Lymphocyte heterogeneity assessment in the context of the clinical course of acute lymphoblastic leukemia during chimeric antigen receptor T cell (CAR-T) immunotherapy.

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INTRODUCTION

Variability in the CD19 CAR-T cell infusion product was previously proven, however there is no sufficient data about the impact on the efficacy and toxicity.

OBJECTIVES

This study reports an **in-depth immunophenotypic characterization of FDA-approved CD19-CAR T cells (tisagenlecleucel, Kymriah®, Novartis),** pre and post infusion, among pediatric patients with BCP-ALL.

METHODS

- Collected samples (Figure 1) were evaluated using **cytometry by time of flight (CyTOF)**
- Labeling was performed with **Maxpar Direct Immune Profiling Assay**
- **An in-house 169Tm-conjugated monoclonal anti-FMC63 scFv antibody** for CAR detection
- **39 populations** of white blood cells were identified using **Cytobank software** (34-marker panel)
- The **tSNE-CUDA algorithm** was implemented to reduce high-parameter data for analysis
- **Paired Exact Wilcoxon tests with FDR p value adjustment** were performed to compare cell subset proportions before and after infusion.

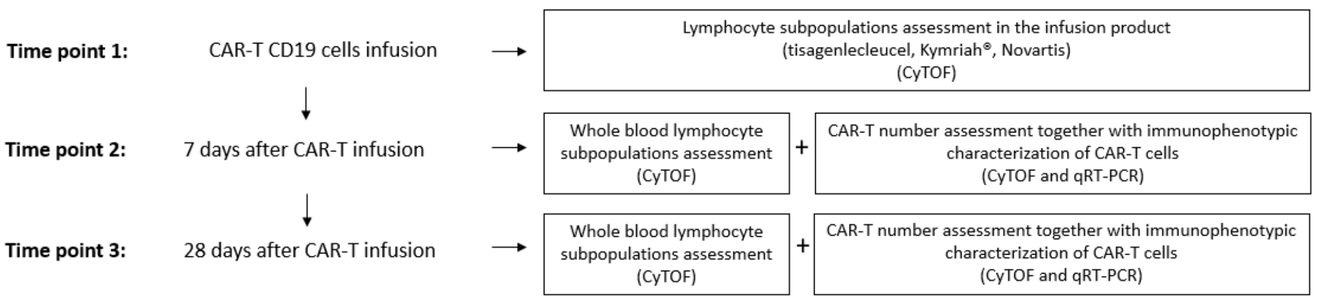

Figure 1. Study scheme.

RESULTS

- All **pediatric BCP-ALL** patients aged **2-17 years** who received tisagenlecleucel starting at September 2022 in Poland (**n=15**, (female to male 8/7)
- **11** patients developed **CRS** (grade ≦2), **4** patients suffered from **ICANS, 13** achieved **CR**, maintaining B-cell aplasia throughout the evaluation period
- CAR expression ranged from **6.85 to 38.23%** of all T cell subsets (mean 18.8%, SD=7.37, n=9), with 95-99% T cell purity in the pre-infusion tisagenlecleucel products (Figure 2)

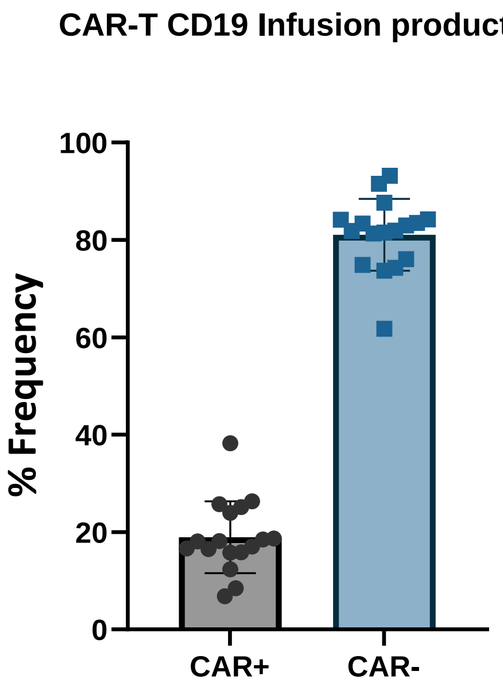

Figure 2. Percentage of CAR+ cells in CAR- cells infusion products.

RESULTS

- **Significant donor variability** among CAR+ and CAR– cells (Figure 3)
- pre-infusion products exhibited high proportions of **CAR+ T-regs** (range: 38-72%), but T-regs were neither detected at day 7 nor day 28 post infusion (day 7: p=0.001; day 28: p=0.011)
- Significant **decrease** in post-infusion blood samples,
  CAR+ Th2-like (day 7: p=0.011; day 28: p=0.015),
  CAR+ Th17-like (day 7: p=0.007),
  CAR+ CD4 total (day 7: p=0.001; day 28: p=0.012),
  CAR+ CD4 Central Memory (day 7: p=0.001; day 28: p=0.012),
  CAR+ CD4 Terminal Effector (day 7: p=0,001; day 28: p=0.02)
  CAR+ CD8 Terminal Effector cells (day 7: p=0,001; day28: p=0.011)
- Significant **increase** in the proportion of **CAR+ CD8 total** (p=0.02), **CAR+ CD8 Naïve** (p=0.011; day 28: p=0.011) and **CAR+ CD8 Central Memory** (p=0.013) between the infusion product and day 7 post infusion (Figure 4)

