Treatment of a patient with severe systemic sclerosis (SSc) using CD19-targeted CAR T cells

Several lines of evidence suggest a role of B cells in severe systemic sclerosis (SSc) pathophysiology: elevated levels of B-cell stimulating factors, disturbed B-cell homeostasis with expansion of naïve and decrease of memory B cells,[1] and anti-fibrotic effects of B-cell depletion in murine fibrosis models. First randomised controlled trials showed promising efficacy of the CD20-targeting antibody rituximab (RTX)[2 3]; however, therapeutic efficacy of RTX in SSc remains controversial: while one trial demonstrated significant skin improvement,[2] RTX slowed forced vital capacity (FVC) decline in another study but with similar effects compared to the cyclophosphamide control arm.[3] As in cases of severe lupus erythematosus (SLE), we speculated that CD20+ B-cell depletion may not be sufficient as B-cell precursors, which are particularly expanded in SSc, and plasmablasts, which may be responsible for autoantibody production, are not targeted via CD20 but via CD19. Of note, autologous stem-cell transplantation overcomes these limitations and has shown remarkable treatment efficacy in severe SSc.[4] However, transplant-related mortality is high. Hence, a more tolerable treatment with deep and broad CD19+ B-cell depletion may be more

effective. Recently, CD19-chimeric antigen receptor (CAR) T cells showed remarkable effects in refractory SLE[5] and first evidence of efficacy in a patient with antisynthetase syndrome,[6] suggesting the principle feasibility to intercept B cell-driven autoimmune diseases via CD19-CAR T cells.

Here, we report for the first time the treatment of a patient with severe, treatment refractory SSc with CD19-CAR T cells. A 60-year-old man presented with diffuse cutaneous SSc with first non-Raynaud's disease manifestation (skin, lung and heart fibrosis) 22 months before baseline and onset of Raynaud's phenomenon 28 months before baseline. At baseline, the patient presented with diffuse myocardial fibrosis (cardiac MRI), lung fibrosis (high-resolution computed tomography), pulmonary hypertension (class I with combined precapillary and postcapillary pulmonary hypertension based on the results of right heart catheterisation: mean pulmorary arterial pressure of 33 mm Hg (>20), pulmonary arterial wedge pressure of 18 mm Hg (>14) and pulmonary vascular resistance of 3 Wood units (>2)), Raynaud's phenomenon and carpal arthritis. He had positive anti-nuclear antibody titres and anti-RNA polymerase III autoantibodies (subunit RP11). Previously failed immunosuppressants included methotrexate (15 mg/week, treatment duration 3 months) and mycophenolate (dose 2 g/day for 23 months). Cyclophosphamide was not used due to inefficacy for arthritis and concerns regarding cardiac involvement. Immunosuppression was tapered before lymphodepletion and stopped 4 weeks before CAR T-cell infusion. CAR T cells were produced from autologous T cells obtained by leucapheresis. T cells were transduced by a lentiviral vector (Miltenyi) and expanded through the automated system (ClinicMACS Prodigy).[5] After dose-reduced lymphodepletion (50%) due to renal impairment with fludarabine (12.5 mg/m^2; days −5, −4 and −3) and cyclophosphamide (500 mg/m^2, day −3), 1×106 CAR T cells/kg were administered. CAR T cells rapidly expanded from day 3 (total: 0.3 cells/μL, 0.1% CARs of CD3+ T cells) until day 9 (1275/μL; 66,35% CARs of CD3+ T cells; figure 1A,B). CAR T cells then rapidly decreased but were still detectable (1%) on day 119 after infusion. Full B-cell depletion was reached on day 7 (figure 1B).

