High-dose TIL product with improved phenotype and functionality

*Christina Friese, Christina Heeke, **Nikolaj Kirketerp-Møller**, Sandra Færch, Maria Juul Nielsen, Amalie Hey, Ulrik Cordes*

Background: Adoptive cell transfer (ACT) with autologous tumor-infiltrating lymphocytes (TILs) has proven to be one of the most successful immune therapies with overall response rates of around 50% in patients with metastatic melanoma including complete responses in up to 20% of the patients.

Current protocols combine a first expansion of TILs from tumor fragments or tumor digest with high-dose IL-2, followed by further expansion with a rapid-expansion-protocol (REP) using allogeneic feeder cells, αCD3 and IL-2. Following this protocol, TIL production takes 4-7 weeks, and many patients deteriorate before they can receive therapy. With success rates of TIL expansion ranging from 70-90%, a TIL product cannot be generated for every patient. Furthermore, clinical response to TIL therapy is lower in other solid tumors such as ovarian cancer, likely due to a lower number of expanded TILs and lower frequencies of tumor reactive CD8⁺ T cells.

Therefore, there is a clinical need for improvement of current TIL expansion protocols to make this therapy available to more cancer patients.

Methods: In this project, TILs were generated from both fresh and frozen tumor fragments of patients with various solid tumors. The TILs have been cultured under three different conditions using novel culture vessels and the standard 24 well plates. TIL yield, viability, composition, phenotype, reactivity and expansion time were compared to TILs expanded following the standard protocol.

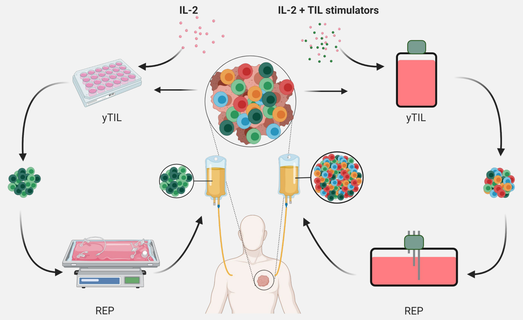

Fig. 1 Comparison of the standard and the improved TIL production process

Left: The standard TIL expansion protocol using 24-well plates, high-dose IL-2 stimulation and the REP in a Xuri bioreactor. This process takes 4-7 weeks. Right: Our improved process using G-Rex vessels, high-dose IL-2, and TIL stimulators decreased the production time to just 3 weeks while still producing a high-dose TIL product. *Illustration created with BioRender*

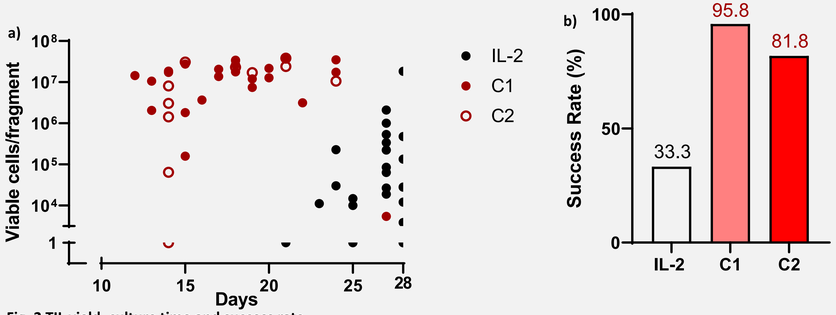

Fig. 2 TIL yield, culture time and success rate

a) The total amount of viable cells obtained after culturing 5 tumor fragments in 24-well plates or G-Rex 6-well plates without TIL stimulators (IL-2) or with TIL stimulators (C1 and C2). The cells were harvested when deemed appropriate by visual inspection or after a maximum of four weeks. The total amount of viable cells were counted on the day of harvest. Cultures with no countable viable cells were set to 1 to show on the log scale b) We observed a highly improved success rate when adding the TIL stimulators to the culture. Cultures were considered successful, if they reached 100,000 cells/fragment within the four weeks of culture.

