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

Pietro Merli,[1,*] Thomas Eichholz,[2] Maria Luigia Catanoso,[1] Peter Lang,[2] and Franco Locatelli[1,3]
[1]Department of Pediatric Hematology/Oncology, Cell and Gene Therapy, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy
[2]Children's University Hospital, Eberhard Karls University Tübingen, Tübingen, Germany
[3]Sapienza, University of Rome, Rome, Italy
*Correspondence: pietro.merli@opbg.net
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We read with great interest the manuscript by Lobo de Figueiredo-Pontes and colleagues (Lobo de Figueiredo-Pontes et al., 2021) on the potential clinical usefulness of NK cell-derived IFNγ inhibition to favor engraftment in allogeneic hematopoietic stem cell transplantation (HSCT). Indeed, the role of IFNγ in regulating hematopoiesis during acute or chronic inflammation through modulation of transcription factor expression and perturbation of cytokine signaling is well characterized (Merli et al., 2021). Thus, we agree that modulation of inflammation of marrow microenvironment may be a useful strategy to favor engraftment in the HSCT setting. We recently confirmed in a clinical setting that IFNγ has an important pathogenic role in immune-mediated graft failure (GF) (Merli et al., 2019), also showing that inhibition of this cytokine with emapalumab (a clinical grade, IFNγ-inhibiting monoclonal antibody developed for the treatment of resistant/relapsed primary HLH; Locatelli et al., 2020) might improve engraftment of selected patients (e.g., those affected by HLH).

However, we do not concur with the authors, who propose that "NK cell depletion may actually favor HSC donor engraftment in the setting of allogeneic transplantation for non-neoplastic diseases in selected patients." In fact, in addition to anti-tumor activity, NK cells may exert control over pathogens (Zuo and Zhao, 2021). These properties have been exploited in the clinic, especially in the haploidentical HSCT field, by optimizing *ex vivo* T cell depletion to obtain "designed" grafts that retain useful immune effector cells (e.g., NK cells, γδ T cells, and memory T cells), while avoiding potentially harmful alloreactive populations (e.g., αβ T cells and naive T cells). These improvements increased the use of haploidentical donors because of improved clinical results (superimposable to those of T cell replete/HLA-matched transplants according to some reports; Locatelli et al., 2017). Moreover, donor alloreactive NK cells are able to kill both T lymphocytes and antigen-presenting cells of the recipients, thus contributing to the prevention of graft rejection and graft-versus-host disease (GvHD) (Ruggeri et al., 2002).

To further corroborate our statement, we retrospectively analyzed 2 cohorts of pediatric patients transplanted after *ex vivo* T cell depletion: TCRαβ/CD19-depletion (thus with a graft containing, among the others, CD34+, NK, and TCRγδ cells) was used in Rome (Locatelli et al., 2017; Merli et al., 2022) while CD3/CD19-depletion (thus with a graft containing CD34+ and NK cells) was used in Tübingen (Lang et al., 2014). These experiences resemble the models used by Lobo de Figueiredo-Pontes and colleagues. One-hundred and fifty patients were transplanted in Rome after TCRαβ/CD19-depletion, 80 because of malignant disease [56 cases of acute lymphoblastic leukemia (ALL) and 24 of acute myelogenous leukemia (AML)] and 70 for a non-malignant condition (primary immunodeficiencies, red blood cell disorders, inherited bone marrow failure syndromes, aplastic anemia, or metabolic diseases) at a median age at HSCT of 7.2 years (range 0.3–20.9). Median CD34+ and NK cell content was 17.3 × 10^6/kg (range 6.0–48.8) and 40.0 × 10^6/kg (range 3.8–156.1), respectively. Cumulative incidence of GF at 100 days was 14.7% (95% CI 9.9–21.5) for the whole cohort and 30.4% (95% CI 21.0–42.7) for patients affected by non-malignant disorders. It did not differ according to NK cell content, with patients who received less than 40 × 10^6/kg NK cells showing a cumulative incidence of GF of 14.2% (95% CI 7.9–24.9), compared to 15.4% (95% CI 8.8–26.2) of patients receiving more NK cells (p = 0.87). Also in the group of non-malignant patients, the cumulative incidence of GF did not differ according to NK cell content [lower NK cell content 36.6% (95% CI 22.2–56.4) versus 29.0% (95% CI 16.2–48.4) for higher NK content, p = 0.49]. Median time to neutrophil and platelet recovery was 13 (range 9–33) and 11 days (range 7–51). The number of NK cells infused with the graft did not influence the time to neutrophil and platelet engraftment, both for the whole cohort and for the non-malignant patient subset. In detail, for the non-malignant group, median time to neutrophil recovery was 14 days (range 9–33) and 16 days (range 11–25) for those with NK cell content below or above the median (p = 0.54), respectively. Concerning platelet recovery, median time to engraftment was 11 days (range 8–51) and 10 (range 7–34) days for those with NK cell content below or above the median (p = 0.8), respectively.