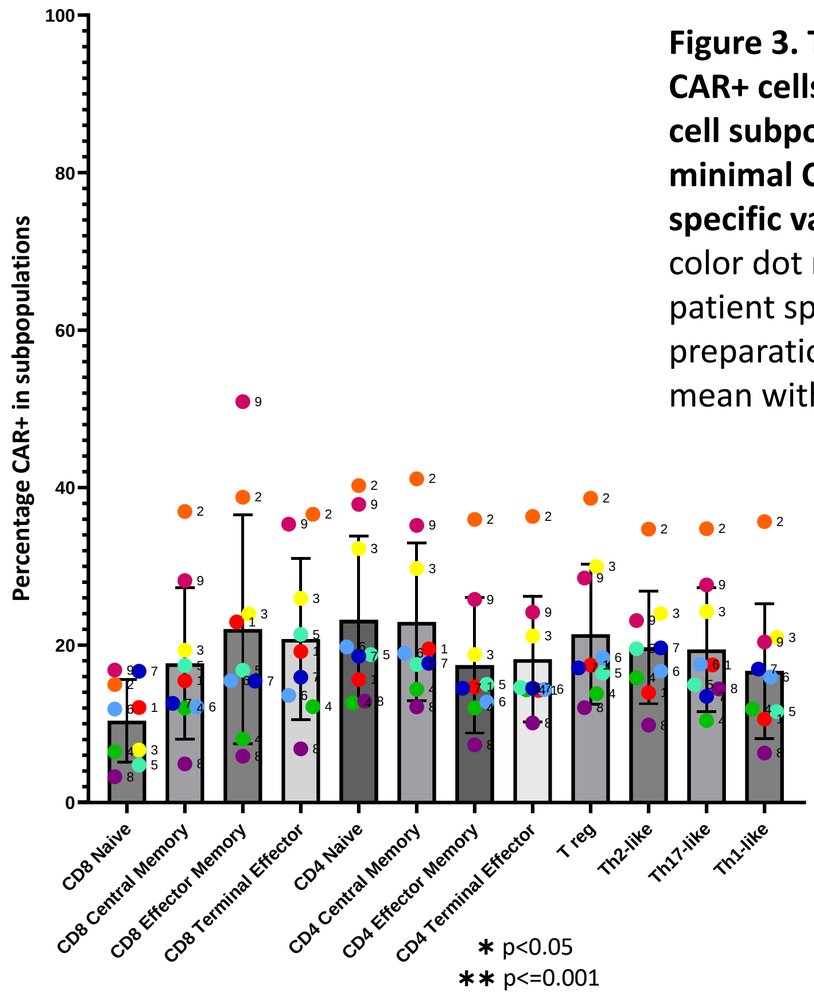

Figure 3. The frequency of CAR+ cells across distinct T cell subpopulations shows minimal CAR product-specific variation. Each color dot represents patient specific individual preparation. Bar shows mean with SD.

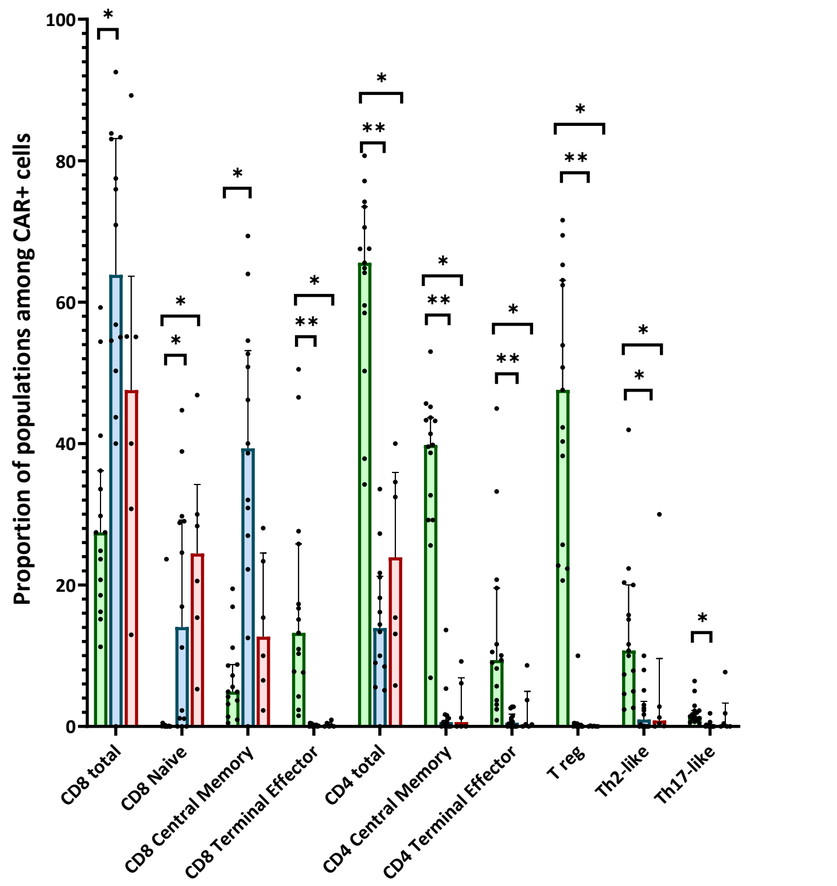

Figure 4. The proportion of the CAR+ T cell subpopulations significantly changed within four weeks after tisagenlecleucel intravenous infusion. Brackets indicate paired exact Wilcoxon tests that were statistically significant after FDR-adjustment for multiple testing (*, p < 0.05; **, p <= 0.001). Green, infusion product; blue, day 7 post infusion; red, day 28 post infusion.

CONCLUSIONS

- **Significant heterogeneity** of CAR+ T cell subpopulations **in infusion tisagenlecleucel products**
- **No detectable of CAR+ Treg cells**, a significant **decrease of CAR+ CD4 cells**, and **an increase of CAR+ CD8 cells** within one to four weeks from treatment.

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