American Journal of Transplantation 26 (2026) 340–348

Contents lists available at ScienceDirect

American Journal of Transplantation

journal homepage: www.amjtransplant.org

Brief Communication

Sustained allogeneic kidney graft operational tolerance despite discontinued conventional immunosuppression after CD19-CAR-T cell therapy for relapsed/refractory posttransplant lymphoproliferative disorder

Helena Hansmann [1,†], Chiara Henkel [2,†], Matthias A. Fante [2,†], Dennis C. Harrer [2], Stefanie Heidemanns [3], Michael Oellerich [4], Julia Beck [5], Ekkehard Schütz [5,6], Verena Biswenger [2], Markus Perl [2], Barbara Zartner [2], Matthias Edinger [2,7], Daniel Wolff [2], Petra Hoffmann [2,7], Wolfgang Herr [2], Bernhard Banas [1], Daniel Zecher [1,‡], Leo Hansmann [2,*,‡]

[1] *Department of Nephrology, Universitätsklinikum Regensburg, Regensburg, Germany*
[2] *Department of Internal Medicine III, Universitätsklinikum Regensburg, Regensburg, Germany*
[3] *Department of Nuclear Medicine, Universitätsklinikum Regensburg, Regensburg, Germany*
[4] *Department of Clinical Pharmacology, University Medical Center Göttingen, Göttingen, Germany*
[5] *Chronix Biomedical GmbH, Göttingen, Germany*
[6] *Insight Molecular Diagnostics, Inc, Nashville, Tennessee, USA*
[7] *Leibniz Institute for Immunotherapy, Regensburg, Germany*

ARTICLE INFO

Keywords:
kidney transplantation
CAR-T cell therapy
tolerance

ABSTRACT

Management of immunosuppression after solid organ transplantation in context of chimeric antigen receptor T cell therapy (CART) is challenging. Although required to prevent graft rejection, systemic immunosuppression can interfere with biological functions of apheresis products and adoptively transferred T cells. We treated a 33-year-old kidney transplant recipient who developed relapsed/refractory posttransplant lymphoproliferative disorder with CD19-directed CART as a fourth-line therapy. Immunosuppression was discontinued before leukapheresis for CART and not reinitiated ever since. Although the posttransplant lymphoproliferative disorder remained in complete remission, we did not

Abbreviations: Axi-Cel, axicabtagene-ciloleucel; CART, chimeric antigen receptor T cell therapy; CD19-CART, CD19-directed chimeric antigen receptor T cell therapy; dd-cfDNA, donor-derived cell-free DNA; ddPCR, droplet digital polymerase chain reaction; CERI, cytomegalovirus, Epstein-Barr virus, respiratory syncytial virus, influenza A; CMV, cytomegalovirus; EBV, Epstein-Barr virus; HLA, human leukocyte antigen; MNC, mononuclear cell; PCR, polymerase chain reaction; PTLD, posttransplant lymphoproliferative disorder; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone; TTV, torque teno virus.

* Corresponding author. Department of Internal Medicine III, Universitätsklinikum Regensburg, Regensburg, Germany.
E-mail address: leo.hansmann@ukr.de (L. Hansmann).

† These authors contributed equally: Helena Hansmann, Chiara Henkel, and Matthias A. Fante.
‡ These authors contributed equally: Daniel Zecher and Leo Hansmann.

https://doi.org/10.1016/j.ajt.2025.09.009
Received 14 July 2025; Received in revised form 30 August 2025; Accepted 7 September 2025
Available online 17 September 2025

observe any signs of graft rejection (clinically and by determination of donor-derived cell-free DNA) until last follow-up at 23 months after CART. Phenotyping of peripheral blood immune cell subsets showed stable recovery of T and B cell compartments with dominant naïve differentiation. Immune responses against foreign antigens were shown by T cell cytokine production after stimulation with virus-derived peptide pools and absence of torque teno virus DNA. The lack of human leukocyte antigen antibodies and absence of T cell proliferation in a mixed leukocyte reaction with peripheral blood cells of the kidney donor confirmed tolerance against donor antigens. We envision CD19-directed CART as a therapeutic option to prevent organ rejection without conventional long-term immunosuppression in selected patients.

1. Introduction

Current strategies for allogeneic kidney transplantation require life-long immunosuppression, which affects quality of life and increases the risk of infections, myelosuppression, and secondary malignancies.[1] Immunosuppression-associated replication of persistent viruses such as Epstein-Barr virus (EBV) can drive the development of life-threatening posttransplant lymphoproliferative disorders (PTLD) underlining the unmet clinical need for the induction of operational tolerance without conventional immunosuppression after solid organ transplantation. We present a case of long-term discontinuation of conventional immunosuppression without kidney graft rejection after CD19-directed chimeric antigen receptor T cell therapy (CD19-CART) for PTLD. Detailed peripheral blood immune phenotyping and functional T cell assays complement our clinical observations and provide immunologic context for the mechanisms potentially underlying absence of graft rejection.

2. Materials and methods

2.1. Participants, cell isolation, and magnetic enrichment

The study was approved by the local ethics committee at University of Regensburg, Germany (Ethikkommission der Universität Regensburg), and conducted in accordance with the Declaration of Helsinki. All participants gave written informed consent.

Mononuclear cells (MNCs) were isolated from peripheral blood or leftover material of leukapheresis products by density gradient centrifugation using Ficoll-Paque PLUS (Merck) according to the manufacturer's instructions. If indicated, cell populations were magnetically separated using CD2 MicroBeads, human (Miltenyi Biotec) according to the manufacturer's instructions. Purity of the separation was confirmed by CD3 staining and flow cytometry (flow cytometry reagents are tabulated in Supplementary Table 1).

