Molecular monitoring of T-cell kinetics and migration in severe neurotoxicity after real-world CD19-specific chimeric antigen receptor T cell therapy

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Supplementary Materials for

Molecular monitoring of T-cell kinetics and migration in severe neurotoxicity after real-world CD19-CAR-T cell therapy

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Abbreviations used in the *Online Supplementary Figures*:

Ana, anakinra; Ara, cytarabine; axi-cel, axicabtagene ciloleucel; BM, bone marrow; CAR, chimeric antigen receptor; CNS, central nervous system; CSF, cerebrospinal fluid; CRS, cytokine release syndrome; Dex, dexamethasone; dPCR, digital PCR; gDNA, genomic DNA; ICANS, immune effector cell-associated neurotoxicity syndrome; IL, interleukin; IP, infusion product; ITT, intrathecal therapy; LDH, lactate dehydrogenase; leu, leukocytes; LP, lumbar puncture; MNC, mononuclear cells; MTX, methotrexate; m, multiple; NGS, next generation-sequencing; PB, peripheral blood; PBMC, peripheral blood mononuclear cells; tisa-cel, tisagenlecleucel; Toci, tocilizumab; TRB, T-cell receptor-β.

Online Supplementary Methods

Study design

A cohort of 48 consecutive patients with advanced relapsed and/or refractory (r/r) B-cell lymphoma (n=47) or leukemia (n=1) was treated with a lymphodepleting regimen followed by commercially available CD19-redirected chimeric antigen receptor-T cells (CD19-CAR-T cells), namely axicabtagene ciloleucel (axi-cel; n=35) and tisagenlecleucel (tisa-cel; n=13). The lymphodepletion consisted of cyclophosphamide (Cy; 500 mg/m^2/day) and fludarabine (Flu; 30 mg/m^2/day) given on 3 consecutive days intravenously (IV) as described.[1-3] Two axi-cel patients received only two doses of Cy (250 or 500 mg/m^2/day) due to preexisting cytopenia, all other patients received the full dose, while the Flu dose was adjusted to reduced kidney function in 5 patients. In our tisa-cel treated B-cell lymphoma patients the lymphodepletion regimen consisted of Cy (250 mg/m^2/day) and Flu (20-25 mg/m^2/day) given on 3 consecutive days. However, patient #21 received only Flu due to prior intense pretreatment. After a wash-out period of 3 days, a single IV infusion of axi-cel (1-2 x 10^6 CD19-CAR-T cells/kg) or tisa-cel (0.9-4.6 x 10^8 CD19-CAR-T cells total) was administered as recommended by the manufacturer. Per our protocol, blood samples were collected at scheduled intervals after infusion on days (± 3 days) 4, 7, 10, 14, 21, 28; month (± 2 weeks) 2, 3, 6, 12, 24, 36, or triggered by clinical events including diagnosis and treatment of complications related to a cytokine release syndrome (CRS) or immune effector cell-associated neurotoxicity syndrome (ICANS). The study was approved by the local ethics committee (PV7081) and written informed consent was obtained from all patients. Patients underwent thorough evaluation of clinical status and laboratory parameter per institutional practice. CRS and ICANS were defined and graded according to *American Society for Transplantation and Cellular Therapy* (ASTCT) guidelines.[4] For ICANS, patients were examined using the immune effector cell-association encephalopathy score. Patients with a suspected ICANS and/or CNS disease received a cranial computerized tomography, cerebral magnetic resonance imaging, electro-encephalogram, and/or a lumbar puncture (LP) as determined by the consulting neurologist.

The LPs were only performed if needed for medical reasons including diagnostic purposes, for drug administration, and/or other therapeutic interventions (for example to release pressure). Efforts were made to restrict the number of LP procedures per patient and we used implanted catheters in some of our patients to facilitate the procedure, including patient #24 who received a Rickham reservoir on day 19 after the CAR-T cell infusion. We obtained approval by the local ethic committee to use an aliquot of the respective diagnostic CSF specimen (single or serial) for the molecular immune monitoring after CAR-T cell therapy. Treatment of CRS and/or ICANS consisted of tocilizumab (8 mg/kg IV), dexamethasone (4-20 mg IV every 6 hours or 4 mg intrathecal every 6 hours), and/or anakinra (100 mg/dose once or twice daily subcutaneously), and in some patients methylprednisone (up to 1,000 mg/m^2 daily for 3 days IV). Four patients of our cohort with grade 2-4 ICANS received an intrathecal chemotherapy consisting of up to 3 injections of cytarabine (40 mg/dose), methotrexate (12-15 mg/dose) and dexamethasone (4 mg/dose).[5] Analyses of complete cell counts, chemistry, and interleukin-6 in peripheral blood or cerebrospinal fluid were performed in accredited diagnostic laboratories at our University Medical Center. Tumor response assessment was performed per institutional practice and based on Lugano criteria.[6]

T-cell receptor-β (TRB) amplification for next generation sequencing (NGS)

Successfully recombined TRB gene segments were amplified and tagged with Illumina adapters (San Diego, USA) and a 3'-seven base pair barcode sequence in a two-step multiplex PCR using BIOMED2-TRB primer pools[7] and 250 ng gDNA as described.[8-10] Primers were purchased from Metabion (Martinsried, Germany) and PCR experiments were performed using Phusion HS II DNA polymerase (Thermo Fisher Scientific Inc., Waltham, USA). All PCR products were size separated by agarose gel electrophoresis and amplicons with the expected size were purified using the NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel, Düren, Germany). Before being used for NGS library preparation, the concentration of the barcoded

amplicons was determined on Qubit (Qiagen, Hilden, Germany) and the quality control was performed on an Agilent 2100 Bioanalyzer (Agilent technologies, Böblingen, Germany).

Illumina-based NGS and calculation of repertoire indices

NGS and demultiplexing were performed on an Illumina MiSeq sequencer (600-cycle single indexed, paired-end run, V3-chemistry). Barcoded amplicons were sequenced with a depth of 50,000 reads per amplicon. TRB sequence analysis was computed using the MiXCR software.[11] A clone was defined as unique complementarity-determining region 3 (CDR3) nucleotide sequence and all clones detected in one sample were considered as the specific TRB repertoire. Only productive CDR3 sequences with a read count ≥2 were considered for the data analysis, which was performed using R,[12] and the package tcR.[13] The TRB diversity, including the inverse Simpson index, was calculated as described.[10,14-16]

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Online Supplementary Table S1. **Characteristics of patients with grade ≥3 neurotoxicity after real-world CD19-CAR-T cell therapy**

