Excellent proliferation and persistence of allogeneic donor-derived 41-BB based CAR-T cells despite immunosuppression with cyclosporine A

The curative potential of allogeneic hematopoietic stem cell transplantation (allo-HSCT) is vastly based on a graft-*versus*-malignancy effect, which is thought to be primarily mediated by donor T cells.[1] Relapse of underlying malignancy remains a major challenge after allo-HSCT. Donor lymphocyte infusions (DLI) have demonstrated efficacy in the treatment of persistent and relapsed malignant disease after allo-HSCT.[2,3] Although escalating-dose strategies have resulted in reduced risks of severe graft-*versus*-host disease (GVHD) after DLI,[4,5] occurrence is still frequent and it remains a major concern. In addition, the good efficacy of DLI as demonstrated for chronic myeloid leukaemia[2] has been less evident in other malignancies, particularly acute leukemia, aggressive lymphoma and multiple myeloma.[2-5]

Clinical trials with autologous CD19-targeted CAR-T cells have shown very promising results and led to the recent approval of two CAR-T cell products for the treatment of acute lymphoblastic leukemia[6] and diffuse large B-cell lymphoma.[7,8] Allogeneic CAR-T-cell therapy bears the potential to combine graft-*versus*-malignancy effects and CAR-directed target killing, but comes with a considerable risk of GVHD. This holds particularly true, when the cells used for CAR-T-cell production are collected directly from the allogeneic donor. Immunosuppression to prevent GVHD may also prevent proliferation and efficacy of the CAR-T cells.

Here we present, for the first time, data on expansion and persistence of 4-1BB based CAR-T cells under immunosuppression with clinically relevant concentrations of cyclosporine.

A 64-year old female patient underwent allo-HSCT from an HLA-matched (10/10) unrelated donor at our centre for refractory follicular lymphoma grade IIIA. Disease manifestation included multiple enlarged lymph nodes in the supraclavicular, axillar, retroperitoneal and parailiac regions with a maximum diameter of 4.4 cm, maximum lactate dehydrogenase (LDH) of 577 U/L and in partial remission at time of conditioning with total-body irradiation (800 cGy), fludarabine (150 mg/m^2) and post-transplant cyclophosphamide. Two weeks after allo-HSCT, she experienced cutaneous acute GVHD stage 3, which was sensitive to systemic corticosteroid therapy. Steroid treatment was tapered off and stopped on day 55 after transplant. Two weeks later, (day 65 after allo-HSCT) she developed cervical lymphadenopathy. Computed tomography imaging confirmed the relapse of the lymphoma. At time of diagnosis of the relapse she had no evidence of GVHD and was under immunosuppression with cyclosporine.

Taking into consideration the early relapse despite GVHD, the low T-cell numbers in the peripheral blood of the patient at the time and the otherwise lack of alternative curative treatment options we considered the possibility of compassionate-use treatment with donor-derived allogeneic CAR-T cells after informed consent of the patient. Informed consent was also obtained for monitoring of CAR-T-cell persistence and phenotype as well as eventual publication and approval obtained from the local ethics committee (PV7091).

CD19-CAR-T cells were manufactured from leucocytes freshly collected from the HSCT allogeneic donor using the CliniMACS Prodigy® system and a lentiviral vector encoding the CD19-CAR containing a 4-1BB costimulatory domain. It took 14 days to manufacture and administer the CAR-T cells.

No treatment was administered between the relapse and lymphodepletion. The patient received lymphodepleting chemotherapy with cyclophosphamide (500 mg/m^2) and fludarabine (30 mg/m^2) on days 5 to 3 and 1x10^6/kg body weight (BW) CAR-expressing cells on day 0 (day 98

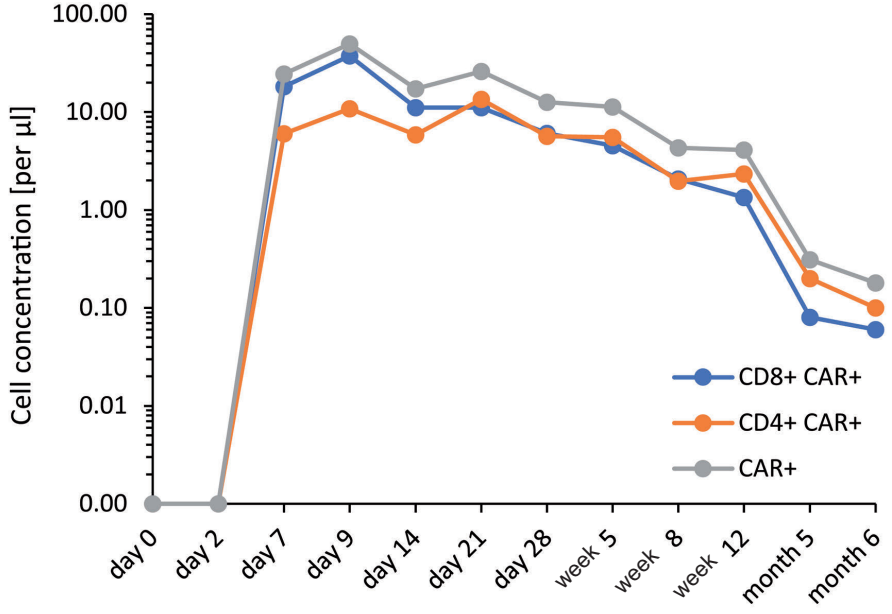

Figure 1. CAR-T cell quantification in peripheral blood samples was performed by flow cytometry using the biotinylated "CD19 CAR detection reagent" followed by streptavidin-PE (Miltenyi Biotec, Germany) according to manufacturer's recommendations. Depicted are the curves for total CD4$^+$, and CD8$^+$ as well as total CAR-T cells.