2.2. In vitro stimulation and intracellular staining

Approximately 1×10^6 MNC/well were cultured in 96-well plates at a final volume of 150 μL medium consisting of RPMI1640 (PAN Biotech) supplemented with 2 mM L-glutamine (Thermo Fisher Scientific), 100 U/mL penicillin, 100 mg/mL streptomycin (Thermo Fisher Scientific), and 10% fetal bovine serum (Capricorn Scientific GmbH). For peptide stimulation, we added CERI-MHC class I control peptide pool (cytomegalovirus [CMV]; EBV, respiratory syncytial virus, influenza A peptide pool; CTL-CERI-300; CTL Europe GmbH) at a final concentration of 1 μg/mL, or phorbol 12-myristate 13-acetate/ionomycin (eBioscience Cell Stimulation Cocktail [500×]; Thermo Fisher Scientific) according to the manufacturer's instructions (positive control), or cells were left unstimulated (negative control). After 2 hours, GolgiPlug (BD Bioscience) was added according to the manufacturer's instructions, and cells were incubated overnight.

Surface staining with multicolor panels (Supplementary Table 1) was done according to the manufacturer's instructions. For fixation, permeabilization and intracellular staining, we used the Intracellular Fixation and Permeabilization Buffer Set (Thermo Fisher Scientific) according to the manufacturer's instructions. Intranuclear FOXP3 staining was done using the Foxp3/Transcription Factor Staining Buffer Set (Thermo Fisher Scientific) according to the manufacturer's instructions. All flow cytometry data were recorded on LSRFortessa and FACSymphony instruments (BD Biosciences).

2.3. Mixed leukocyte reaction

CD2-enriched MNC isolated from the patient were labeled with carboxyfluorescein at a final concentration of 2 μM in phosphate-buffered saline (Thermo Fisher Scientific) for 4 minutes at room temperature in the dark. CD2-depleted cells were irradiated with 30 Gy (IBL 437C; Schering CIS Bio International) and subsequently labeled with CellTrace Violet at a final concentration of 2 mM for 20 minutes at 37 °C, 5% CO_2.

Approximately 1×10^5/well CD2-enriched cells were cocultured with 1×10^5/well irradiated, CD2-depleted cells at a final volume of 200 μL medium (as described earlier) in 96-well U-bottom plates for 7 days. For analysis, cells were stained with a surface marker panel (Supplementary Table 1) according to the manufacturer's instructions and measured on a FACSymphony instrument.

2.4. Determination of CAR-T cell frequencies

MNCs were stained with a PE-conjugated anti-FMC63 antibody (ACROBiosystems) in conjunction with a BV421-labeled CD3 antibody (BioLegend) to determine frequencies of CD19-CAR$^+$ T cells. The viability dye eFluor 780 (ThermoFisher) was used for live/dead cell discrimination. Gating was based on fluorescence-minus-one controls. Data were recorded on a FACSLyric (BD Biosciences) instrument.

2.5. Determination of donor-derived cell-free DNA

Blood samples for cell-free DNA analyses were collected in Cell-Free DNA BCT Tubes (Streck) and handled according to the manufacturer's instructions. Kidney donor–derived cell-free DNA (dd-cfDNA) was quantified by digital polymerase chain reaction (PCR) with hybridization probes following chaotropic extraction of cfDNA from plasma.[2,3] For the first 2 collection time points, dd-cfDNA quantification was performed using a nested assay targeting 4 informative loci on the 2 channel QX200 droplet digital polymerase chain reaction (ddPCR) system (Bio-Rad).[2] Samples from collection time points 3 and 4 were analyzed using the improved version 2 of the assay (GraftAssure), incorporating 11 informative loci in a nested multiplex ddPCR format using the 6-channel QX600 system (Bio-Rad). The expanded set of informative loci increases the analytical sensitivity of the assay version 2 and results in an improved lower limit of quantification (0.05% and 3 copies/mL) for dd-cfDNA determination. The decision limits were determined as previously described.[4]

2.6. Determination of other routine laboratory parameters

All other laboratory parameters were determined by certified routine clinical diagnostics.

2.7. Statistics and data visualization

Statistics and data visualization were done using R software,[5] version 4.5.0 (R Foundation for Statistical Computing). Applied tests are stated in the respective figure descriptions.

3. Results

3.1. Sustained remission of relapsed/refractory PTLD and hematologic recovery after CD19-CART

A 33-year-old woman (Table 1) with perinuclear antineutrophil cytoplasmic antibody–associated vasculitis and end-stage kidney disease received an ABO-compatible living donor kidney transplantation from her human leukocyte antigen (HLA)-haploidentical father in August 2011. In January 2021, the patient developed abdominal pain that was attributed to preexisting Crohn's disease. However, positron emission tomography/computed tomography showed a conglomerate tumor in the lower abdomen (Fig. 1 and Supplementary Fig. S1), and routine workup revealed stage IV EBV-positive polymorphic PTLD with plasmocytic differentiation. First-line treatment consisted of reduction of immunosuppression (tacrolimus 4-6 ng/mL target level; prednisolone 5 mg/d, discontinuation of azathioprine) and weekly intravenous administration of rituximab (375 mg/m^2) (Fig. 1).

Table 1
Patient characteristics.

Characteristic	Value
Age (y) at time of transplantation	19
Gender	Female
Race	White
Underlying kidney disease	p-ANCA–associated vasculitis
Body mass index (kg/m^2), August 2023	18.9
Hypertension	Yes (medication: candesartan and metoprolol)
Serum cholesterol (mg/dL), August 2023	198
Serum triglycerides (mg/dL), September 2023	233
Smoking	Never
Pregnancies	None
Previous transplantation	No
Donor age (y) at time of transplantation	51
Donor HLA typing	A*01, *32; B*40, *44; DRB1*04, *11; DQB1*03
Recipient HLA typing	A*01, *03; B*40, *07; DRB1*04, *15; DQB1*03, *06
HLA mismatch	Haploidentical
Preformed anti-HLA donor-specific antibodies	None

HLA, human leukocyte antigen; p-ANCA, perinuclear antineutrophil cytoplasmic antibody.