Patient ID	03	12	15	16	24	25	26	28	31	43
Age (years)	78	79	77	76	59	70	74	80	63	62
Gender	male	male	female	male	female	female	male	male	female	male
Diagnosis	DLBCL	DLBCL	DLBCL	DLBCL	DLBCL	DLBCL	DLBCL	DLBCL	DLBCL	DLBCL
Type	non-GCB	na	na	na	non-GCB	non-GCB	na	non-GCB	na	na
BM involvement	no	no	no	no	no	no	no	no	no	yes
CNS involvement	no	no	no	no	no	no	yes	no	yes	yes
Prior Therapies (number)	4	5	6	6	5	4	5	4	4	4
Prior autologous SCT (number)	no	no	no	no	yes (1)	no	no	no	yes (1)	yes (1)
Bridging	R-ICE, R-Pix	R-Pix	no	R-Pix	R-ICE, R-Pix	R-ICE, R-GemOx, R-Pola	Ibrutinib	R-GemOx	R-DHAOx	R-GemOx
Stage at enrollment	St 4, PD/ Refractory	St 4, PD/ Refractory	St 3, PD/ Refractory	St 4, PD/ Refractory	St 3-4, PD/ Refractory	St 4, PD/ Refractory	CNS Relapse, PD	St 4, PD/ Refractory	St 4 (CNS), PR	St 4, PD/ Refractory
CD19 CAR	axi-cel	axi-cel	axi-cel	axi-cel	tisa-cel	tisa-cel	axi-cel	tisa-cel	axi-cel	axi-cel
CAR-T cell peak/µL (day)	3.2 (26)	23.2 (15)	31.3 (11)	131.7 (7)	5.9 (36)	230.5 (8)	967.0 (7)	3.2 (5)	271.6 (9)	101.7 (7)
CRS (grade)[$]	2	2	2	2	2	2	3	1	2	1
CRS treatment	Supportive	Supportive	Toci (2x)	Toci (2x)	Toci (3x)	Toci (5x)	Toci (3x)	Toci (3x)	Toci (6x)	Toci (1 x)
ICANS (grade)[$]	4	3	3	3	4	4	3	4	3	3
ICANS treatment	none	none	IV Dex (m)	IV Dex	IV/IT Dex (m) MP, Ana IT Ara, MTX, Dex	IV Dex (m) MP IV, Ana	IV Dex (m) Ana IT Ara, MTX, Dex	IV Dex (m) Ana	IV Dex (m)	IV Dex (m), IT Ara, MTX, Dex
Response, early (day)	PD (33)	CR (60)	PR (27)	PR (32)	PD (64)	PD (23)	PR (48)	PD (22)	PR (43)	PR (42)
Response, latest (day)	PD	PD	CR	CR	PD	PD	PR	PD	CR	PR
Status (day)	deceased (95)	deceased (726)	alive (964)	alive (974)	deceased (66)	deceased (24)	alive (602)	deceased (30)	alive (391)	alive (175)
Cause of Death	PD	PD			PD	PD		PD		

Ana, anakinra; Ara, cytarabine; axi-cel, axicabtagene ciloleucel; BM, bone marrow; CAR, chimeric antigen receptor; CNS, central nervous system; CR: complete remission; CRS, cytokine release syndrome; Dex, dexamethasone; DLBCL, diffuse large B-cell lymphoma; GCB, germinal center B cell; ICANS, immune effector cell-associated neurotoxicity syndrome; IT, intrathecal; IV, intravenously; m, multiple; MP, methylprednisone; MTX, methotrexate; na, not available; PD, progressive disease; PR, partial remission; R, rituximab; R-DHAOx rituximab, dexamethasone, high-dose cytarabine, oxaliplatin; R-GemOx, rituximab, gemcitabine, oxaliplatin; R-ICE, rituximab, ifosfamide, carboplatin, etoposide; R-Pix, rituximab, pixantron; R-Pola, rituximab, polatuzumab; SCT, stem cell transplantation; tisa-cel, tisagenlecleucel; St, stage; Toci, tocilizumab. [$]CRS and ICANS grading per ASTCT criteria. Updated as of 31.05.2022

Online Supplementary Table S2. **Absolute numbers of CAR-T cells in diagnostic samples of cerebrospinal fluid or peripheral blood**

Absolute numbers of CAR-T cells for results shown in Figure 2.

Patient ID	Day	Cerebrospinal Fluid		Peripheral Blood		Fold change
		(Leu/μL)	(CAR-T/μL)	(Leu/μL)	(CAR-T/μL)	(CSF vs PB)
#12	11	6.0	**0.40**	2,900	**1.46**	**0.274**
#15	12	3.3	**1.09**	1,400	**31.28**	**0.035**
#26	49	2.3	**0.43**	5,300	**2.67**	**0.161**
#31	15	3.0	**1.27**	700	**5.78**	**0.220**
#43	7	19.0	**2.89**	1,100	**101.72**	**0.028**
#24	14	11.7	**1.54**	2,700	**3.40**	**0.453**
#25	9	3.3	**2.59**	2,400	**230.51**	**0.011**
#28	23	1.3	**0.29**	13,800	**2.94**	**0.100**

Absolute numbers of CAR-T cells for results shown in *Online Supplementary Figure S3*.

Patient ID	Day	Cerebrospinal Fluid		Peripheral Blood		Fold change
		(Leu/μL)	(CAR-T/μL)	(Leu/μL)	(CAR-T/μL)	(CSF vs PB)
#35	5	30	**21.20**	700	**24.51**	**0.865**
#09	10	65.3	**26.56**	7,000	**16.14**	**1.646**
#32	33	6	**4.72**	1,100	**4.49**	**1.051**
#36	10	2	**1.67**	1,500	**171.03**	**0.010**
#42	6	1	**0.35**	800	**33.93**	**0.010**
#33	14	2	**0.77**	2,700	**145.54**	**0.005**
#45	5	2.0	**0.17**	500	**112.79**	**0.002**
#46	8	14	**9.99**	1,700	**640.22**	**0.016**

Absolute numbers of CAR-T cells for results shown in Figure 3.

Patient ID	Day	Cerebrospinal Fluid		Peripheral Blood		Fold change
		(Leu/μL)	(CAR-T/μL)	(Leu/μL)	(CAR-T/μL)	(CSF vs PB)
#31*	2	<5	**0.00**	190	**0.21**	**0.00**
#31	6	2.0	**0.98**	500	**12.19**	**0.08**
#31	15	3.0	**1.27**	700	**5.78**	**0.22**
#28*	5	1.7	**0.13**	190	**3.20**	**0.04**
#28	13	1.0	**0.43**	500	**2.32**	**0.18**
#28	23	1.3	**0.29**	13,800	**2.94**	**0.10**

Absolute numbers of CAR-T cells for results shown in Figure 4.

Patient ID	Day	Cerebrospinal Fluid		Peripheral Blood		Fold change
		(Leu/μL)	(CAR-T/μL)	(Leu/μL)	(CAR-T/μL)	(CSF vs PB)
#24	8	12.3	**0.23**	600	**1.54**	**0.15**
#24	14	11.7	**1.54**	2,700	**3.40**	**0.45**
#24*	19	22.7	**5.83**	190	**1.37**	**4.26**
#24	36	13.0	**0.60**	1,100	**5.61**	**0.11**
#24	43	84.0	**3.21**	7,300	**0.61**	**5.26**
#24	63	0.3	**0.01**	800	**0.11**	**0.09**
#26	9	6.3	**3.35**	3,900	**223.87**	**0.01**
#26	13	66.7	**4.65**	3,200	**27.68**	**0.17**
#26	23	5.0	**2.97**	3,000	**6.40**	**0.46**
#26	31	1.3	**0.27**	8,900	**1.31**	**0.21**
#26	49	2.3	**0.43**	5,300	**2.67**	**0.16**

Absolute numbers of CAR-Tcells for results shown in *Online Supplementary Figure S5*.

Patient ID	Day	Cerebrospinal Fluid		Peripheral Blood		Fold change
		(Leu/μL)	(CAR-T/μL)	(Leu/μL)	(CAR-T/μL)	(CSF vs PB)
#32	9	2	**0.58**	300	**8.13**	**0.07**
#32	20	27	**20.78**	2,300	**29.00**	**0.72**
#32	23	19	**10.59**	1,100	**14.70**	**0.72**
#32	26	20	**14.91**	1,000	**8.22**	**1.81**
#32	33	6	**4.72**	1,100	**4.49**	**1.05**

CAR-T, chimeric antigen receptor modified-T cells; CSF, cerebrospinal fluid; Leu, leukocyte; PB, peripheral blood.

