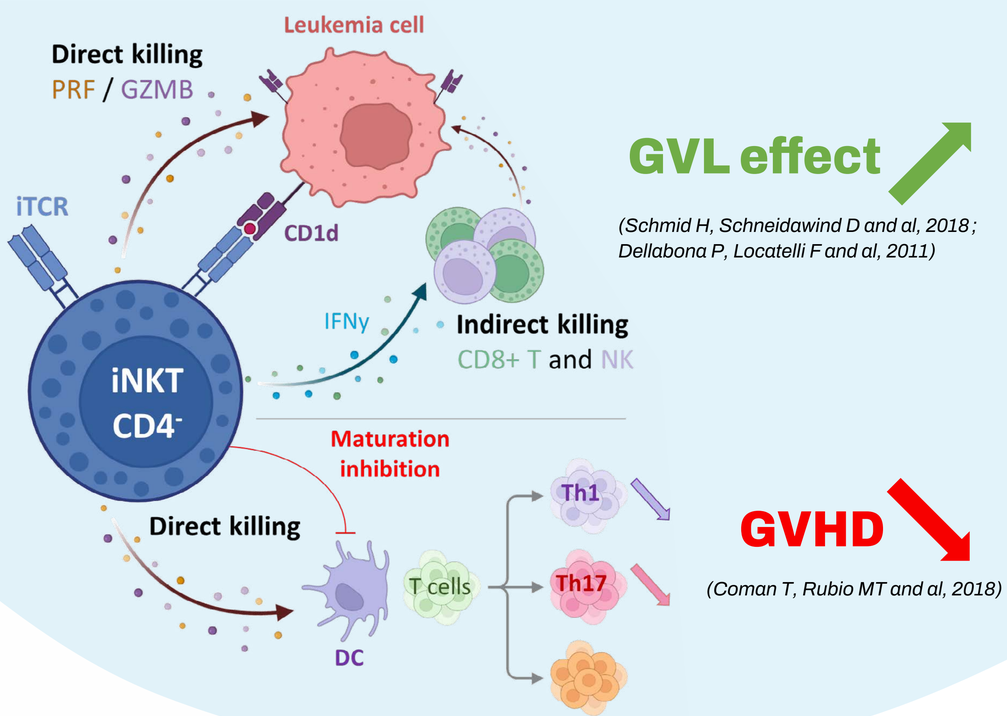
Development of an efficient expansion protocol for human CD4⁻ iNKT

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INTRODUCTION

- iNKT cells **regulate GVHD** in hematopoietic stem cell transplantation (HSCT).
- CD4⁻ iNKT cells **reduce alloreactivity** by decreasing dendritic cells number and maturation.
- Also a natural cytotoxicity against CD1d-expressing tumor cells, **enhancing the GVL effect**.
- iNKT cells are promising for cancer treatment and improving HSCT outcomes.
- Their clinical use is limited by challenging expansion and enrichment methods.