After 3 cycles of rituximab, the patient presented with progressive disease. The abdominal conglomerate tumor was resected, and histology, in contrast to the initial diagnosis, revealed EBV-negative monomorphic PTLD of diffuse large B cell lymphoma type (germinal center B cell type).

Second-line treatment with 4 courses of R-CHOP (rituximab, 375 mg/m^2; cyclophosphamide, 750 mg/m^2; doxorubicin, 50 mg/m^2; vincristine, 1.4 mg/m^2 [courses 1 and 2 only]; prednisolone, 100 mg) followed by a fifth course due to residual lymphoma resulted in complete remission (Fig. 1). Intermittent neutropenia was attributed to autoimmune neutropenia in combination with chemotherapy-induced bone marrow damage and was temporarily treated with granulocyte colony-stimulating factor.

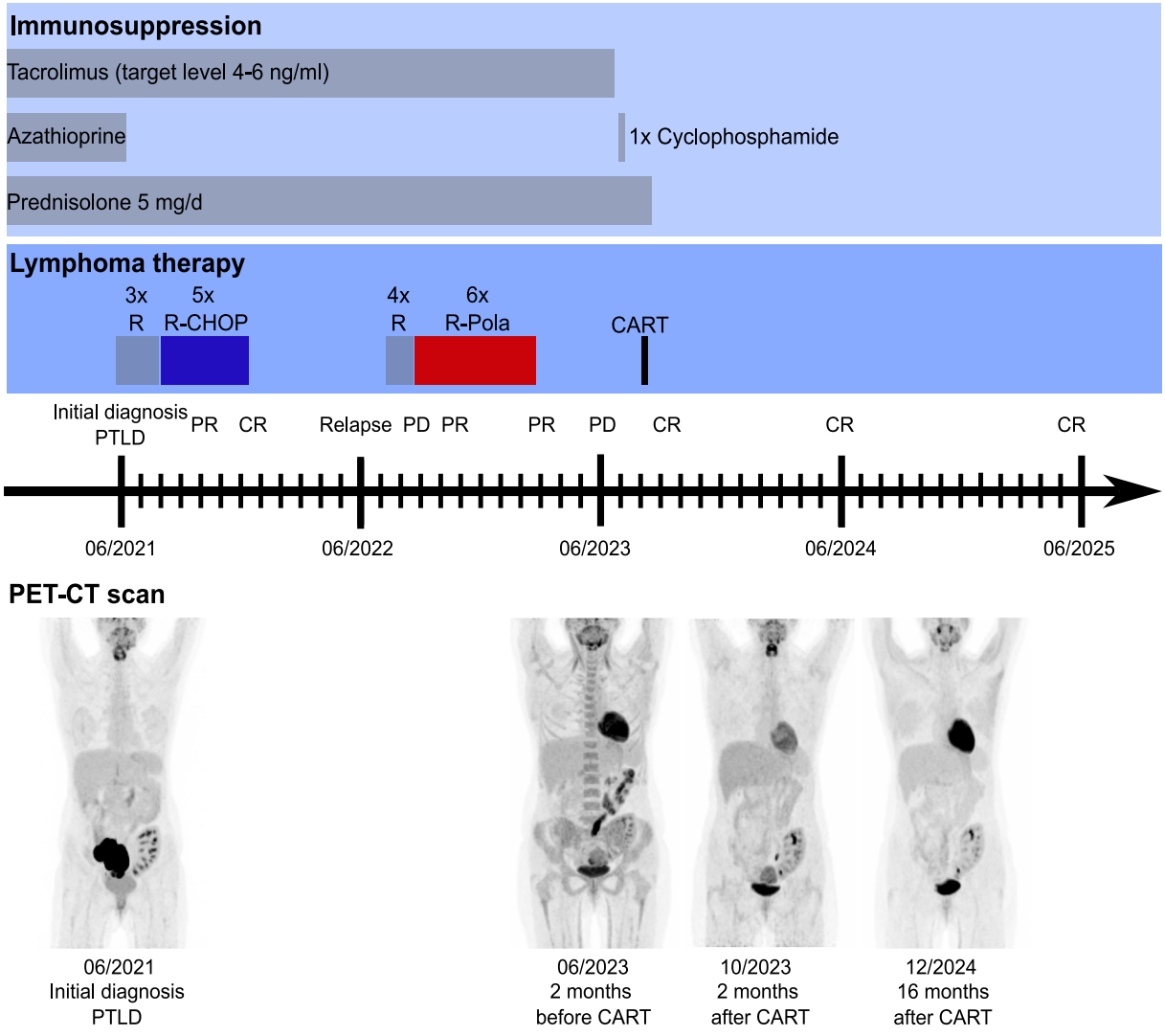

Figure 1. Graphical summary of immunosuppression, lymphoma therapy, and remission status. CART, chimeric antigen receptor T cell therapy; CR, complete remission; PD, progressive disease; PR, partial remission; PTLD, posttransplant lymphoproliferative disorder; R, rituximab; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisolone; Pola, polatuzumab-vedotin.

Five months after the end of R-CHOP, lymphoma relapsed as stage IV disease. Another 4 cycles of rituximab resulted in progressive lymphoma (Fig. 1). Subsequent third-line treatment with 6 courses of rituximab (375 mg/m^2) in combination with polatuzumab-vedotin (1.8 mg/kg) induced partial remission that lasted for 3 months.

Given the aggressive disease course, we initiated leukapheresis for chimeric antigen receptor T cell therapy (CART) with commercially manufactured axicabtagene-ciloleucel (Axi-Cel; YESCARTA; GILEAD Sciences GmbH). The patient received lymphodepleting chemotherapy at a kidney function–adapted dosage (3 $\times$ 250 mg/m^2 cyclophosphamide; 3 $\times$ 15 mg/m^2 fludarabine), followed by Axi-Cel, in August 2023 (Fig. 1).