* Leukocytes were < 200/μL and estimated 190/μL for calculation.

Online Supplementary Table S3. **Characteristics of patients with grade 0-2 neurotoxicity after CD19-CAR-T cell therapy**

Patient ID	09	32	33	35	36	42	45	46
Age (years)	44	77	79	54	52	63	48	37
Gender	male	male	female	male	female	female	male	male
Diagnosis	PMBCL	DLBCL	DLBCL	DLBCL	DLBCL	DLBCL	DLBCL	DLBCL
Type		non-GCB	na	na	non-GCB	non-GCB		na
BM involvement	no	no	yes	no	yes	no	no	yes
CNS involvement	no	no	yes	no	no	no	no	no
Prior Therapies (number)	4	5	5	7	5	6	2	3
Prior autologous SCT (number)	no	yes (1)	yes (1)	yes (1) and allogeneic SCT (1)	yes (1)	yes (1)	no	no
Bridging	R-ICE	Radiatio	Ibrutinib	R-Pola	GemOx,R-Pola-Bendamustin	R-GemOx/Pola-Vedotin	Radiatio	R-GemOx/ Pola-
Stage at enrollment	PD/ Refractory/ Bulk	St 4, PD/ Refractory	St 4, CR	St 4, PD/ Refractory	St 4, PD/ Refractory	St 4, PD/ Refractory	St 4, PD/ Refractory	St 4, PD/ Refractory
CD19 CAR	axi-cel	axi-cel	tisa-cel	axi-cel	axi-cel	axi-cel	axi-cel	axi-cel
CAR-T cell peak/µL (day)	16.1 (9)	127.6 (15)	325.4 (10)	351.6 (8)	193.8 (6)	44.6 (7)	388.5 (7)	640.2 (8)
CRS (grade)[$]	3	1	2	2	2	2	2	1
CRS treatment	Toci	Toci	Toci, Dex (m)	Toci	Toci	Toci	Toci (3 x)	Toci
ICANS (grade)[$]	2	2	0	1	2	2	3	2
ICANS treatment	IV Dex	IV Dex (m) IT Ara, MTX, Dex		IV Dex	IV Dex (m)	IV Dex	IV Dex (m) Ana	IV Dex (m)
Response								
Initial (day)	PR (48)	PR (69)	PR (34)	PR (55)	PR (99)	PR (52)	PR (42)	PR (30)
Latest	CR	PD	CR	PR	CR	PR	PR	PR
Status (day)	alive (1036)	alive (378)	alive (354)	alive (307)	alive (294)	alive (190)	alive (127)	alive (63)

Ara, cytarabine; axi-cel, axicabtagene ciloleucel; BM, bone marrow; CAR, chimeric antigen receptor; CNS, central nervous system; CR: complete remission; CRS, cytokine release syndrome; Dex, dexamethasone; DLBCL, diffuse large B-cell lymphoma; GCB, germinal center B cell; ICANS, immune effector cell-associated neurotoxicity syndrome; IT, intrathecal; IV, intravenously; m, multiple; MTX, methotrexate; na, not available; PD, progressive disease; PR, partial remission; R, rituximab; GemOx, gemcitabine, oxaliplatin; R-ICE, rituximab, ifosfamide, carboplatin, etoposide; R-Pola, rituximab, polatuzumab; SCT, stem cell transplantation; tisa-cel, tisagenlecleucel; St, stage; Toci, tocilizumab; [$]CRS and ICANS grading per ASTCT criteria. Updated as of 31.05.2022.

Online Supplementary Table S4. **Absolute numbers of recovered leukocytes in diagnostic cerebrospinal fluid specimen**

		Cerebrospinal Fluid		
Patient ID	**Day**	**Leukocytes/μL**	**Volume (mL)**	**Number of cells**
#24	8	12.3	0.98	12,005
#24	14	11.70	2.00	23,400
#24	19	22.70	1.40	31,780
#24	36	13	2.60	33,800
#24	43	84.00	6.20	520,800
#24	63	0.33	3.50	1,155
#26	9	6.3	2.00	12,600
#26	13	66.7	1.50	100,005
#26	23	5.0	3.00	15,000
#26	31	1.3	1.80	2,340
#26	49	2.3	0.70	1,631
#32	9	2	1.30	2,600
#32	20	27	na*	na*
#32	23	19	1.20	22,800
#32	26	20	0.90	18,000
#32	33	6	1.00	6,000

*not available

Online Supplementary Table S5. **Analysis of both IL-6 and protein in corresponding serum and diagnostic cerebrospinal fluid specimen**

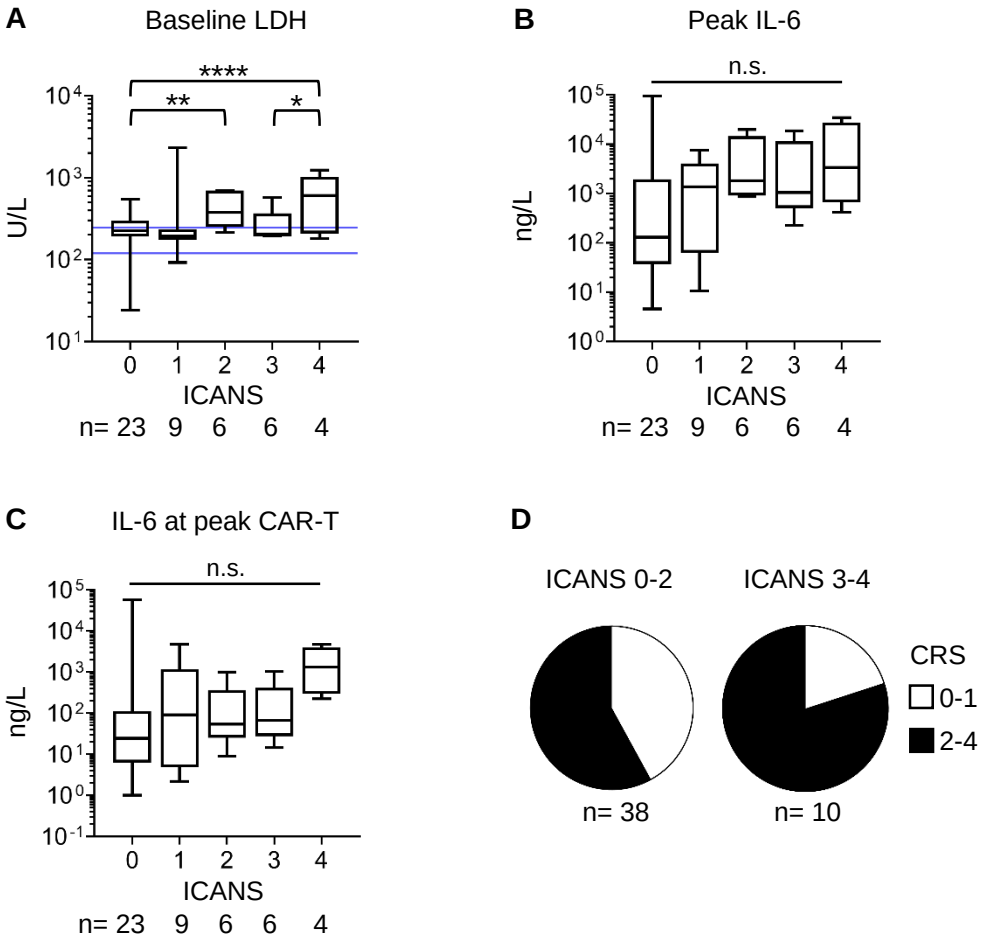
				IL-6			Protein	
Patient ID	**CAR-T**	**ICANS**	**Day**	**CSF (ng/L)**	**Serum (ng/L)**	**Fold (CSF/Serum)**	**CSF (mg/L)***	**Serum (g/L)****
#24	tisa	4	36	993	502.2	1.98	1,362	48
#24	tisa	4	43	376	94.7	3.97	1,501	49
#25	tisa	4	9	111.8	1,946	0.06	439	48
#31	axi	3	2	76	5,504.6	0.01	188	58
#32	axi	2	9	15.6	573.7	0.03	455	56
#32	axi	2	20	162.8	183.8	0.89	563	44
#32	axi	2	23	83.3	113.0	0.74	524	45
#35	axi	1	5	2,215.5	340.9	6.50	759	60
#35	axi	1	7	460.4	144.2	3.19	854	51
#36	axi	2	5	1,077	162.7	6.62	3,144	55
#36	axi	2	10	39.7	36.2	1.10	861	47
#45	axi	2	5	790.1	142.7	5.54	1,105	66

