SUPPLEMENTARY APPENDIX

Toxicity and efficacy of chimeric antigen receptor T-cell therapy in patients with diffuse large B-cell lymphoma above the age of 70 years compared to younger patients – a matched control multicenter cohort study

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Supplement Data

Referral and eligibility

All 3 CAR-T centers are large tertiary centers for both allogeneic/autologous hematopoietic cell transplantations and CAR-T therapy. All referred patients underwent full assessment based on institution eligibility criteria (**Supplement Table 1**). Once patients were deemed to be eligible for CAR-T, they were scheduled for lymphopheresis. Screening for eligibility included serology tests, review of comorbidities, and echocardiography.

Lymphopheresis

Lymphopheresis was performed using the Spectra-Optia Apheresis System (Terumo BCT) and the continuous mononuclear cell (CMNC) program (software version 11). Typical lymphopheresis setting included an inlet/anticoagulant ratio of 12:1. The collection flow rate was set at 1.0 mL/min. An acid citrate dextrose ("ACDA") was used as the anticoagulant. The inlet flow rate was between 50-100 mL/minute. The patient's blood volume was processed 2-4 times through an indwelling central or peripheral venous line. According to the manufacturer requests for tisagenlecleucel, target apheresis material specification was set according to 3 parameters: viable total nucleated cells ≥ $2x10^9$ cells, viable CD3+ cells ≥ $1x10^9$, and percentage of CD3+ cells ≥ 3% of total nucleated cells. Axicabtagene ciloleucel products were not requested to pass predefined criteria, but collection volume was requested to be between 12 and 15 liters. Product was either cryopreserved and then transferred (tisagenlecleucel) or transferred as a fresh product (axicabtagene ciloleucel).

Bridging therapy

During the manufacturing process and after completion of lymphophersis, majority of patients received bridging treatment, (mainly in the referring centers) with the aim to maximize disease control without substantial toxicities. Post bridging therapy PET-CT was performed as a routine staging.

Preparative regimen and supportive care

Once product was available for infusion, patients were admitted to the Bone Marrow Transplantation ward in designated rooms with high efficiency particulate air (HEPA) filters. Prior to infusion of CAR-T, patients were given cyclophosphamide (250-500 mg/m^2) and Fludarabine (25-30 mg/m^2) for 3 days (days -5 to -3). Prophylactic therapy was administered according to center policy, however majority received acyclovir from the start of the preparative regimen, and ciprofloxacin and fluconazole upon development of aplasia (defined as a neutrophil count below 500/microL). Red blood cell and platelet transfusions were administered in the case of hemoglobin (Hb) below 7 gr/dL and platelets below 10,000/dL, respectively. Premedication for product infusion included IV promethazine 6.25mg and PO paracetamol 1000 mg. Cells are thawed and infused according to manufacturer recommendations. GCSF was given from day 14 in cases of prolonged aplasia. Neutrophil recovery was defined as the first of three consecutive days of the absolute neutrophil count exceeded 500cells/microL.

Evaluation of pre-treatment T cell compartment and assessment of CAR-T product and persistency