Cytokine-release syndrome grade 1 was treated with 4 doses of tocilizumab (8 mg/kg each) due to persisting fever. Immune cell–associated neurotoxicity syndrome grade 1 was observed on day 8 after Axi-Cel and successfully managed with dexamethasone (3 $\times$ 10 mg/d).

Positron emission tomography/computed tomography 2 months after Axi-Cel revealed uterine fluorodesoxyglucose uptake that was histologically confirmed as myoma, and the patient remained in complete remission ever since with last follow-up 23 months after Axi-Cel (Fig. 1).

Since hospital discharge, no severe Common Terminology Criteria of Adverse Events grade of $\geq$3 cytopenia (leukocytopenia, thrombocytopenia, or anemia) occurred (Fig. 2A-C). Total T cell counts within CD4$^+$ and CD8$^+$ compartments recovered shortly after lymphodepleting chemotherapy (Fig. 2D, E). T cell immune phenotyping revealed dominant naïve and effector differentiation in CD4$^+$ and CD8$^+$ compartments; regulatory T cells accounted for 6.5% of CD4$^+$ T cells (Table 2; Supplementary Fig. S2) 663 days after CART. B cells were not detectable in peripheral blood during immunochemotherapy, absent after

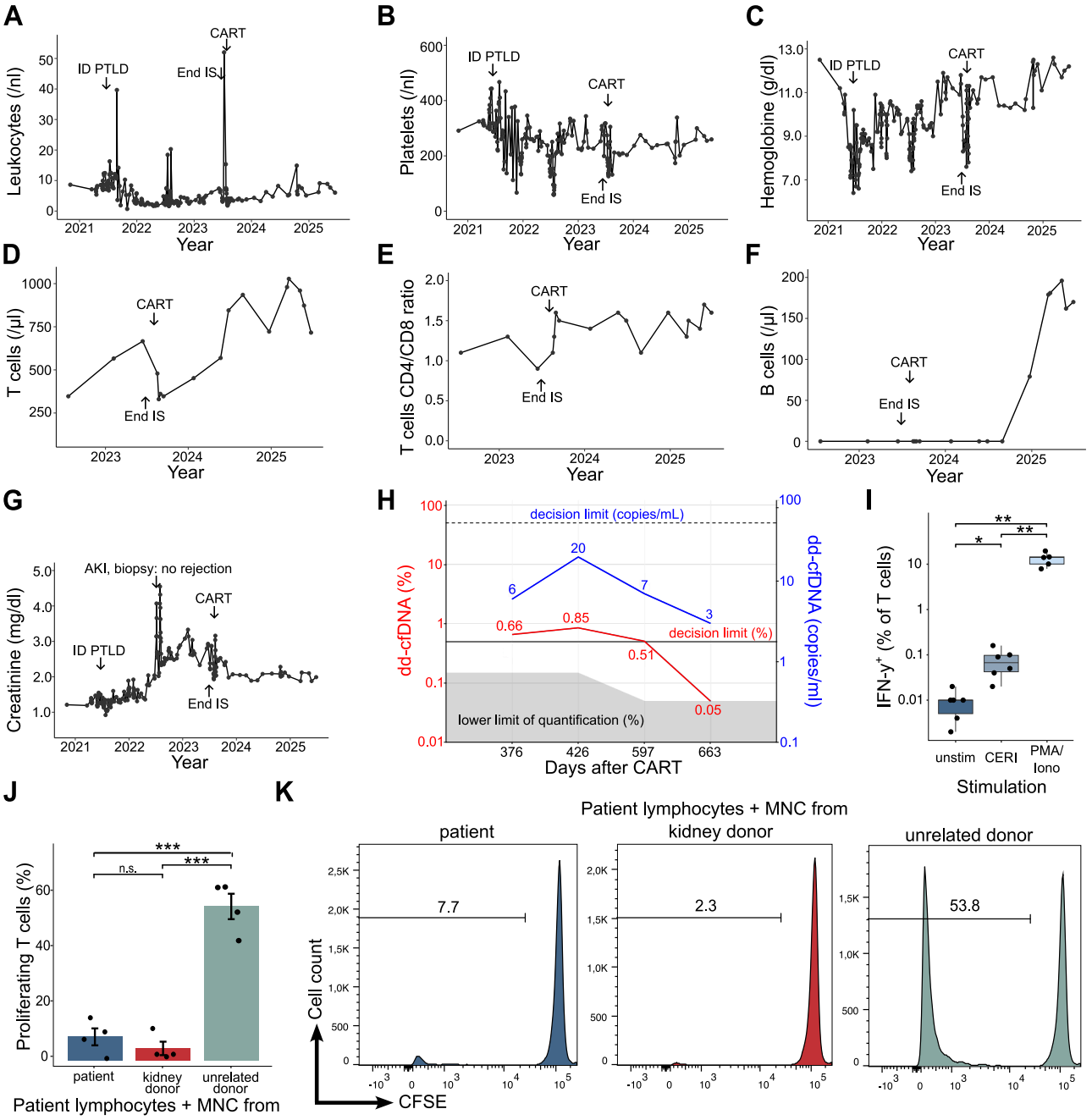

Figure 2. Immune monitoring and functional determination of immunosuppression and operational tolerance. (A-G) Selected laboratory parameters determined in peripheral blood. (H) dd-cfDNA determined in peripheral blood plasma of the patient at indicated time points. (I) Interferon gamma production in patient T cells after in vitro stimulation. Detailed gating is shown in Supplementary Figure S3. Points indicate individual data of 5 (phorbol 12-myristate 13-acetate/ionomycin) or 6 (all other conditions) replicates from 4 independent experiments performed on days 588, 597, and 663 after CART (2 experiments at the first time point, 1 experiment at each of the 2 other time points). Boxes reach from first to third quartiles, lines within boxes indicate medians. The upper whisker extends from the hinge to the largest value, no further than 1.5 x inter-quartile range (IQR). The lower whisker extends from the hinge to the smallest value, at most 1.5 × IQR. Statistics were calculated using a 2-sided unpaired *t* test. (J-K) CD2-enriched patient lymphocytes were incubated with CD2-depleted and irradiated MNC from the indicated donors. CFSE was detected by flow cytometry to determine proliferation. Histograms are pregated on live single patient lymphocytes (detailed gating in Supplementary Fig. S5). Numbers next to gates indicate percentages. The bar chart shows mean ± standard error. Data points indicate 4 replicates of 1 experiment. Statistics were calculated by 1-way analysis of variance and subsequent TukeyHSD. *$P < .05$; **$P < .01$; ***$P < .001$; n.s. not significant. AKI, acute kidney injury; CART, chimeric antigen receptor T cell; CERI, cytomegalovirus, Epstein-Barr virus, respiratory syncytial virus, influenza A; CFSE, carboxyfluorescein; dd-cfDNA, donor-derived cell-free DNA; ID, initial diagnosis; IFN, interferon; IS, immunosuppression; MNC, mononuclear cell; PTLD, posttransplant lymphoproliferative disorder.

Table 2
Frequencies of immune cell subsets in peripheral blood.

Parent population	Subset	Frequency within parent population (%) at days after CART	
		597	663
$CD4^+$ T cells	Naïve	58.5	57.3
	Central memory	4.0	6.2
	Effector	21.3	18.7
	Effector memory	16.2	17.8
	Regulatory	ND	6.5
	$CD28^+$	99.4	99.2
	TCRαβ	99.7	99.2
$CD8^+$ T cells	Naïve	49.3	49.3
	Central memory	0.1	0.1
	Effector	47.5	48.9
	Effector memory	3.0	1.6
	$CD28^+$	54.1	63.5
	TCRαβ	95.4	90.5
B cells	$CD20^+$	99.6	99.6
	Memory	0.5	20.2
	Naïve	99.3	77.7
	IgD^+	90.0	93.1
	IgG^+	0.1	0.0
	Plasmablast	0.0	0.2