CAR-T, chimeric antigen receptor modified-T cells; CSF, cerebrospinal fluid; ICANS, immune effector cell-associated neurotoxicity syndrome; IL, interleukin.
*normal range in CSF: 80-320 mg/L ** normal range in serum: 57-82 g/L.

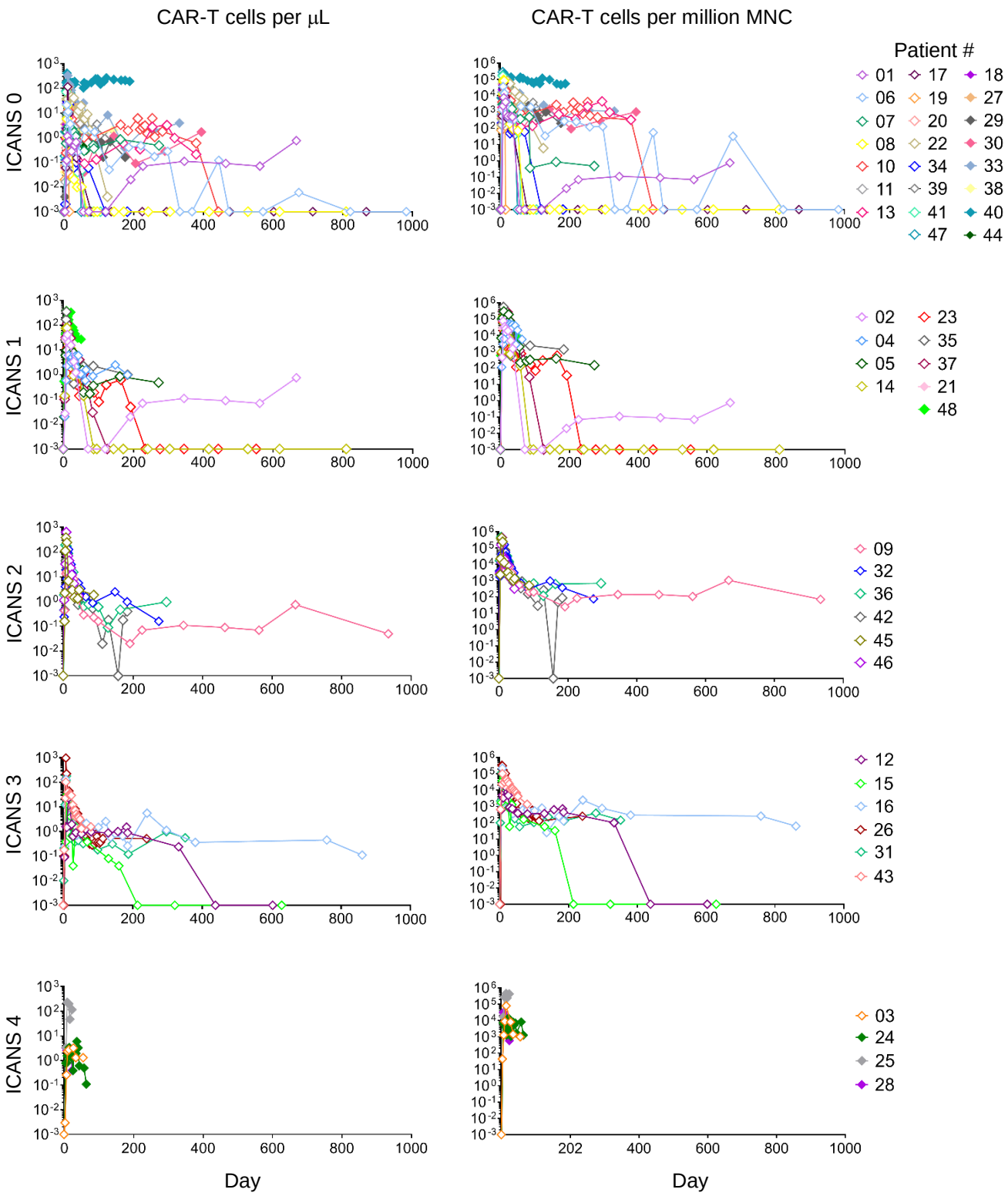
Online Supplementary Table S6. **Detection of unique hyperexpanded T cell clones in cerebrospinal fluid specimen from patient #24**

