studies that include the genetic variability of patient samples. In their letter, Merli and colleagues employed 2 cohorts of pediatric patients suffering from a large variety of conditions, such as AML, ALL, advanced myelodysplastic syndrome, solid tumors, and a variety of non-malignant conditions. However, we do not favor NK cell removal from any graft, but rather, "we would like to propose that NK cell depletion may actually favor HSC donor engraftment in the setting of allogeneic transplantation for non-neoplastic diseases in selected patients. Such cases would include, for instance, but are not limited to, patients with idiopathic severe aplastic anemia or sickle cell anemia" (see our original manuscript by Lobo de Figueiredo-Pontes et al., 2021). Therefore, we would not expect that NK cell depletion would exhibit beneficial effects in a mixed and random cohort of transplanted patients.

Second, the discrepancies between both studies could also be caused by the HSC and NK cell numbers in the grafts. While Merli et al. compare patients that received NK cell contents below and above the median, we mostly compare presence or absence of NK cells in our experimental conditions. Further, based on the median CD34+ and NK cell content, the ratio in the cohort of patients transplanted in Rome was 1:2 and, in the patients transplanted in Tübingen, it was between 1:4 and 1:5. In our experimental settings, we observe slight effects using an HSC:NK cell ratio of 1:10, while we demonstrated more pronounce differences with a 1:50 ratio. Thus, as also suggested by Merli et al., the effect of NK cells in the clinical setting could be missed due to the high doses of CD34+ cells infused. Consequently, it is very important to remember that we suggested in our original manuscript that NK depletion could gain great value when clinicians deal with limited donor HSC numbers. We believe that rather than comparing patients that received NK cell content below and above the median, it would be more relevant to determine whether differences on the transplantation outcome exist when comparing high and low CD34+:NK cell ratios.

Finally, another relevant aspect that would explain the distinct conclusions of both studies is the experimental readout. The data presented by Merli and colleagues suggest that the time to neutrophil and platelet engraftment after transplantation are not affected by the number of NK cells infused with the graft. Nevertheless, neutrophil and platelet recovery derives from hematopoietic progenitor cells at this time point and does not measure HSC function or engraftment as described in our work.

In conclusion, while we appreciate the alternative perspective by Merli and colleagues, we consider that their approach can be narrowed down to correlations in a complex cohort of patients and cannot be equal to causation. The interpretation of our data and the translation into clinical relevance is of course up for debate. However, without a clinical trial targeting NK cells in a transplant setting in patients, the letter by Merli and colleagues relies on data mining and correlations, and it does not speak directly to our study and our specific conclusions.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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