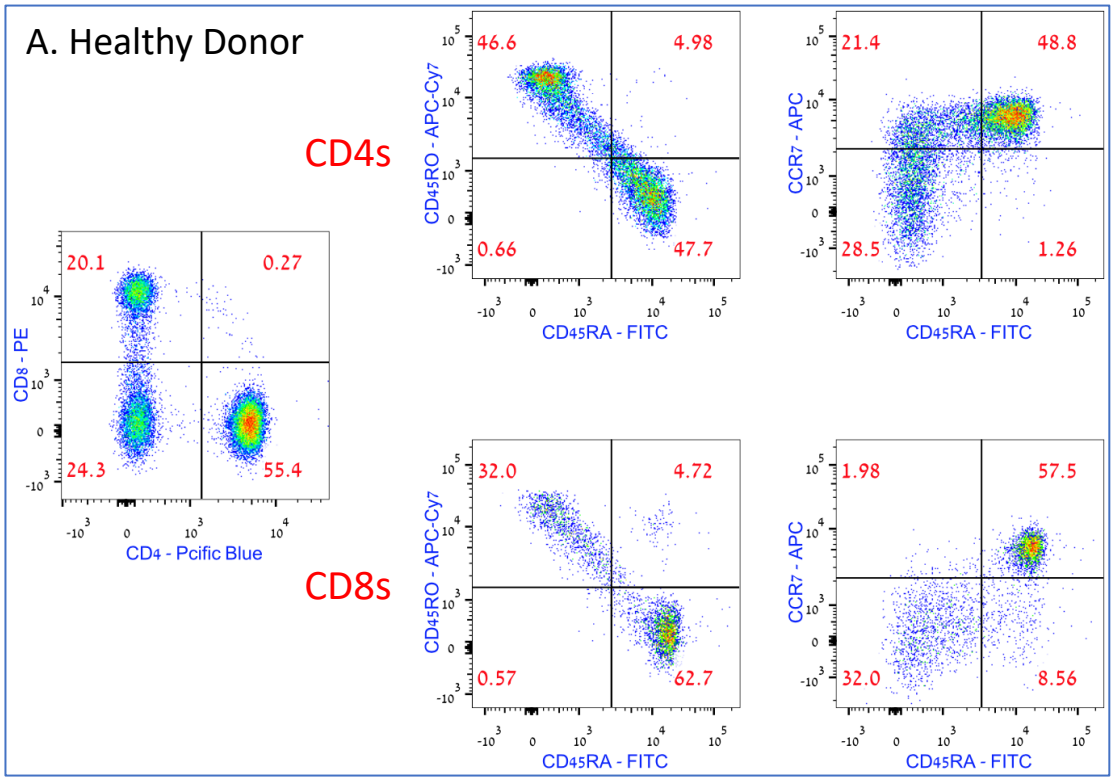
Evaluation of the T-cell compartment was performed with flow-cytometry-based T cell immunophenotyping on samples of both the apheresed cells and the post manufactured CAR-T products. For evaluation of only the apheresis T-cells, a control group was used with peripheral blood mononuclear cells samples (PMBCs) obtained from healthy adult donors. Since we do not have healthy donor CAR-T cells, we only compared CAR-T products of young vs. older patients. All cell samples were stained with the following conjugated-antibodies: CD4 PB (clone RPA-T4), CD8a PE (clone RPA-T8), CD45RA FITC (clone HI100), CD45RO APC/Cy7 (clone UCHL1), HLA-DR PE/Cy7 (clone L243), CCR7 AF®647 (clone G043H7) and anti-PD1 (Clone PD1.3.1.3 Miltenyi Biotec). All antibodies were purchased from BioLegend (San-Diego, CA). Cells were acquired using BD FACSCanto II flow cytometer and analyzed using FlowJo software (V10.0, TreeStar).

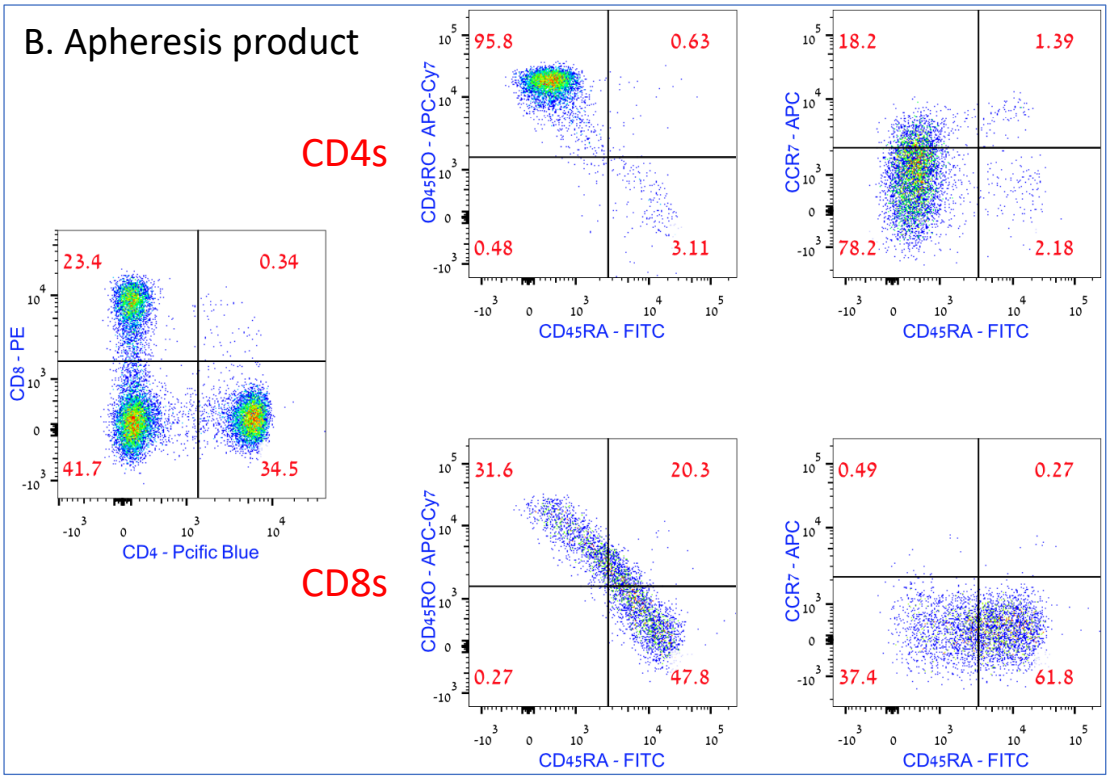
Persistency of CAR-T was evaluated on day 7 post infusion of CAR-T, using FITC-conjugated recombinant human CD19 protein (CD19-Ig, Ab246020, Abcam, Cambridge, UK). The recombinant FITC-conjugated CD19 could be identified by the anti-CD19 CAR-T and was used to track anti-CD19 CAR-T persistence in peripheral blood.