Clone ID	CDR3 aa sequence	Clone count	V gene	D gene	J gene	V gene*	NT sequence of CDR3 region
H14-1	CASRTLGTGELFF	9800	TRBV6-5		TRBJ2-2	Vbeta6-5	TGTGCCAGCAGAACTCTGGGAACCGGGGAGCTGTTTTTT
H14-2	CASSFRGNEQFF	4209	TRBV27	TRBD1	TRBJ2-1	Vbeta27	TGTGCCAGCAGTTTCAGGGGGAATGAGCAGTTCTTC
H14-3	CSGSPGGEQFF	4133	TRBV29-1	TRBD1	TRBJ2-1	Vbeta4/Vbeta4.3	TGCAGCGGCAGCCCGGGGGGTGAGCAGTTCTTC
H14-4	CSVGVAGPNTGELFF	4077	TRBV29-1	TRBD1	TRBJ2-2	Vbeta4/Vbeta4.3	TGCAGCGTTGGGGTGGCGGGGCCGAACACCGGGGAGCTGTTTTTT
H14-5	CASTPSGSRGGELFF	3668	TRBV19	TRBD1	TRBJ2-2	Vbeta17.1	TGTGCCAGTACCCCCTCGGGTTCGAGAGGCGGGGAGCTGTTTTTT
H36-1	CASSLEADYEQYF	67284	TRBV5-5	TRBD2	TRBJ2-7	Vbeta5/Vbeta5.3a	TGTGCCAGCAGCTTGGAAGCGGACTACGAGCAGTACTTC
H36-2	CSVEDQARGGEQFF	3943	TRBV29-1	TRBD1	TRBJ2-1	Vbeta4/Vbeta4.3	TGCAGCGTTGAAGATCAGGCCAGGGGGGGCGAGCAGTTCTTC
H36-3	CSAGGQGGSYEQYF	3404	TRBV29-1	TRBD1	TRBJ2-7	Vbeta4/Vbeta4.3	TGCAGCGCCGGAGGACAGGGTGGGTCCTACGAGCAGTACTTC
H36-4	CASSYSETRGAGELFF	3094	TRBV6-5	TRBD2	TRBJ2-2	Vbeta6-5	TGTGCCAGCAGTTACTCGGAGACTAGGGGGGCCGGGGAGCTGTTTTTT
H36-5	CASSDGLAGWETQYF	2734	TRBV9	TRBD2	TRBJ2-5	Vbeta1.1	TGTGCCAGCAGTGACGGACTAGCGGGGTGGGAGACCCAGTACTTC
H36-6	CASSLGRQGSYEQYF	2418	TRBV12-3	TRBD1	TRBJ2-7	Vbeta8.1	TGTGCCAGCAGTTTGGGGCGCCAGGGATCCTACGAGCAGTACTTC
H36-7	CASTRLGDYEKLFF	2417	TRBV19	TRBD1	TRBJ1-4	Vbeta17.1	TGTGCCAGTACCCGGCTGGGTGACTATGAAAAACTGTTTTTT
H36-8	CAISGPSNEQFF	2255	TRBV12-3	TRBD2	TRBJ2-1	Vbeta8.1	TGTGCCATTAGCGGGCCGTCCAATGAGCAGTTCTTC
H36-9	CASSEEWDRGNTDTQYF	2132	TRBV6-9	TRBD1	TRBJ2-3	Vbeta13.4	TGTGCCAGCAGTGAGGAGTGGGACAGGGGAAACACAGATACGCAGTATTTT
H36-10	CASSPNGGPYGYTF	1781	TRBV12-3	TRBD1	TRBJ1-2	Vbeta8.1	TGTGCCAGCAGCCCGAATGGGGGTCCCTATGGCTACACCTTC
H36-11	CASSYSYEQYF	1458	TRBV6-5		TRBJ2-7	Vbeta6-5	TGTGCCAGCAGTTACAGCTACGAGCAGTACTTC
H36-12	CSVWTVTNYGYTF	1378	TRBV29-1		TRBJ1-2	Vbeta4/Vbeta4.3	TGCAGCGTGTGGACAGTTACAAACTATGGCTACACCTTC
H43-7	CSVQTGAGELFF	1000	TRBV29-1	TRBD1	TRBJ2-2	Vbeta4/Vbeta4.3	TGCAGCGTCCAAACAGGGGCCGGGGAGCTGTTTTTT
H43-8	CSGETADTQYF	908	TRBV29-1	TRBD2	TRBJ2-3	Vbeta4/Vbeta4.3	TGCAGCGGGGAGACGGCAGATACGCAGTATTTT
H43-9	CASSLVGTENTEAFF	879	TRBV12-3	TRBD1	TRBJ1-1	Vbeta8.1	TGTGCCAGCAGTTTAGTCGGGACAGAGAACACTGAAGCTTTCTTT
H63-2	CASSEGLWGQQVGNQPQHF	6287	TRBV6-1	TRBD1	TRBJ1-5	Vbeta13.3	TGTGCCAGCAGTGAAGGGCTTTGGGGACAGCAAGTAGGCAATCAGCCCCAGCATTTT
H63-3	CASSLGGRTGTDTQYF	6074	TRBV13		TRBJ2-3	Vbeta22	TGTGCCAGCAGCTTAGGCGGTAGGACGGGCACAGATACGCAGTATTTT
H63-4	CAIRTSGEQYF	5039	TRBV10-3	TRBD2	TRBJ2-7	Vbeta12.2	TGTGCCATCAGGACTAGCGGGGAGCAGTACTTC
H63-5	CASSSYRVFEKLFF	5014	TRBV27	TRBD1	TRBJ1-4	Vbeta27	TGTGCCAGCAGTTCGTACAGGGTTTTTGAAAAACTGTTTTTT
H63-6	CASSFGGAGEQYF	3456	TRBV5-6		TRBJ2-7	Vbeta5-6	TGTGCCAGCAGCTTTGGCGGAGCCGGCGAGCAGTACTTC
H63-7	CASRHGAEGNQPQHF	2649	TRBV27	TRBD1	TRBJ1-5	Vbeta27	TGTGCCAGCCGCCACGGGGCGGAAGGGAATCAGCCCCAGCATTTT
H63-8	CASSLGETVRGTDTQYF	2204	TRBV7-9	TRBD1	TRBJ2-3	Vbeta6.5	TGTGCCAGCAGCTTGGGGGAGACAGTGAGGGGCACAGATACGCAGTATTTT
H63-9	CASSQALGTNYGYTF	2083	TRBV4-2		TRBJ1-2	Vbeta7	TGTGCCAGCAGCCAAGCTCTGGGCACTAACTATGGCTACACCTTC
H63-10	CASSWLTGGTEAFF	1854	TRBV27	TRBD1	TRBJ1-1	Vbeta27	TGTGCCAGCAGCTGGTTGACAGGGGGCACTGAAGCTTTCTTT
H63-11	CASSPGVTGANVLTF	1629	TRBV7-9		TRBJ2-6	Vbeta6.5	TGTGCCAGCAGCCCAGGTGTAACGGGGGCCAACGTCCTGACTTTC
H63-12	CASSGHLGLGSYGYTF	1231	TRBV6-1	TRBD1	TRBJ1-2	Vbeta13.3	TGTGCCAGCAGTGGCCACCTGGGGCTGGGGTCCTATGGCTACACCTTC
H63-14	CASSLAAGVVYEQYF	1039	TRBV12-3	TRBD1	TRBJ2-7	Vbeta8.1	TGTGCCAGCAGTTTGGCGGCAGGGGTAGTCTACGAGCAGTACTTC
H63-15	CASTTGGVLYNEQFF	518	TRBV12-3	TRBD2	TRBJ2-1	Vbeta8.1	TGTGCCAGCACCACGGGGGGGGTCCTCTACAATGAGCAGTTCTTC