CAR T-cell therapy was well tolerated. Following CAR T-cell infusion, the patient had mild fever for less than 24 hours (Cytokine Release Syndrome (CRS) grade 1) and no signs of immune effector cell-associated-neurotoxicity syndrome. No anti-interleukin-6 receptor antibody treatment was given as there was no higher-grade CRS. Immune cells reconstituted quickly: CD4+ T cells were above 200/μL on day 37 and cells reoccurred on day 77. IgG levels remained above 700 mg/dL in the following 6 months, while ANA reactivity (titre 1:320 at baseline, speckled pattern) was abrogated, and RP11 autoantibodies were no longer detectable (figure 1C) on follow-up of 3 and 6 months. Seroconversion was paralleled by reduced molecular fibroblast activation particularly in the myocardium by 32.6% as determined by ^{68}Ga-FAPI-04-positron emission tomography–CT (septobasal maximal standard uptake value prior to CAR T cell therapy: 8.6, on follow-up: 5.8)[7] (figure 1D). Pulmonary fibrosis remained stable as assessed by CT on follow-up of 3 months and pulmonary function tests (figure 1H) on follow-up of 3 and 6 months. Echocardiography was performed at baseline and 6 months after CAR T-cell therapy as part of the non-invasive PAH screening: left ventricular ejection fraction remained stable, while signs of right ventricular strain showed a tendency of improvement (figure 1I). Carpal arthritis improved 3 months after CAR T-cell therapy (figure 1E). Consistently, tender joint counts improved from n=22 at baseline to n=3. Tendon friction rubs were no longer detectable on follow-up of 3 months as reflected in the European Scleroderma Trials and Research Group (EUSTAR)- activity index (figure 1F) at three and six months follow-up. Skin fibrosis showed a tendency of improvement as analysed by two independent assessors (figure 1G) during three and six months follow-up. Moreover, the patients subjectively reported less frequent and less severe attacks of Raynauds' phenomenon.

These data provide first evidence that CD19-targeting CAR T-cell treatment might be effective in severe SSc. The fast improvement of heart, joint and skin manifestations paralleled by seroconversion supports a central role of B cell-mediated autoimmunity in SSc. It cannot be completely ruled out that conditioning therapy contributed to the short-term effects of CAR T-cell therapy in this patient, and further observation of the drug-free course of SSc is warranted. Level of immunosuppression is limited with the current conditioning therapy as low doses are used compared with standard SSc treatment regimens. Thus, future controlled studies, if not done in life-threatening disease, may include an arm with conditioning therapy only in order to tease out the effect of conditioning therapy. Moreover, SSc patients with other known risk factors for a poor prognosis, such as positivity for anti-Scl antibodies, will need to be studied.

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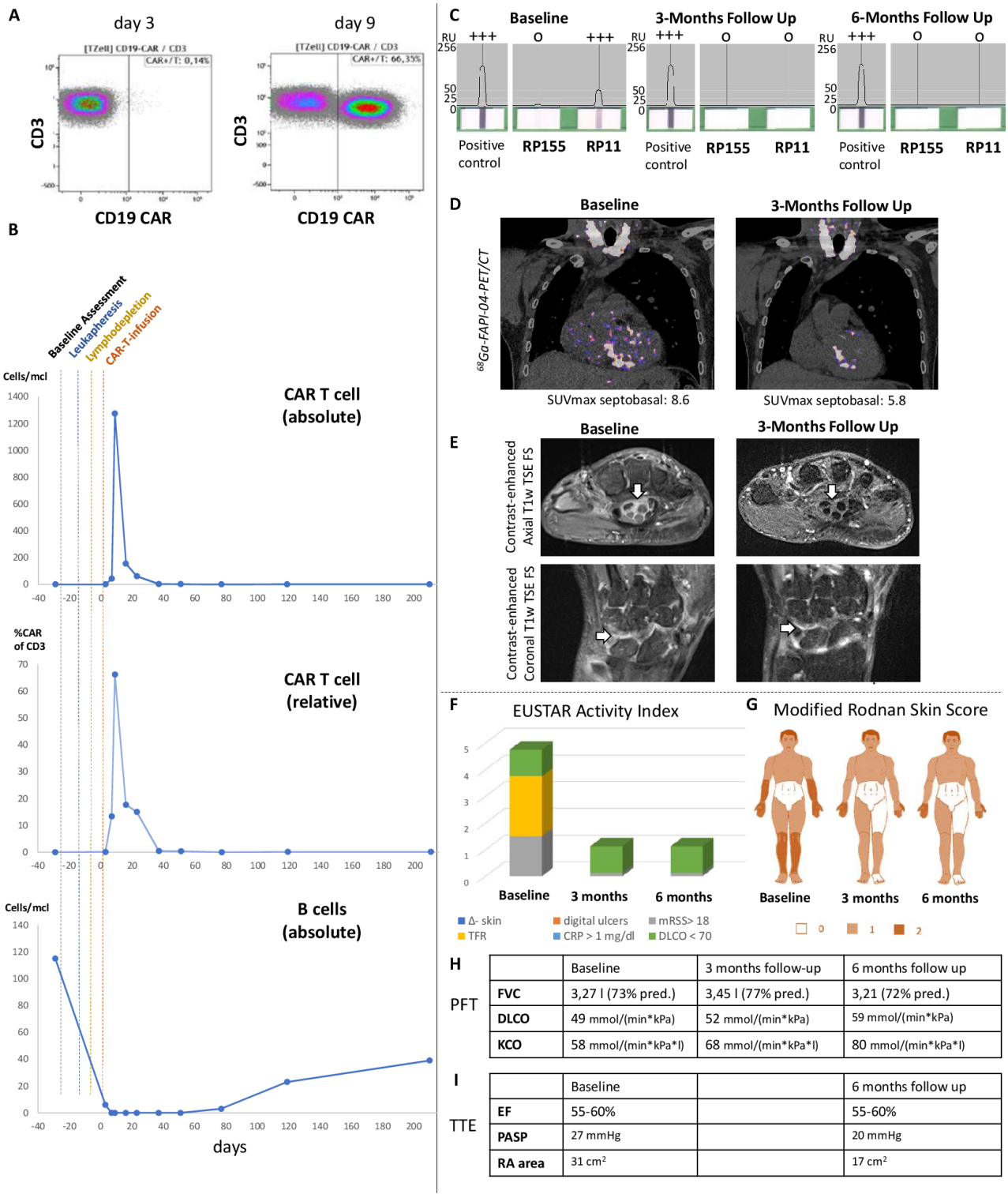
Competing interests None declared.