after allo-HSCT). In accordance with the transduction efficiency with the CAR vector of 23%, the cell product contained approximately 3.35x10^6/kg BW non-modified allogeneic T cells. Given the early time point relative to allo-HSCT, the history of GVHD and the risk of GVHD associated with the dose of donor T cells, we maintained her on GVHD prophylaxis with cyclosporine A at a mean cyclosporine A trough level of 170 µg/L (range 122-206 µg/L) until day +21 after CAR-T-cell infusion.

The patient developed intermittent fever from day +1 to day +14 and required oxygen support up to 4 L/min as from day +6 to +29 after CAR-T-cell infusion. Blood cultures were done at every fever episode, vital signs were monitored at least every 4 hours, CT scans of the lungs on days +2 and +14 and bronchoscopy was done on day +15. Though initially considered cytokine release syndrome (CRS), infections work-up revealed a bacterial infection of the port-catheter (blood culture on day +2) and pneumonia with the detection of respiratory syncytial virus (RSV) (in the sputum on day +2) and aspergillus DNA in bronchial fluid (day +15) as cause of fevers and oxygen requirement. The port catheter was explanted and antimicrobial medication administered according to our standard practice. No immune effector cell-associated neurotoxicity syndrome (IECANS) was observed. Haematopoietic recovery was expectedly slow despite the use of granulocyte colony-stimulating factor (G-CSF) as from day +21. Stable neutrophil counts >1,000/µL without further G-CSF support was attained on day +32, platelet count >50,000/µL on day +150 after CAR-T cell infusion.

A fluorodeoxyglucose positron-emission tomography (FDG-PET) scan on day +30 revealed partial response of the lymphoma manifestations seen in the computerized tomography (CT) scan, but also showed new bone lesions in both sacrum and femur. A follow-up FDG-PET scan on day 120 showed complete resolution of all lymphoma manifestations, with only a 2.6 cm (initial 4.6 cm) residual manifestation in the left sacrum.

With no evidence of active GVHD cyclosporine A was tapered off from day +21 and stopped on day +56 after CAR-T cell infusion. Three months later (five months after the CAR-T infusion) the patient developed moderate chronic GVHD of the skin and mouth, which was treated with systemic steroid. Unfortunately the patient died nine months after the CAR-T infusion from a severe bacterial infection with no signs of progression of the lymphoma.

Quantification of CAR-T-cell persistence was performed by flow cytometry (CD19 CAR detection reagent, Biotin, human; Miltenyi Biotec, Germany). Highest CAR-T-cell numbers were measured on day +9 (57 CAR-T cells/µL) with continuous persistence of CAR-T cells up to the last examination six months after infusion (Figure 1). While overall T-cell reconstitution was well evident (375.6 CD3$^+$/µL at month 5), B-cell aplasia was persistent at all time points (0 CD19+/µL at month 5).

Further analysis of CAR-T cell subtypes by flow cytometry revealed a decline in the fraction of effector and effector memory T cells and a relative predominance of central memory T cells amongst CD4$^+$ CAR-T cells as from day 14 after infusion, while effector memory T cells predominated in the CD8$^+$ fraction (Figure 2).