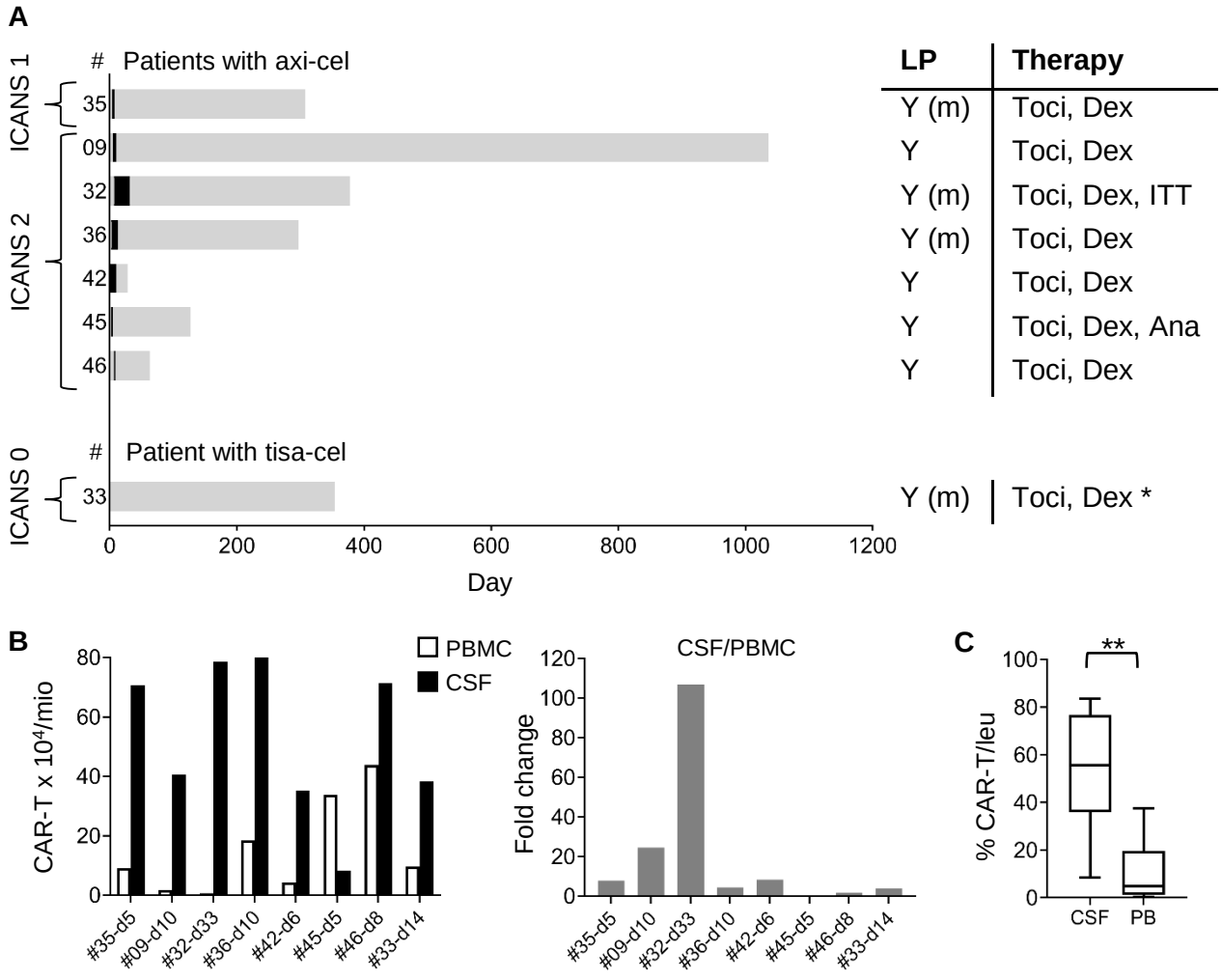
Aa, amino acid; CDR, complementarity-determining region 3, H, hyperexpanded clone; NT, nucleotide sequence; TRB, T-cell receptor-β. * alternative V name.
The 15 hyperexpanded clones that occupied 94% of the clonal space on day 63 are highlighted (box with bold borders).



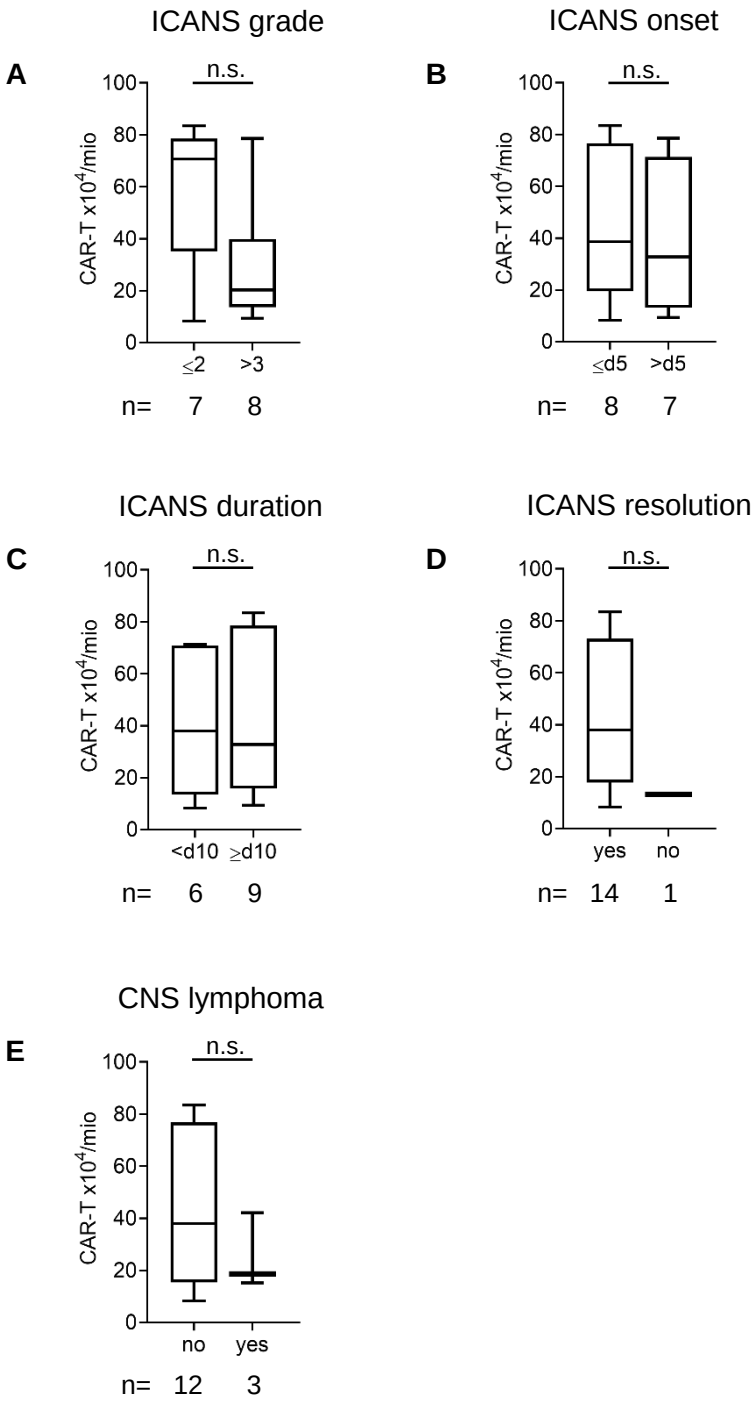
Online Supplementary Figure S1. **Biomarker analysis for tumor burden, inflammation, and CAR-T cell expansion in real-world B-cell tumor patients.** 48 patients were treated with axicel (n=35) or tisa-cel (n=13) and evaluated for symptoms of neurotoxicity. (A-C) Boxplots showing serum levels of (A) baseline LDH, (B) peak IL-6, and (C) IL-6 on the day of the CAR-T peak level in correlation with ICANS. Blue lines: (A) normal LDH range. Boxplots show median (line), 25th and 75th percentiles (upper and lower box borders), and range (whiskers). (D) Pie charts showing the proportion of patients with grade ≤1 CRS versus >1 CRS in correlation with ICANS. Statistical significance: *, $P \leq 0.05$; **, $P \leq 0.01$; ****, $P \leq 0.0001$; n.s. not significant.