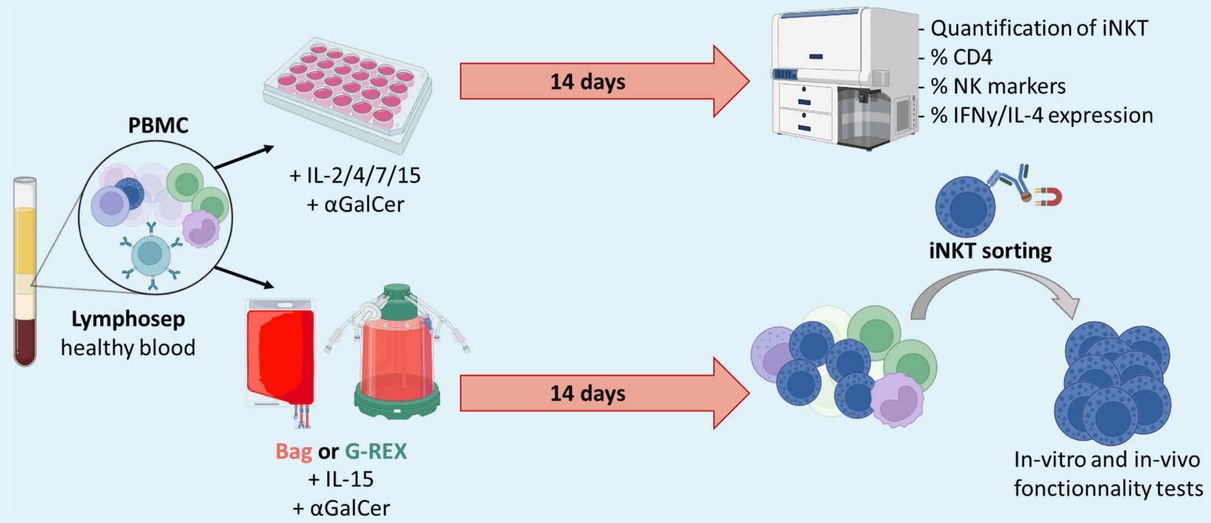
GVL effect ↗

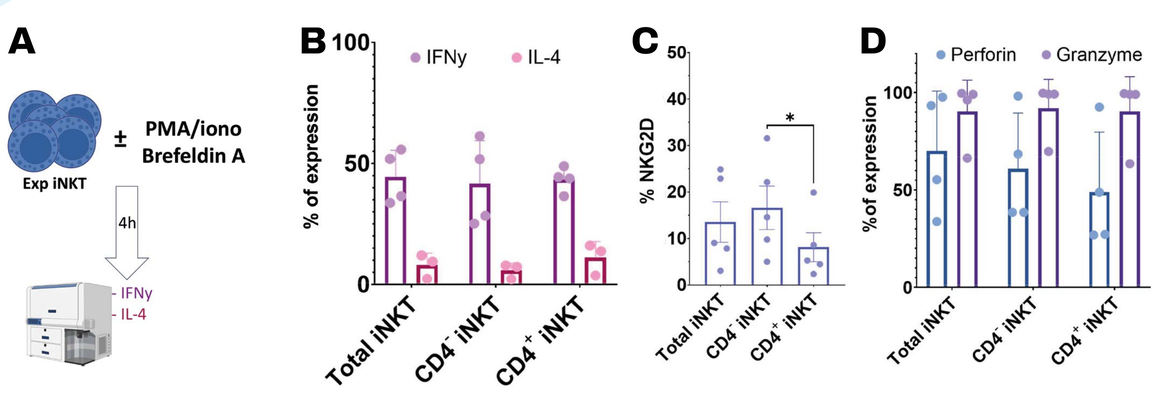
(Schmid H, Schneidawind D and al, 2018 ; Dellabona P, Locatelli F and al, 2011)

GVHD ↘

(Coman T, Rubio MT and al, 2018)