In Tübingen 157 HSCTs after CD3/CD19-depletion were performed; of those, 132 HSCTs were performed in leukemia or solid tumors and 25 in non-malignant diseases. Patient age ranged from 0.3 to 26.5 years (median 8.1). Median CD34+ content was 14.87 × 10^6/kg (range 1.8 – 55.98). Data on NK cells were available in 126 transplantations with a median 73.9 × 10^6/kg (range 9.1–379.0) and 77.77 × 10^6/kg (range 9.63–275.56) in the non-malignant cohort.

Neutrophil and platelet recovery occurred after a median time of 10 days (range 7–40) and 11 days (range 2–188), respectively, in the whole group. Number of infused NK cells below or above the median value of 73.9 × 10^6/kg did not affect neutrophil and platelet recovery. Neutrophils recovered after a median of 10 days (range 8–29) and platelets after a median of 12 days (range 6–27) in patients receiving less than 73.9 × 10^6/kg NK cells compared to neutrophil recovery after 10 days (range 9–40, p = 0.65) and platelet recovery after 9 days (range 4–188, p = 0.45) in patients receiving more NK cells.

Likewise, in non-malignant diseases neutrophil and platelet recovery were independent from NK cell content below (neutrophils, median 9 days, range 9–11 and platelets, 13 days, range 7–17) or above (neutrophils, 9 days, range 9–11, p = 0.57 and platelets, median 13 days, range 6–28, p = 0.61) 73.9 × 10^6/kg NK cells.

At 100 days the cumulative incidence of GF in the whole group was 15.5% (95% CI 6.2–28.7) and 28% (95% CI 6.9–54.6) in the non-malignant patients. There was no significant difference in patients receiving less than 73.9 × 10^6/kg NK cells or more in both the whole group (NK cell content low 12.7%, 95% CI 1.6–35.7; NK cell content high, 14.7%, 95% CI 2.5–37.0, p = 0.9) and the non-malignant cohort (NK cell content low, 20%, 95% CI 0.2–66.9; NK cell content high, 30.8%, CI 4.3–64.4, p = 0.68).

The discrepancies between the clinical results and those from the experiments conducted by Lobo de Figueiredo-Pontes and colleagues may be due to variables not included in the model tested (e.g., the presence in the grafts of myeloid-derived suppressor cells). Additionally, the number of CD34+ and NK cells used for experiments may not reflect that used in the clinic, where mega-doses of CD34+ cells are infused, thus making the negative effect of donor NK cells not clinically significant. However, it has to be mentioned that this interpretation may be further confounded due to the depletion of TCRαβ ± TCRγδ cells that can also produce IFNγ.

Removal of NK cells from the graft may increase the risk of infections immediately after transplant (i.e., in the time period more at risk for this type of complication). Moreover, in the malignant setting, depriving the graft from mature NK cells may impair the graft-versus-leukemia effect; indeed, it would require 6 to 8 weeks from HSCT to obtain the recovery of KIR+ alloreactive NK cells. Thus, not surprisingly, the outcome of haploidentical HSCT using more recent methods of *ex vivo* T cell depletion demonstrated superior post-HSCT outcomes as compared to CD34+ positive selection (Lum et al., 2021).

In summary, despite the interesting results showed by Lobo de Figueiredo-Pontes and coauthors, we think that, from the available data, the proposal of NK cell-depletion of the graft is questionable; nonetheless, targeted inhibition of specific cytokines detrimental for HSC engraftment, such as IFNγ, may be a preferable strategy for patients at high risk of rejection.

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