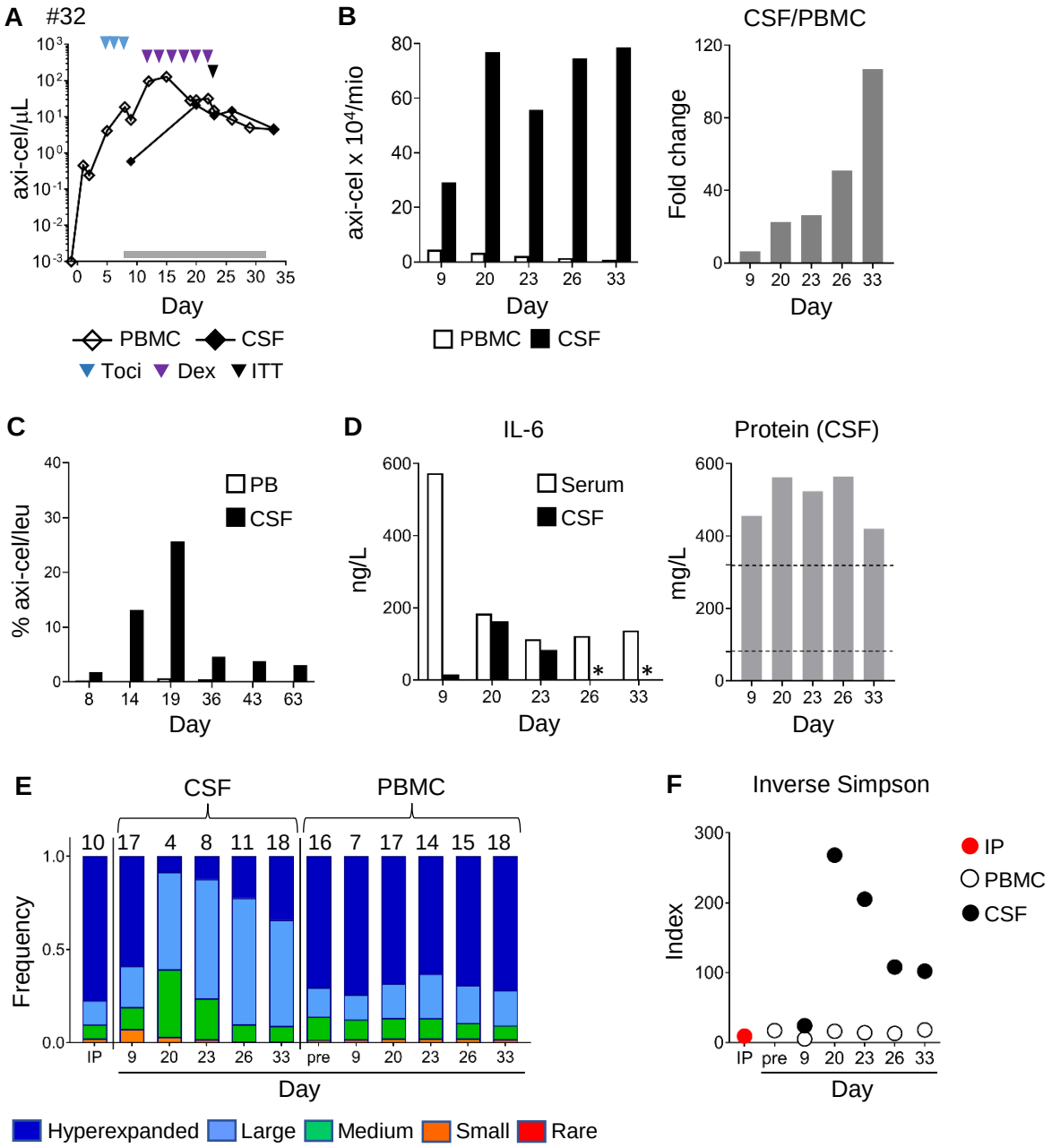
Online Supplementary Figure S2. **Analysis of CAR-T cell kinetics in the peripheral blood.** 48 patients with advanced B-cell tumors received axi-cel (n=35, open diamond) or tisa-cel (n=13; closed diamond) and were evaluated for CAR-mediated neurotoxicity. PBMC were obtained at the indicated days and examined by dPCR for the presence of CAR-T cells. Left panels: CAR-T cells per μL. Right panels: CAR-T cells per million MNC.



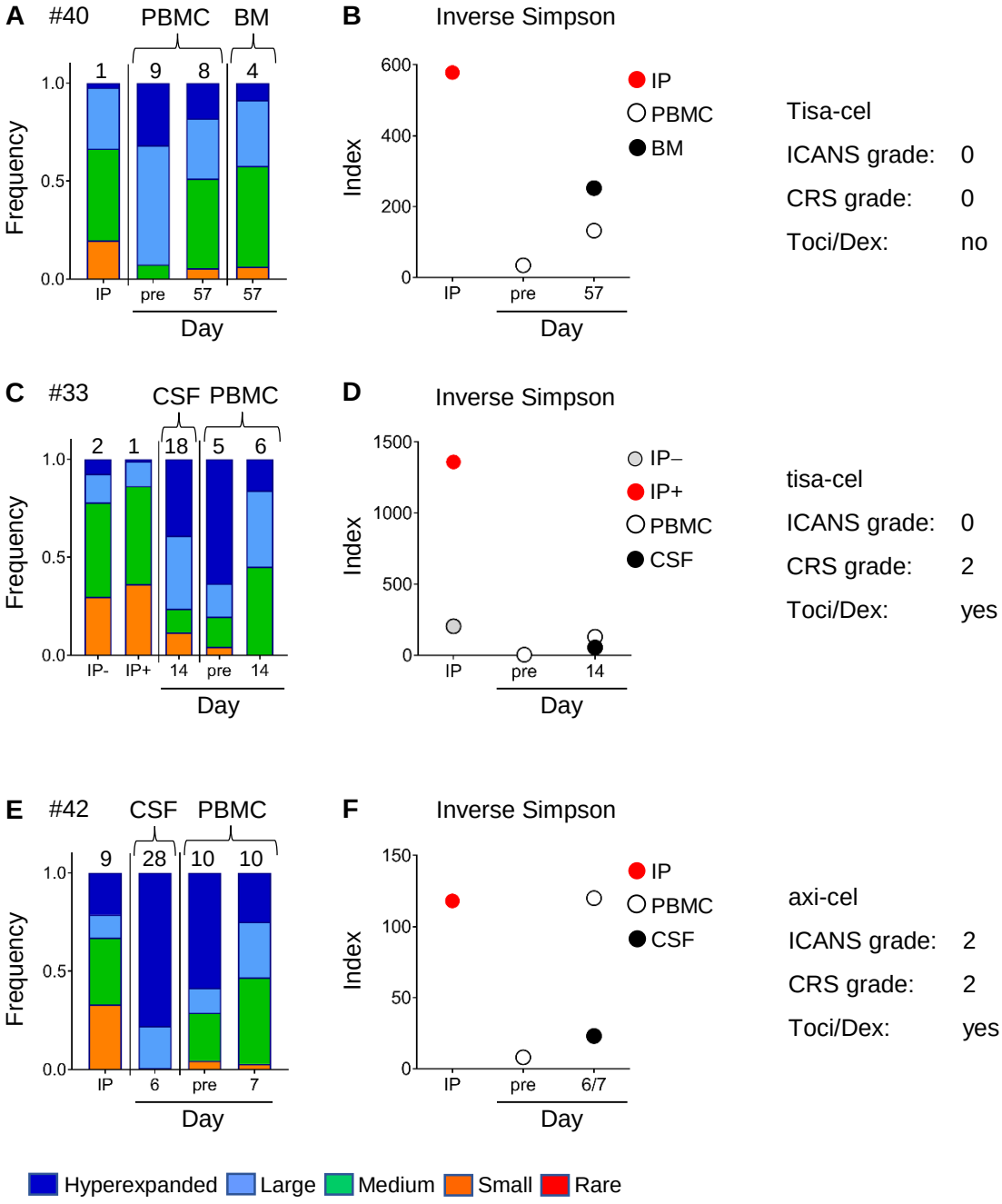
Online Supplementary Figure S3. **Detection of CAR-T cells in the CSF in B-cell lymphoma patients with grades 0-2 neurotoxicity.** (A) Schematic overview of patients. Days of ICANS are highlighted in black. ITT with Dex, Ara, and MTX. *CRS-directed treatment. Last updated May 31st, 2022. (B-C) CAR-T cell kinetics in PBMC (□) and CSF (■). Aliquots of diagnostic CSF and corresponding PB were obtained at indicated days (d) after CAR-T cell infusion and examined by CAR-specific dPCR. (B) Left panel: Absolute frequencies of CAR-T cells per million MNC. Right panel: Fold change (CSF versus PBMC). Shown are results of the maximal enrichment if serial diagnostic specimen were obtained. (C) Proportion of absolute CAR-T cell numbers within white blood cells in CSF versus PB. Statistical significance: **, $P \leq 0.01$.



