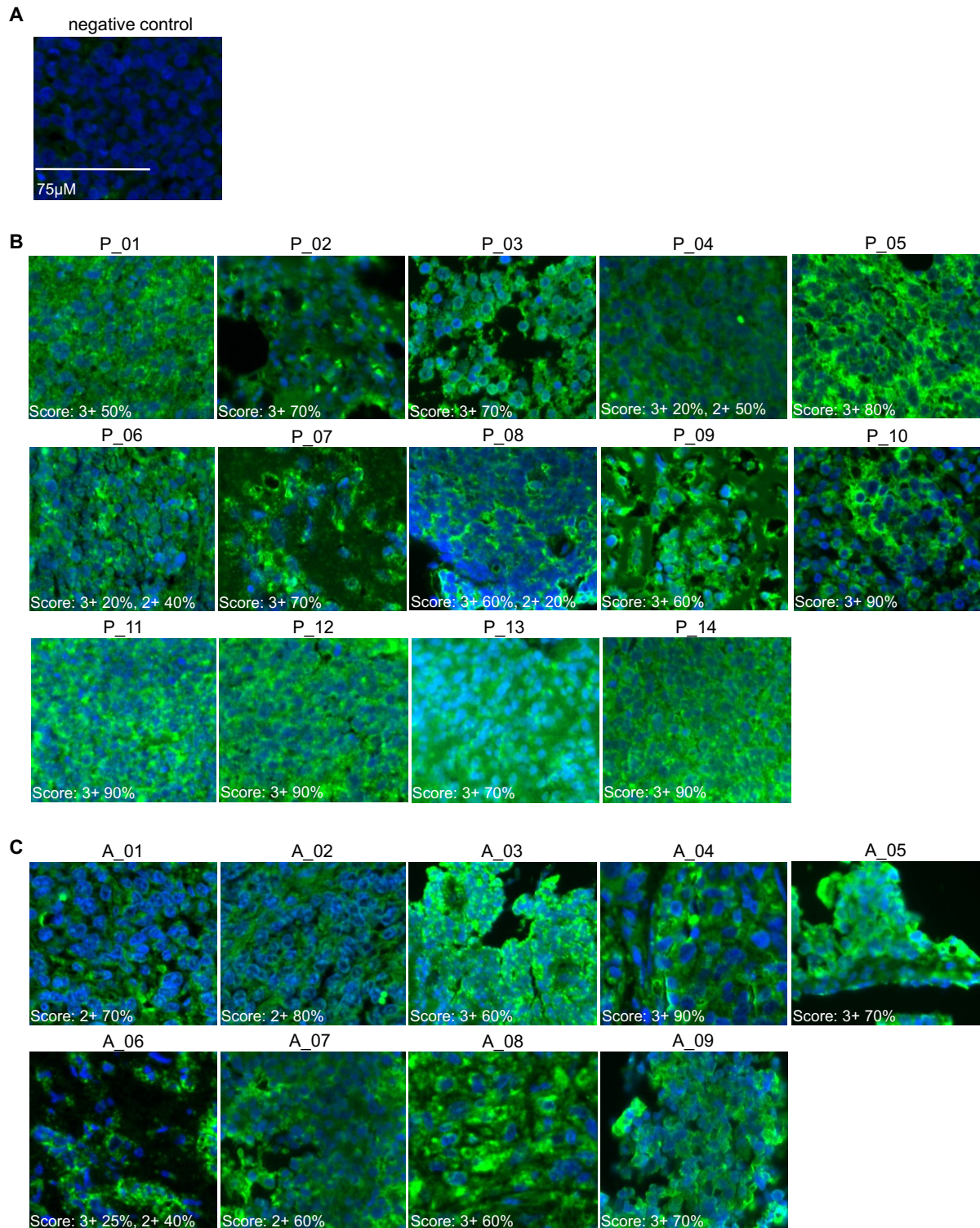


Extended Data

To: Mackensen A, Fischer-Riepe L et al. GD2 CAR T cells with inducible IL18 for patients with refractory solid cancers