Frequencies of immune cell subsets were determined by flow cytometry. T cell subset characteristics: naïve ($CCR7^+CD45RA^+$), central memory ($CCR7^+CD45RA^-$), effector ($CCR7^-CD45RA^+$), effector memory ($CCR7^-CD45RA^-$), and regulatory ($CD25^+FOXP3^+$). B cell subset characteristics: naïve ($CD27^-$), memory ($CD27^+$), and plasmablast ($CD20^-CD27^+CD38^+$). Representative gating examples are provided in Supplementary Figure S2.
ND, not determined.

CART, and recovered 16 months after CART (Fig. 2F) with almost exclusively (99% of B cells) naïve immune phenotypes and plasmablasts of <0.5% of total B cells (Table 2; Supplementary Fig. S2). Over time, we observed increasing frequencies of memory and decreasing frequencies of naïve B cells as part of B cell reconstitution (Table 2). CAR-T cells were present in peripheral blood with 0.34% and 0.10% of $CD3^+$ T cells 65 and 330 days after Axi-Cel, respectively, but were no more detectable at day 596 after Axi-Cel (Supplementary Table 2).

3.2. Kidney function and absence of rejection despite long-term discontinuation of immunosuppression after CD19-CART

The initial posttransplant course was uncomplicated. A potential borderline allograft rejection in September 2016 was not treated, and there were no further suspected rejection events. In September 2019, HLA class II antibodies (DQA1*05, unclear specificity in absence of DQA donor typing) were detectable with a median fluorescence intensity of 1123 for a single time. Owing to stable graft function, neither donor retyping nor additional kidney biopsies were performed at that time. HLA antibodies were not detectable at any following time point.

At manifestation of PTLD, kidney function remained stable during reduction of immunosuppression and immunochemotherapy (Fig. 2G). In July 2022, in the context of neutropenic fever, the patient developed acute kidney injury stage 3 according to Kidney Disease Improving Global Outcome (KDIGO),[6] with a maximum creatinine level of 4.6 mg/dL. Kidney biopsy showed no signs of rejection. Afterward, creatinine remained stable at approximately 2.0 to 3.3 mg/dL (estimated glomerular filtration rate, 18-32 mL/min/1.73 m^2), which is the serum creatinine baseline before CART (Fig. 2G).

To ensure harvest of functionally unaffected T cells for CD19-CART, tacrolimus was discontinued 7 days before leukapheresis (Fig. 1). As bridging of immunosuppression to CART, the patient received 1 infusion of cyclophosphamide (600 mg/m^2) 1 day after leukapheresis (27 days before CAR-T cell infusion). Prednisolone (5 mg/d) was stopped 13 days after CAR-T cell infusion, and the patient has not received any other immunosuppressive therapy ever since (Fig. 1). Graft function remained stable beyond 693 days after CART (creatinine, 1.99 mg/dL; estimated glomerular filtration rate, 32 mL/min/1.73 m^2) (Fig. 2G). Proteinuria after discontinuation of tacrolimus (7 days before leukapheresis) until last follow up was stable (median albuminuria, 126 mg/g creatinine; median proteinuria, 211 mg/g creatinine), compared with the time from 2021 until discontinuation of tacrolimus (median albuminuria, 114 mg/g creatinine; median proteinuria, 228 mg/g creatinine), and considered clinically not relevant regarding potential rejection. Luminex screen for HLA antibodies showed negative results throughout the entire period after CART (last determination 663 days after CART). Repeated measurements of dd-cfDNA[3] until day 663 after CART (698 days after discontinuation of tacrolimus) showed no evidence for graft injury (Fig. 2H). The initially elevated fractional results (in percentage) were due to a low amount of total cell-free DNA. Absolute dd-cfDNA results (copies per milliliter) were always below the decision limit.