H

PFT

	Baseline	3 months follow-up	6 months follow up
FVC	3,27 l (73% pred.)	3,45 l (77% pred.)	3,21 (72% pred.)
DLCO	49 mmol/(min*kPa)	52 mmol/(min*kPa)	59 mmol/(min*kPa)
KCO	58 mmol/(min*kPa*l)	68 mmol/(min*kPa*l)	80 mmol/(min*kPa*l)

I

TTE

	Baseline		6 months follow up
EF	55-60%		55-60%
PASP	27 mmHg		20 mmHg
RA area	31 cm^2		17 cm^2

Figure 1 Patient with severe diffuse systemic sclerosis treated with CD19-CAR T cells. (A) Dot plot showing expression of CD3 (y-axis) and CD19-CAR (x-axis) quantifying the circulating CD19-CAR T cells by FACS on days 3 and 9 after infusion. (B) Time course of absolute and relative numbers of circulating CD19-CAR T cells and CD19+ B cells; d0=01.080.2022. (C) Densitometric analysis of autoantibodies against RNAPIII, subunit RP11 at baseline (1 month prior to CAR T-cell infusion) and 3 months after CAR T-cell therapy. (D) PET showing resolution of fibroblast activation protein inhibitor (^{68}Ga-FAPI-04) tracer accumulation in the heart at baseline and 3 months after CAR T-cell therapy. (E) Axial and coronal sections of T1-weighted contrast-enhanced MRI of the hands at baseline and 3 months after CAR T-cell therapy. (F) EUSTAR activity index, (G) mRSS and (H) lung function parameters at baseline and 3 months after CAR T-cell therapy. mRSS changes (G) were visualised using a modified version of the scheme described in Khanna D, Furst de, *et al*. J scleroderma relat Disord.2017 Jan-Apr;2 (1):11–18.doi:10.5301/jsrd.5000231.[8] CAR, chimeric antigen receptor; CRP, C reactive protein; DLCO, diffusing capacity or transfer factor of the lung for carbon monoxide; EF, ejection fraction; FACS, fluorescence-activated cell scanner; KCO, carbon monoxide transfer coefficient; mRSS, modified Rodnan Skin Score; PASP, pulmonary artery systolic pressure; PET, positron emission tomography; PFT, pulmonary function tests; RA, right atrium; SUV, standard uptake value; TFR, tendon friction rubs; TTE, transthoracic echocardiography.

Patient and public involvement Patients and/or the public were not involved in the design, conduct, reporting or dissemination plans of this research.

Patient consent for publication Consent obtained directly from patient(s).

Ethics approval This study involves human participants. CAR T-cell treatment was performed as named patient use. The collection and analysis of data is covered by the ethics vote of the local Ethics Committee of the University of Erlangen (334_18 B). Participants gave informed consent to participate in the study before taking part.

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