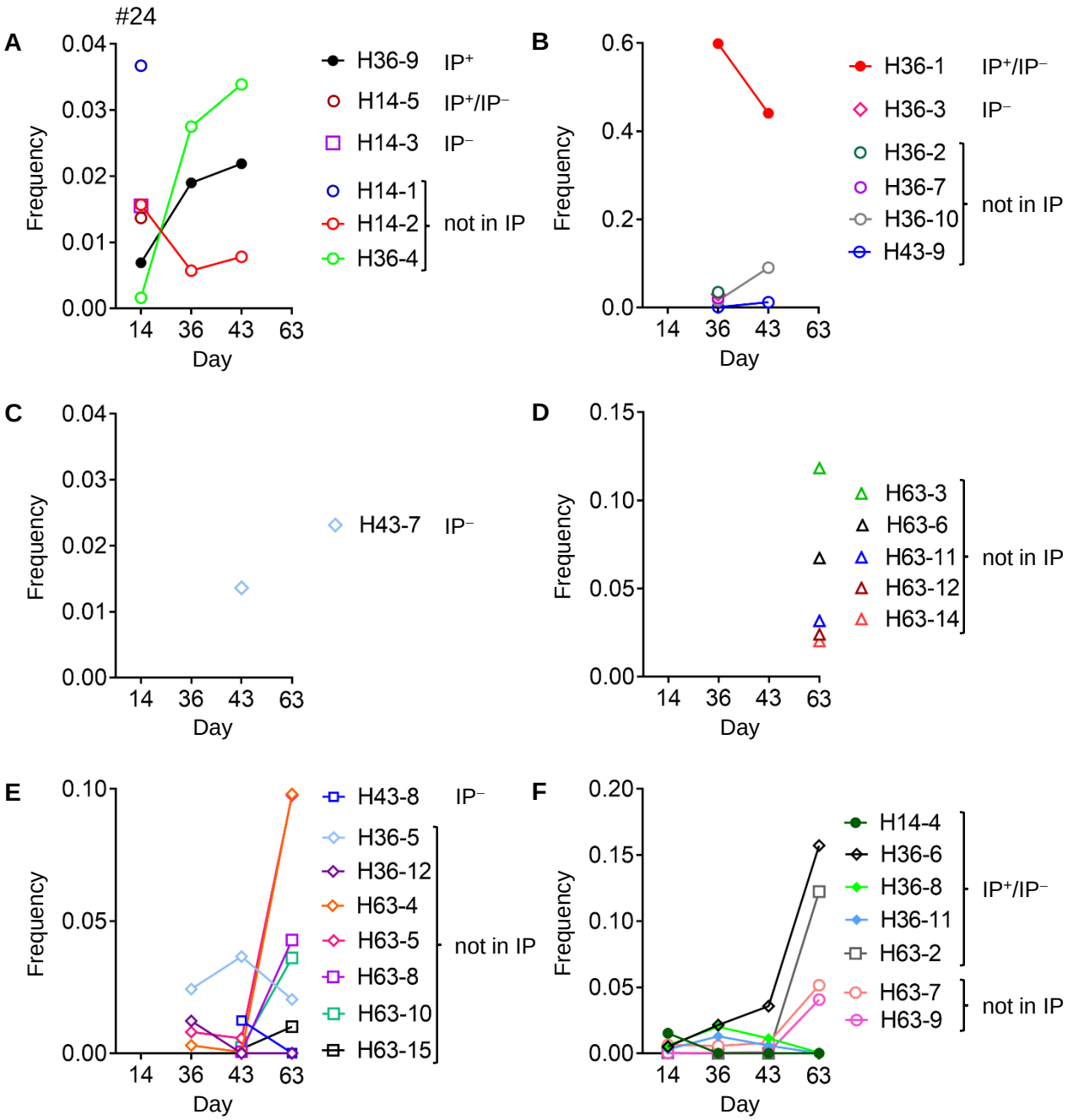
Online Supplementary Figure S4. **Correlation of CAR T-cell enrichment in CSF with clinical features in ICANS patients.** Diagnostic CSF samples were available from 15 patients with grades 1-4 neurotoxicity after treatment with axi-cel (n=12) or tisa-cel (n=3) and examined by dPCR for the frequency of CAR-T cells. The results were correlated with selected clinical parameters including the (A) ICANS grade, (B) ICANS onset either ≤ or > day (d) 5, (C) ICANS duration, (D) ICANS resolution, and (E) history or presence of CNS lymphoma. Statistical significance: n.s. not significant.



Online Supplementary Figure S5. **Axi-cel kinetics in patient #32 with grade 2 ICANS.** (A-C) Samples of PBMC and CSF were examined by dPCR for the presence of CAR-T cells. (A) Absolute CAR-T cell numbers in PBMC (◇) and CSF (◆). Arrow heads: blue, Toci; purple, Dex; black, ITT with Dex, Ara, MTX. Grey bar: ICANS-duration. (B) Left panel: frequency of CAR-T cells per million MNC in PB (□) and CSF (■). Right panel: fold change in CSF versus PBMC. (C) Proportion of absolute CAR-T numbers in leu of CSF versus PB. (D) Inflammatory marker. Left panel: IL-6 in serum (□) or CSF (■), right panel: total protein in CSF. Dotted lines: normal range. *, not done. (E, F) Longitudinal TRB-repertoire analysis. (E) Clonal space distribution. TRB-NGS analysis of gDNA from CSF, PBMC, or the IP. Dark blue: hyperexpanded clones ($0.01 \leq X \leq 1$); blue: large ($1^{-03} \leq X \leq 0.01$); green: medium ($1^{-04} \leq X \leq 1^{-03}$); orange, small ($1^{-05} \leq X \leq 1^{-04}$), red: rare ($0 \leq X \leq 1^{-05}$). The inset values above the bars represent the number of hyperexpanded clones. (F) Repertoire metrics shown as inverse Simpson index.



Online Supplementary Figure S6. **Longitudinal changes in the TRB repertoire in patients without or with low grade ICANS.** TRB-NGS analysis of gDNA from CSF, PBMC, BM, or the IP subsets. (A, C, E) Clonal space distribution and number of hyperexpanded clones on the indicated days. Dark blue: hyperexpanded clones ($0.01 \leq X \leq 1$); blue: large ($1^{-03} \leq X \leq 0.01$); green: medium ($1^{-04} \leq X \leq 1^{-03}$); orange, small ($1^{-05} \leq X \leq 1^{-04}$), red: rare ($0 \leq X \leq 1^{-05}$). The inset values above the bars indicate the number of hyperexpanded clones. (B, D, F) Repertoire metrics. Shown is the corresponding inverse Simpson index.



Online Supplementary Figure S7. **Detection of multiple unique hyperexpanded T-cell clones in the CSF.** Analysis of the TRB repertoire in patient #24 with a treatment-resistant grade 4 ICANS after tisa-cel therapy. gDNA was extracted from serial samples of CSF and examined by TRB-NGS. Shown are the frequencies of unique hyperexpanded clones ($0.01 \leq X \leq 1$) that were detectable over time on single or multiple time points in CSF.