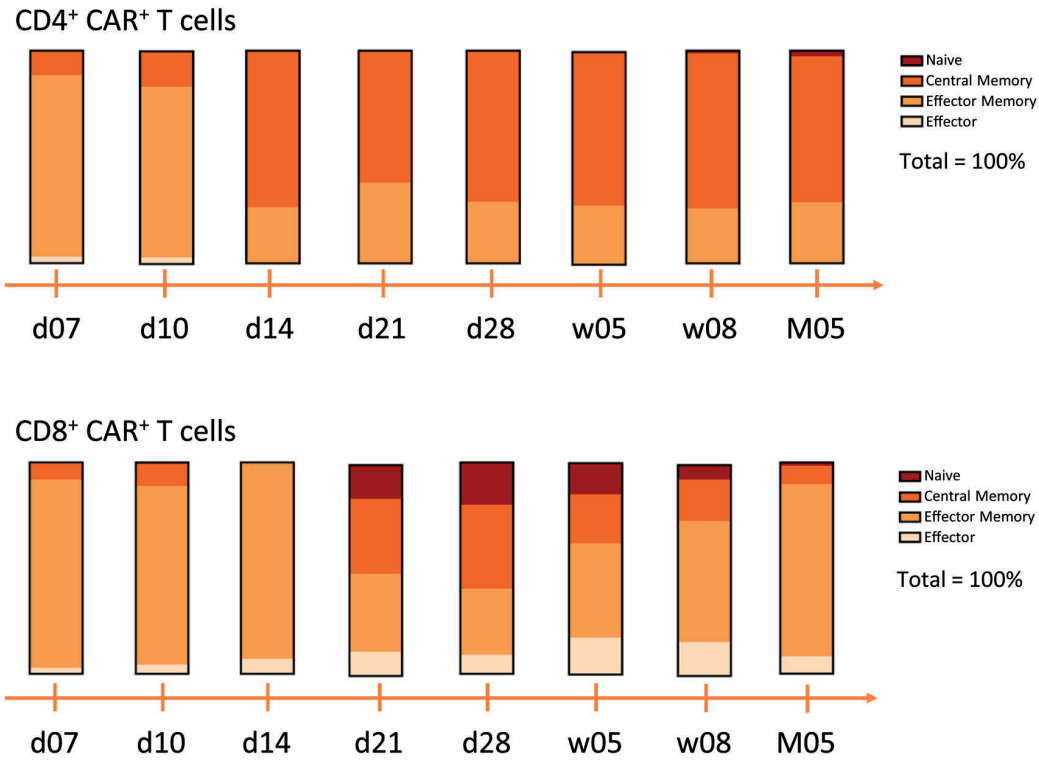

Figure 2. Analysis of CAR-T cell subtypes in peripheral blood samples was performed by flow cytometry after additional staining for CD62L and CD45RO. Depicted are the fractions of naïve (CD62L^{pos}, CD45RO^{neg}), central memory (CD62L^{pos}, CD45RO^{pos}), effector memory (CD62L^{neg}, CD45RO^{pos}) as well as effector (CD62L^{neg}, CD45RO^{neg}) T cells.

There have been several reports on the use of allogeneic donor-derived CAR-T cell with varying incidences of GVHD.[9] T cells can be collected from the recipient, if sufficient reconstitution of donor T cells has been reached, usually beyond six months after allo-HSCT. For patients with early relapse of malignant disease, patient derived CAR-T cell based strategies may therefore not be feasible. In such cases, CAR-T cells derived directly from the donor are an alternative, and several strategies to reduce the risk of GVHD while maintaining efficacy of the CAR-T cells are under investigation: use of virus-specific T cells as well as other more complex approaches such as T-cell receptor (TCR)-less CAR-T cells, use of regulatory T cells or lymphoid progenitors.[9] The latter strategies require extensive multiple-step manipulation of T cells, which may make the production process longer and more expensive.

Animal studies indicate that the risk of GVHD after CAR-T infusion from an allogeneic donor may also depend on the costimulatory domain used. Using CD28-based second-generation CAR-T cells, cumulative CAR and TCR signalling in alloreactive cells led to cell death or accelerated exhaustion of these cells, while non-alloreactive cells maintained their CAR driven antitumor potential.[10] In the same report, first generation and 4-1BB-based CAR-T cells increased the incidence of GVHD. In the so far largest patient cohort reported, which included 27 children and young adults who received patient-derived donor 4-1BB costimulated CD19$^+$ CAR-T cells for treatment of relapsed/ refractory B lineage acute lymphoblastic leukemia, only one patient (3.7%) developed GVHD.[11] A similarly low incidence of GVHD (about 10%) was reported, when donor-derived CAR-T cells with a CD28 costimulatory domain were used for treatment of patients with relapsed/refractory B-cell malignancies.[12,13] Interestingly, in the only report on donor-derived CAR-T cells with a 4-1BB costimulatory domain, both patients developed acute GVHD.[14] While costimulation of T cells *via* CD28 increases the IC50 of cyclosporine A, 4-1BB costimulation does not affect sensitivity of cells to cyclosporine A.[15] 4-1BB-based allogeneic donor derived CAR-T cells therefore bear the potential to cause GVHD and are sensitive to immunosuppression with cyclosporine A. Our findings indicate, however, that these cells still proliferate extensively under immunosuppression with therapeutic levels of cyclosporine A. Based on experience after allo-HSCT, where T-cell proliferation and reconstitution is observed under cytostatic immunosuppression with calcineurin inhibitors, mTOR-inhibitors or mycophenolic acid, we believe or observation may not be restricted to cyclosporine. The use of high doses of lymphocytotoxic agents such as corticosteroids, may on the other hand kill the CAR-T cells and prevent persistence.

In conclusion, we for the first time, present data on proliferation and persistence of allogeneic donor-derived 4-1BB based CAR-T cells under therapeutic levels of cyclosporine A. This indicates that for patients with early relapse after allo-HSCT with ongoing immunosuppression donor CAR-T cell therapy may be a feasible treatment option.

Francis Ayuk, Boris Fehse, Dietlinde Janson, Carolina Berger, Kristoffer Riecken and Nicolaus Kröger

Department of Stem Cell Transplantation with Research Department Cell and Gene Therapy, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Correspondence: FRANCIS AYUK - ayuketang@uke.de

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