MATERIALS AND METHODS





RESULTS

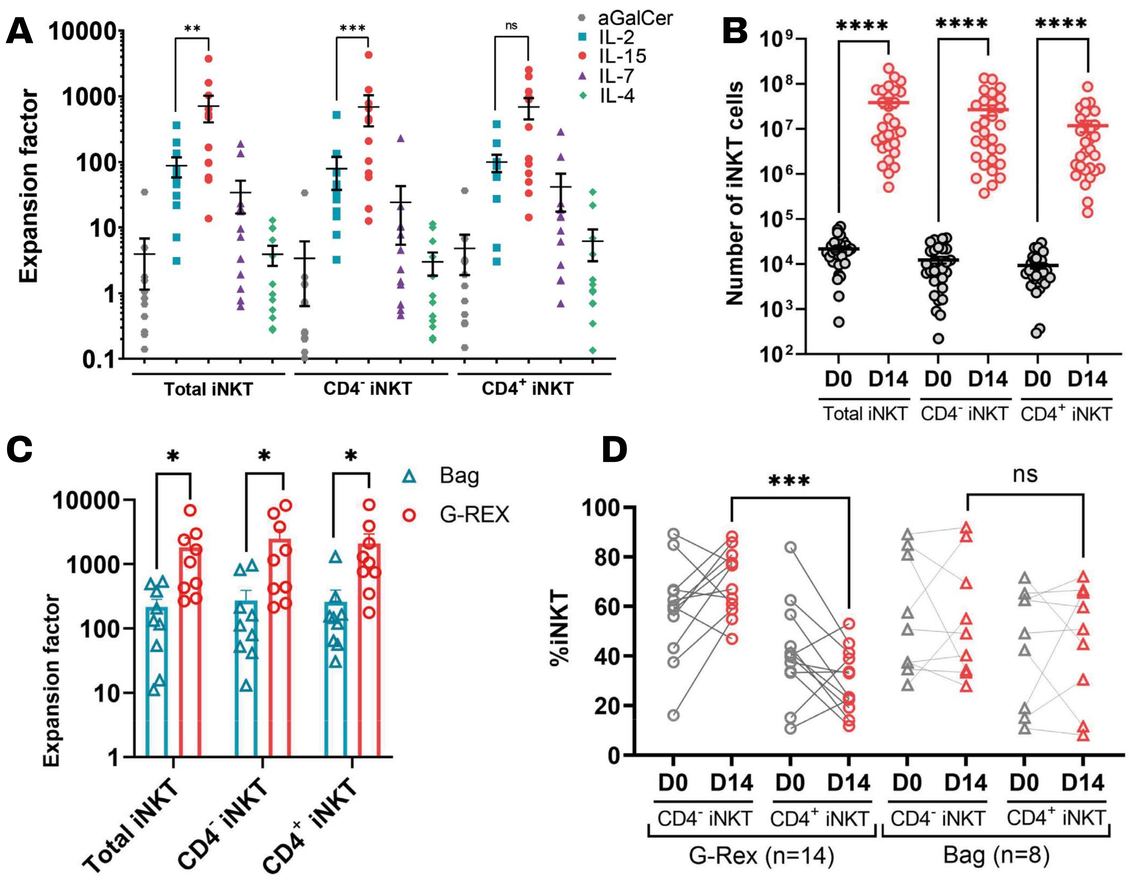


Figure 1. IL-15 combined with the G-Rex bioreactor optimizes human CD4⁻ iNKT cell expansion.

(**A**) IL-15 supplementation at day 0 significantly enhances total and subset-specific iNKT expansion compared to IL-2, IL-4, and IL-7. (**B**) Absolute numbers of total, CD4⁻, and CD4⁺ iNKT cells increase significantly from day 0 (D0) to day 14 (D14). (**C**) The G-Rex bioreactor supports superior iNKT expansion compared to expansion bags at D14. (**D**) The percentage of CD4⁻ iNKT cells increases significantly in G-Rex, while CD4⁻ iNKT remain stable in bags.

Data are presented as mean ± SEM. Statistical significance is indicated as : p < 0.05 (*), p < 0.01 (**), p < 0.001 (***), and p < 0.0001 (****)