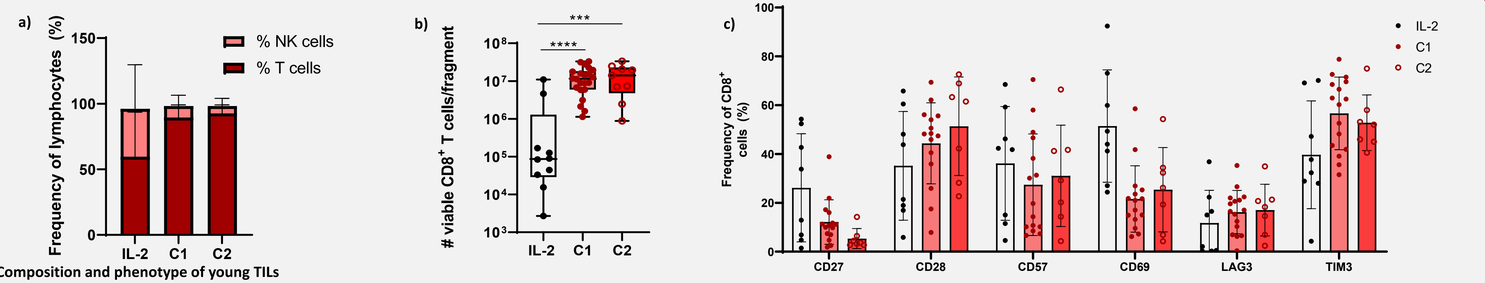

Fig. 3 Composition and phenotype of young TILs

a) Condition 1 (C1) and condition 2 (C2) generally yielded a higher frequency of CD3⁺ cells compared to the control (IL-2). b) In addition to more CD3⁺ cells in general, C1 and C2 favors the expansion of CD8⁺ T cells compared to the standard condition (IL-2), which results in significantly higher frequency (not shown) and higher total numbers of CD8⁺ cells. c) The T cells were also analyzed for activation and exhaustion markers by flow cytometry. We observed a tendency toward a lower expression of CD27 and CD69 and a higher expression of CD28 and TIM3 in C1 and C2 compared to the IL-2 condition.

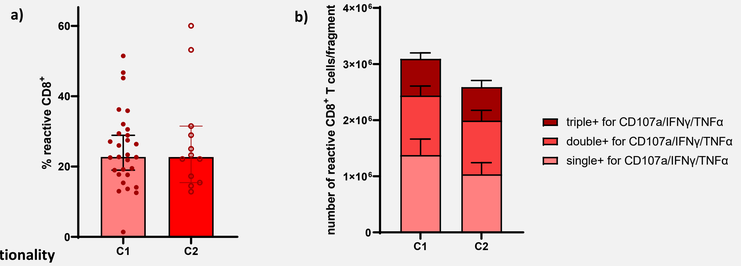

Fig. 4 CD8⁺ T-cell functionality

a) There was no significant difference between the functionality of the TILS produced in C1 or C2. The functionality of the T cells was measured by stimulating the cells with anti-CD3/CD28/CD137 beads and staining for CD107a, IFNγ and TNFα. b) Cells that did not stain were defined as not activated and the rest was divided into single, double, or triple positive for either of the three markers of cytotoxicity. It was not possible to stain the standard condition (IL-2) due to low yield of cells.

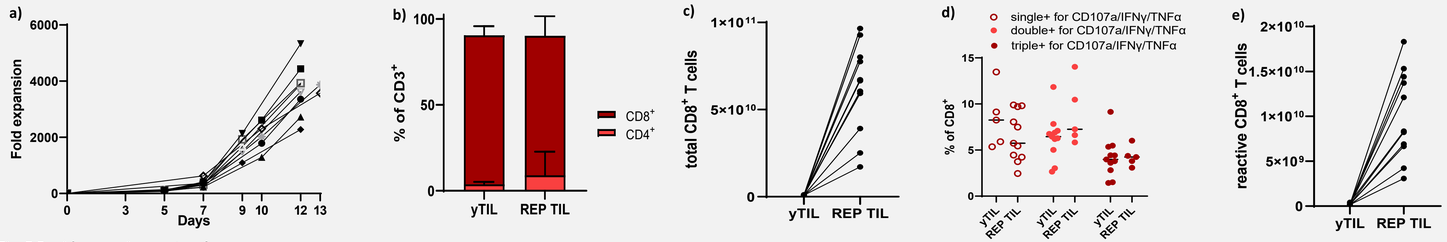

Fig. 5 Rapid expansion protocol

a) After the first expansion, the TILs were subjected to the rapid expansion protocol (REP) in G-RexM 6-well plates by culturing them with allogenic feeder cells, anti-CD3 and high-dose IL-2. Each line represents a patient sample counted on multiple days throughout the REP. The mean expansion at day 12 was 3695 fold. b) After the REP, the TILs were subjected to flow analysis. The CD4⁺/CD8⁺ ratio was largely maintained after the REP, and we observed a strong expansion of CD8⁺ cells (c). d) In addition, the reactivity of the cells (in %) was maintained after the REP, measured by stimulating the cells with anti-CD3/CD28/CD137 beads and staining for CD107a, IFNγ and TNFα. e) The maintained percentage of activated cells resulted in an increased total number of reactive CD8⁺ cells in the final product.

Conclusions: With this study, we show that by improving the initial culture conditions, we can shorten expansion time while simultaneously improving the characteristics of the TIL product with a high dose of functional CD8⁺ T cells with potential anti-tumor activity.