3.3. Kidney graft operational tolerance after CD19-CART

Absence of kidney graft rejection despite long-term discontinuation of conventional immunosuppression and recovery of peripheral blood immune cell compartments raised the question whether CD19-CART induced a state of general immunosuppression or indeed mediated operational tolerance. Serum torque teno virus (TTV) DNA levels indicated adequate immunosuppression until 330 days after CART (1.3 × 10^6 copies/mL)[7] but were below the detection limit upon next determinations 588 and 663 days after CART (Supplementary Table 3), suggesting functional immune reconstitution. The patient never received immunoglobulin replacement

therapy, and serum immunoglobulin levels were in the lower range until last follow-up (Supplementary Table 4). Peripheral blood T cells showed robust interferon gamma production upon stimulation with peptide pools of common persistent viruses (CERI) indicating reactivity against foreign antigens 588, 597, and 663 days after CART (Fig. 2I; representative flow cytometry data of 1 experiment as an example in Supplementary Fig. S3). To investigate tolerance toward donor antigens, we tested proliferation of CD2-enriched patient T cells in a mixed reaction with CD2-depleted, irradiated peripheral blood leukocytes of the kidney donor and an unrelated donor (positive control) or the patient herself (negative control) 663 days after CART (purity of CD2-enriched or CD2-depleted cell populations in Supplementary Fig. S4). Patient T cells showed strong proliferation in response to the unrelated but not to the haploidentical kidney donor (Fig. 2J; representative histograms of 1 experiment as an example in Fig. 2K; flow cytometry gating in Supplementary Fig. S5).

4. Discussion

Data on immunosuppressive therapy in context of CD19-CART and solid organ transplantation are limited. In most cases, immunosuppression was reduced or suspended around CAR-T cell infusion and reinitiated during the following months with variable outcomes regarding graft survival and hematologic remission.[8-16] Immunosuppression was not reinitiated in our patient, which may be especially critical for therapeutic success in the case of PTLD.

We observed stable kidney function for more than 24 months after discontinuation of tacrolimus and ongoing—a finding that could not be expected. Absence of graft rejection was supported by stable kidney function throughout the entire period after CART, dd-cfDNA levels, absence of donor-specific HLA antibodies, and stable proteinuria with microalbuminuria. A kidney biopsy for confirmation of absence of rejection was clinically not indicated.

Our clinical observations raised the question whether absence of graft rejection resulted from a state of general immunosuppression or operational tolerance. Notably, the patient received numerous cycles of lymphocyte-targeted immunochemotherapy before CD19-CART, which complicated the interpretation of our findings. Operational tolerance would require efficient immune responses to foreign antigens other than donor antigens. Although initially present at high levels, TTV DNA was repeatedly below the detection limit, indirectly indicating an elevated risk for organ rejection due to low immunosuppression.[17] Accordingly, we confirmed low but robust T cell responses (interferon gamma production) in response to peptides of highly prevalent viruses (CERI). These responses met our expectations because the patient was CMV seronegative, CMV peptides comprised approximately one-quarter of CERI pool peptides (30/124 peptides), and CMV-specific T cells can be expected in higher frequencies in peripheral blood than EBV-specific T cells in seropositive healthy individuals.[18] Adequate function of the patient's immune system was confirmed by a strong response in a mixed leukocyte reaction with unrelated third-party immune cells. However, in the very same mixed leukocyte reaction, T cells of the patient showed a lack of direct alloresponse toward donor cells. Considering our data on immune reconstitution, immune function after CART, and absence of rejection, the patient achieved kidney allograft operational tolerance as defined by Ansari et al[19] and Roussey-Kesler et al.[20] To set our observations into an immunologic context, we performed detailed peripheral blood immune phenotyping at different time points after CART. T cell compartments showed dominant naïve and effector differentiation with regular frequencies of Treg. B cell compartments were dominated by naïve, non–class-switched B cells with almost absent plasmablasts. These differentiation states indicate an immunologic reset that, along with eradication of autoantibodies, has already been observed and held responsible for the beneficial effects of CD19-CART in patients with autoimmune diseases.[21,22] Naïve B cell phenotypes with plasmablasts at the lower detection limit are in line with the continuous absence of donor-specific HLA antibodies. Whether decreasing frequencies of naïve B cells and increasing frequencies of memory B cells as part of immune reconstitution pose a potential risk for future rejection in this special situation after CD19-CART cannot be concluded from our data and has to be determined in larger biomarker trials.

Despite the profound impact of CD19-CART on the B cell compartment, the mechanisms leading to operational tolerance in the presented patient are not clear. Organ rejection is not solely B cell or antibody dependent but rather the consequence of complex immunologic processes involving a variety of different cell types. Moreover, operational tolerance after kidney transplantation has been observed in selected patients independent of CAR-T cell therapy and lymphodepleting immunochemotherapies.[20,23-28] The exact contribution of CD19-CART to the development of operational tolerance and whether our findings can be applied to larger cohorts or selected subsets of patients has to be determined in future clinical trials.

As a treatment for B cell malignancies, CD19-CAR-T cells can persist for years (at least 11 months in case of the presented patient) and our observations do not allow conclusions whether B cell malignancy–driven CD19-directed CAR-T cell persistence was critical for induction of operational tolerance and long-term kidney graft survival. Furthermore, our patient was treated with Axi-Cel 12 years after kidney transplantation, a scenario that is immunologically different from time points within weeks to months after transplantation.

In context of current therapeutic strategies against kidney graft rejection, Mayer et al[29] showed promising results applying the CD38 antibody felzartamab against antibody-mediated kidney allograft rejection. Felzartamab did not substantially affect serum levels of donor-specific HLA antibodies and graft rejections recurred after treatment discontinuation.[29] We expect effects of CD19-CART to be complementary to CD38 antibodies and beneficial for mediating long-term operational graft tolerance.