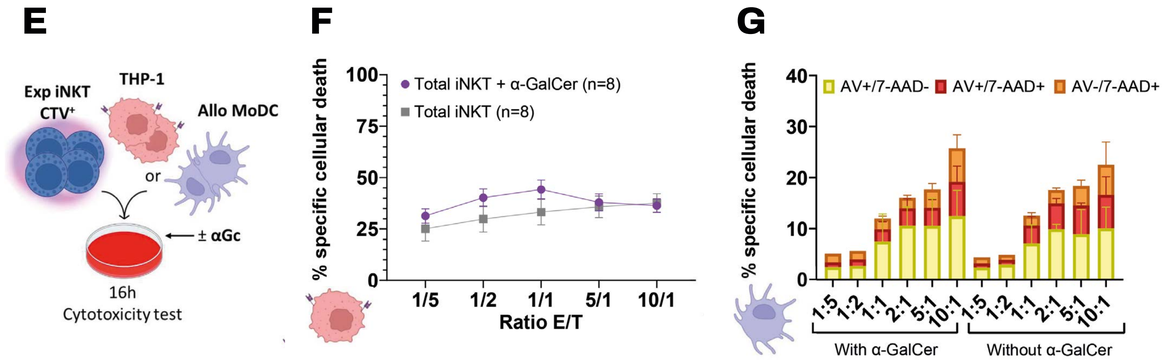


Figure 2. Expanded hu-iNKT conserve functional activity

(**A**) Schematic of the stimulation protocol used to assess cytokine production (IFN-γ, IL-4) shown in (**B**). (**B**) Expanded hu-iNKT cells produce IFN-γ and IL-4, with higher IFN-γ expression. (**C**) NKG2D is mainly expressed on CD4⁻ hu-iNKT cells. (**D**) Expanded hu-iNKT cells retain high intracellular levels of perforin and granzyme B. (**E**) Schematic of cytotoxicity assays on (**F**) THP-1 cells and (**G**) allogeneic MoDCs (n=3) with or without α-GalCer at different E:T ratios. (**F**) Expanded hu-iNKT cells show strong cytotoxicity against THP-1, regardless of α-GalCer. (**G**) They effectively induce apoptosis in allogeneic MoDCs, as shown by Annexin V/7-AAD staining (early: yellow, late: red, necrotic: orange). Data are mean ± SEM (n indicated in B, C, D).

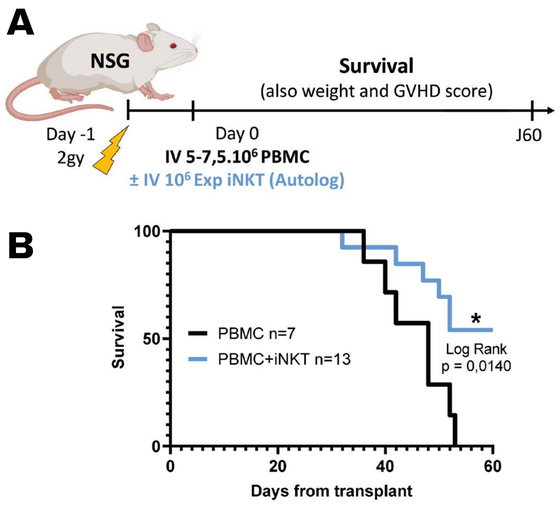


Figure 3. Expanded iNKT cells improve survival in a xeno-GVHD mouse model.

(**A**) Experimental design: NSG mice were irradiated (2 Gy) one day before receiving 5×10⁶ human PBMCs, with or without 10⁶ autologous expanded iNKT cells (Exp-iNKT). Survival, weight loss, and GVHD scores were monitored until Day 60. (**B**) Kaplan-Meier survival curve showing that co-transplantation with Exp-iNKT (blue, n=13) significantly improves survival compared to PBMCs alone (black, n=7; p = 0.014, Log-rank test).

CONCLUSION

- Simple, reproducible, and clinically GMP-ready protocol to **efficiently expand iNKT cells** from human blood.
- Expanded iNKT cells are **predominantly CD4⁻** and **highly functional**.
- **Cytotoxic activity** against leukemia cells, and allogeneic MoDCs.
- Expanded iNKT cells **control GVHD** mortality in a humanized xeno-GVHD model.

1st CiRT meeting 14-15 february 2025, Prague

Acknowledgments to the PRT-K grant (ExpiNKT 2017), the SATT- Sayens maturation grant, the UMS IBSLor Flow Cytometry facility, the «ACBS» animal facility (Collin M, Poussier S), and BioRender for the illustrations.