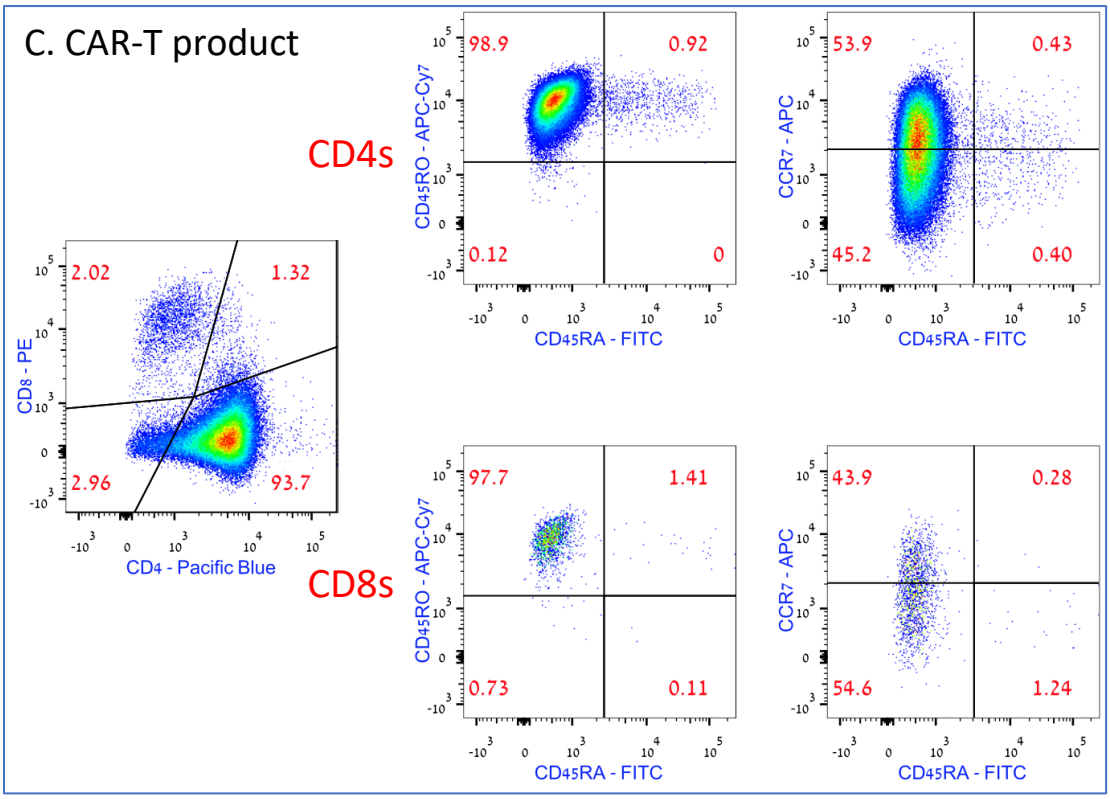
Supplemental Table 1 – Eligibility Criteria for chimeric antigen receptor T cell therapy

Domain	Definition
Age limit (DLBCL)	No limit
ECOG performance status	<4
History of malignancy	Not on current chemotherapy and no impending organ dysfunction
Prior allogeneic hematopoietic cell transplantation	Eligible
Prior anti CD19 therapy	Eligible
History of autoimmune disease	Eligible but individualized assessment required
Current systemic immune-suppressive therapy	Eligible but individualized assessment required
Existing/suspected infection	Active infection should be controlled prior to apheresis
History of central nervous involvement	Eligible
Cardiac ejection fraction	>30% - individualized assessment required in cases of lower than 30%
Caregiver	Mandatory for all patients

Supplemental Figure 1 -Representative flow cytometry T cell phenotype of A. Healthy donor, B. apheresis product, and C. CAR-T product






C. CAR-T product
CD4s
CD8s
2.02
1.32
2.96
93.7
CD8 - PE
CD4 - Pacific Blue
98.9
0.92
0.12
0
CD45RO - APC-Cy7
CD45RA - FITC
53.9
0.43
45.2
0.40
CCR7 - APC
CD45RA - FITC
97.7
1.41
0.73
0.11
CD45RO - APC-Cy7
CD45RA - FITC
43.9
0.28
54.6
1.24
CCR7 - APC
CD45RA - FITC