In the future posttransplantation setting, we envision CD19-CART as a therapeutic option to prevent chronic organ rejection

especially in scenarios where conventional immunosuppression should be avoided (eg, PTLD) or is considered inefficient. Our data have to be interpreted with caution, as long as they rely on a single case; however, they encourage application of CD19-directed CAR-T cells in selected conditions in clinical trials.

Acknowledgments

The authors thank Rüdiger Eder for critical technical advice and Bruno L. Cadilha for helpful scientific discussions.

Author contributions

H.H., S.H., C.H., M.O., J.B., E.S., and L.H. created and designed the figures. C.H., D.C.H., V.B., P.H., and L.H. performed the experiment design. C.H., D.C.H., and V.B. performed experimentation. M.F., S.H., M.P., B.Z., M.E., D.W., W.H., B.B., D.Z., and L.H. were responsible for patient care. H.H., C.H., M. F., and L.H. drafted the manuscript. All authors: edited the manuscript. H.H., D.Z., and L.H. conceptualized the study. L.H. was responsible for acquisition of funding.

Funding

The study was supported by funding from Deutsche Krebshilfe e.V. (70115705 to L.H.) and German Research Foundation (Project number 545913134 to L.H.).

Declaration of competing interest

The authors of this manuscript have conflicts of interest to disclose as described by *American Journal of Transplantation*. L.H. reports financial support by Deutsche Krebshilfe e.V. (70115705) and German Research Foundation (545913134); consulting or advisory fees Bristol Myers Squibb, Gilead, Johnson & Johnson, Pierre-Fabre, and Sanofi; and travel support from Amgen, Gilead, and Johnson & Johnson. D.W. reports research support from Novartis and honoraria from Novartis and Kite-Gilead. D.Z. reports advisory board participation in Apellis and Novartis and travel support from Boehringer Ingelheim. E.S. and J.B. are employees at Chronix Biomedical GmbH (an Oncocyte Company); hold stock or option ownership in Oncocyte, Inc; and are inventors on patents US11155872B2 and 10570443B2 and EP3004388B2 and 3201361B1. M.A.F. reports travel grants from Sanofi and honoraria from Sanofi and Novartis. M.O. is consultant of Insight Molecular Diagnostics, Inc Nashville TN, USA. M.P. reports travel grants and honoraria from Kite and Takeda. W.H. reports research support from Johnson & Johnson and travel grants from Amgen, Johnson & Johnson, and Servier.

Data availability

All presented data are included within the article and the respective supplemental information.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajt.2025.09.009.

ORCiD

Helena Hansmann https://orcid.org/0000-0002-0934-7620
Chiara Henkel https://orcid.org/0009-0004-3688-1396
Matthias A. Fante https://orcid.org/0009-0008-5662-2630
Dennis C. Harrer https://orcid.org/0000-0001-7512-572X
Stefanie Heidemanns https://orcid.org/0000-0001-7708-5864
Michael Oellerich https://orcid.org/0000-0002-9255-2261
Julia Beck https://orcid.org/0000-0002-4143-1363
Ekkehard Schütz https://orcid.org/0000-0002-9639-4870
Verena Biswenger https://orcid.org/0000-0002-8002-6387
Markus Perl https://orcid.org/0000-0002-3464-0722
Barbara Zartner https://orcid.org/0009-0004-4894-4614
Matthias Edinger https://orcid.org/0000-0003-2889-2533
Daniel Wolff https://orcid.org/0000-0001-7015-3190
Petra Hoffmann https://orcid.org/0009-0003-3541-6196
Wolfgang Herr https://orcid.org/0009-0001-3991-2256
Bernhard Banas https://orcid.org/0000-0003-2076-2195
Daniel Zecher https://orcid.org/0000-0002-2123-0310
Leo Hansmann https://orcid.org/0000-0003-3790-0128

References

1. Kidney Disease: Improving Global Outcomes Transplant Work Group. KDIGO clinical practice guideline for the care of kidney transplant recipients. *Am J Transplant*. 2009;9(Suppl 3):S1–S155. https://doi.org/10.1111/j.1600-6143.2009.02834.x.
2. Beck J, Oellerich M, Schütz E. A universal droplet digital PCR approach for monitoring of graft health after transplantation using a preselected SNP set. *Methods Mol Biol*. 2018;1768:335–348. https://doi.org/10.1007/978-1-4939-7778-9_19.
3. Oellerich M, Sherwood K, Keown P, et al. Liquid biopsies: donor-derived cell-free DNA for the detection of kidney allograft injury. *Nat Rev Nephrol*. 2021;17(9):591–603. https://doi.org/10.1038/s41581-021-00428-0.
4. Oellerich M, Shipkova M, Asendorf T, et al. Absolute quantification of donor-derived cell-free DNA as a marker of rejection and graft injury in kidney transplantation: results from a prospective observational study. *Am J Transplant*. 2019;19(11):3087–3099. https://doi.org/10.1111/ajt.15416.
5. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing; 2024. http://www.R-project.org/. accessed july 4, 2025
6. Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group. KDIGO clinical practice guidelines for acute kidney injury. *Kidney Int Suppl*. 2012;2(1):1–138.
7. Haupenthal F, Rahn J, Maggi F, et al. A multicentre, patient- and assessor-blinded, non-inferiority, randomised and controlled phase II trial to compare standard and torque teno virus-guided immunosuppression in kidney transplant recipients in the first year after transplantation: TTVguideIT. *Trials*. 2023;24(1):213. https://doi.org/10.1186/s13063-023-07216-0.
8. Guy P, Marion O, Oberic L, et al. CAR T-cell therapy for refractory posttransplantation lymphoproliferative disorder in a kidney transplant patient. *Transplant Direct*. 2024;10(3):e1584. https://doi.org/10.1097/TXD.0000000000001584.
9. Hernani R, Sancho A, Amat P, et al. CAR-T therapy in solid transplant recipients with post-transplant lymphoproliferative disease: case report

and literature review. *Curr Res Transl Med*. Oct 2021;69(4):103304. https://doi.org/10.1016/j.retram.2021.103304.
10. Luttwak E, Hagin D, Perry C, et al. Anti-CD19 CAR-T therapy for EBV-negative posttransplantation lymphoproliferative disease-a single center case series. *Bone Marrow Transplant*. 2021;56(5):1031–1037. https://doi.org/10.1038/s41409-020-01145-1.
11. McKenna M, Epperla N, Ghobadi A, et al. Real-world evidence of the safety and survival with CD19 CAR-T cell therapy for relapsed/refractory solid organ transplant-related PTLD. *Br J Haematol*. 2023;202(2):248–255. https://doi.org/10.1111/bjh.18828.
12. Mamlouk O, Nair R, Iyer SP, et al. Safety of CAR T-cell therapy in kidney transplant recipients. *Blood*. 2021;137(18):2558–2562. https://doi.org/10.1182/blood.2020008759.
13. Krishnamoorthy S, Ghobadi A, Santos RD, et al. CAR-T therapy in solid organ transplant recipients with treatment refractory posttransplant lymphoproliferative disorder. *Am J Transplant.* 2021;21(2):809–814. https://doi.org/10.1111/ajt.16367.
14. de Nattes T, Camus V, Francois A, et al. Kidney transplant T cell-mediated rejection occurring after anti-CD19 CAR T-cell therapy for refractory aggressive burkitt-like lymphoma with 11q aberration: a case report. *Am J Kidney Dis*. 2022;79(5):760–764. https://doi.org/10.1053/j.ajkd.2021.07.012.
15. Ibrahim U, Bassil C, Chavez JC, et al. CAR T cell therapy for post-transplant lymphoproliferative disorder after solid organ transplantation: a safe and feasible therapy for an orphan disease. *Clin Lymphoma Myeloma Leuk*. 2023;23(10):772–778. https://doi.org/10.1016/j.clml.2023.05.007.
16. Portuguese AJ, Gauthier J, Tykodi SS, et al. CD19 CAR-T therapy in solid organ transplant recipients: case report and systematic review. *Bone Marrow Transplant*. 2023;58(4):353–359. https://doi.org/10.1038/s41409-022-01907-z.
17. Doberer K, Schiemann M, Strassl R, et al. Torque teno virus for risk stratification of graft rejection and infection in kidney transplant recipients—a prospective observational trial. *Am J Transplant*. Aug 2020;20(8):2081–2090. https://doi.org/10.1111/ajt.15810.
18. Sukdolak C, Tischer S, Dieks D, et al. CMV-, EBV- and ADV-specific T cell immunity: screening and monitoring of potential third-party donors to improve post-transplantation outcome. *Biol Blood Marrow Transplant*. 2013;19(10):1480–1492. https://doi.org/10.1016/j.bbmt.2013.07.015.
19. Ansari MJ, Sayegh MH. Clinical transplantation tolerance: the promise and challenges. *Kidney Int*. May 2004;65(5):1560–1563. https://doi.org/10.1111/j.1523-1755.2004.05411.x.
20. Roussey-Kesler G, Giral M, Moreau A, et al. Clinical operational tolerance after kidney transplantation. *Am J Transplant*. 2006;6(4):736–746. https://doi.org/10.1111/j.1600-6143.2006.01280.x.
21. Mackensen A, Müller F, Mougiakakos D, et al. Anti-CD19 CAR T cell therapy for refractory systemic lupus erythematosus. *Nat Med*. 2022;28(10):2124–2132. https://doi.org/10.1038/s41591-022-02017-5.
22. Müller F, Taubmann J, Bucci L, et al. CD19 CAR T-cell therapy in autoimmune disease—a case series with follow-up. *N Engl J Med*. 2024;390(8):687–700. https://doi.org/10.1056/NEJMoa2308917.
23. Sagoo P, Perucha E, Sawitzki B, et al. Development of a cross-platform biomarker signature to detect renal transplant tolerance in humans. *J Clin Invest*. 2010;120(6):1848–1861. https://doi.org/10.1172/JCI39922.
24. Newell KA, Asare A, Kirk AD, et al. Identification of a B cell signature associated with renal transplant tolerance in humans. *J Clin Invest*. 2010;120(6):1836–1847. https://doi.org/10.1172/JCI39933.
25. Brouard S, Le Bars A, Dufay A, et al. Identification of a gene expression profile associated with operational tolerance among a selected group of stable kidney transplant patients. *Transpl Int*. 2011;24(6):536–547. https://doi.org/10.1111/j.1432-2277.2011.01251.x.
26. Rebollo-Mesa I, Nova-Lamperti E, Mobillo P, et al. Biomarkers of tolerance in kidney transplantation: are we predicting tolerance or response to immunosuppressive treatment? *Am J Transplant*. 2016;16(12):3443–3457. https://doi.org/10.1111/ajt.13932.
27. Christakoudi S, Runglall M, Mobillo P, et al. Development and validation of the first consensus gene-expression signature of operational tolerance in kidney transplantation, incorporating adjustment for immunosuppressive drug therapy. *EBioMedicine*. 2020;58:102899. https://doi.org/10.1016/j.ebiom.2020.102899.
28. Strober S, Benike C, Krishnaswamy S, Engleman EG, Grumet FC. Clinical transplantation tolerance twelve years after prospective withdrawal of immunosuppressive drugs: studies of chimerism and anti-donor reactivity. *Transplantation*. 2000;69(8):1549–1554. https://doi.org/10.1097/00007890-200004270-00005.
29. Mayer KA, Schrezenmeier E, Diebold M, et al. A randomized phase 2 trial of felzartamab in antibody-mediated rejection. *N Engl J Med*. 2024;391(2):122–132. https://doi.org/10.1056/NEJMoa2400763.