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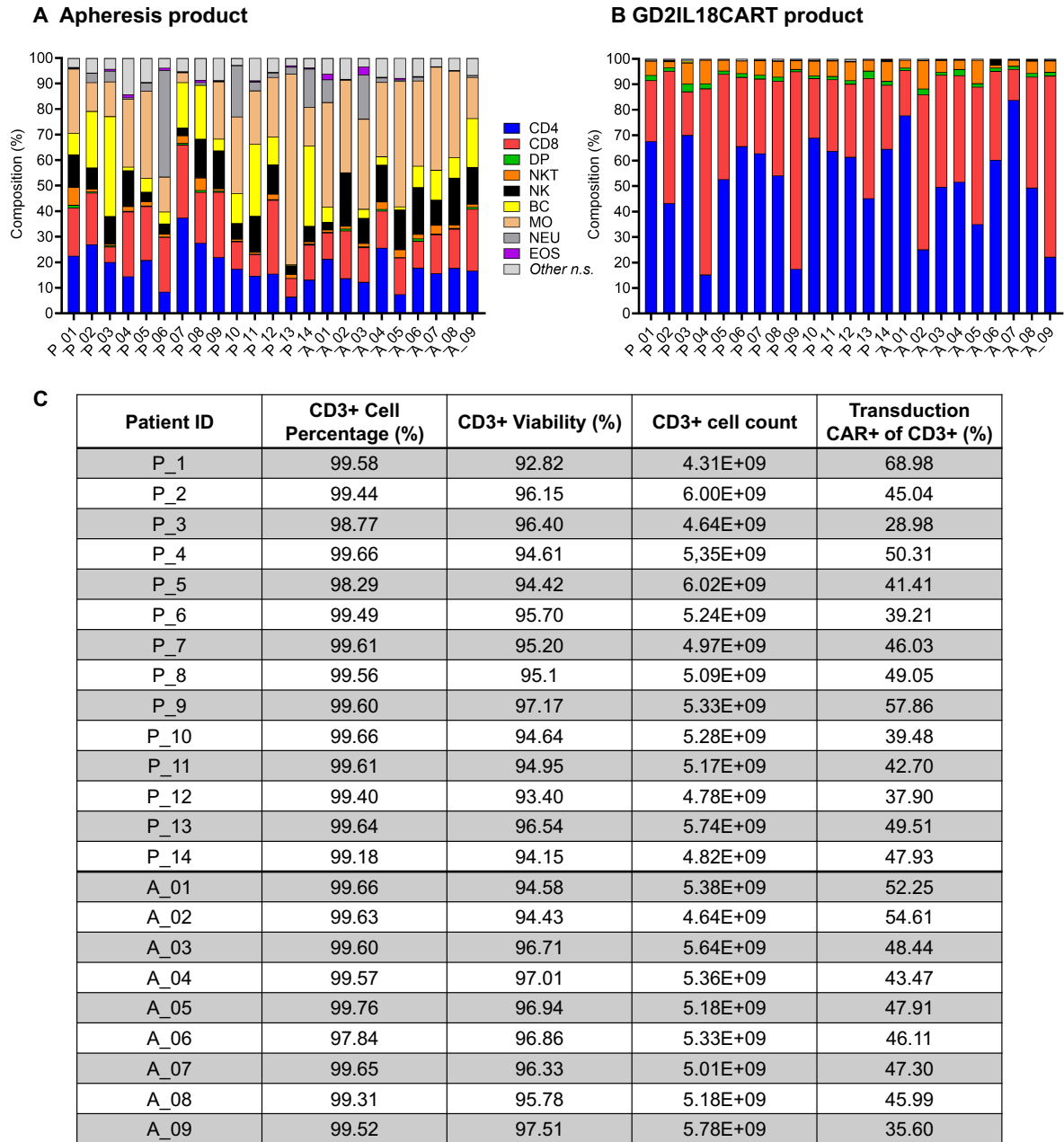
Extended Data Figure 1. GD2 expression in tumor tissue from all enrolled patients.

GD2 expression was assessed by immunofluorescence staining of archival formalin-fixed, paraffin-embedded (FFPE) biopsies in a central reference laboratory using a standardized assay²⁸. GD2 expression was evaluated by direct microscopic assessment according to a semiquantitative scoring system, with GD2 positivity defined according to clinical trial protocol criteria as $\geq 50\%$ of tissue expressing $\geq 2+$ GD2²⁸. The most recent available biopsy for each patient was analyzed. Images shown are representative regions of interest (ROIs) from the entire tissue section and were acquired for documentation purposes only. The timing and origin of individual biopsies are summarized below. (A) GD2 staining of a murine tumor

xenograft derived from the Ewing sarcoma cell line SK-ES-1 as a negative control. (B, C) GD2 staining of pretreatment tumor biopsies from pediatric (B) and adult (C) patients.

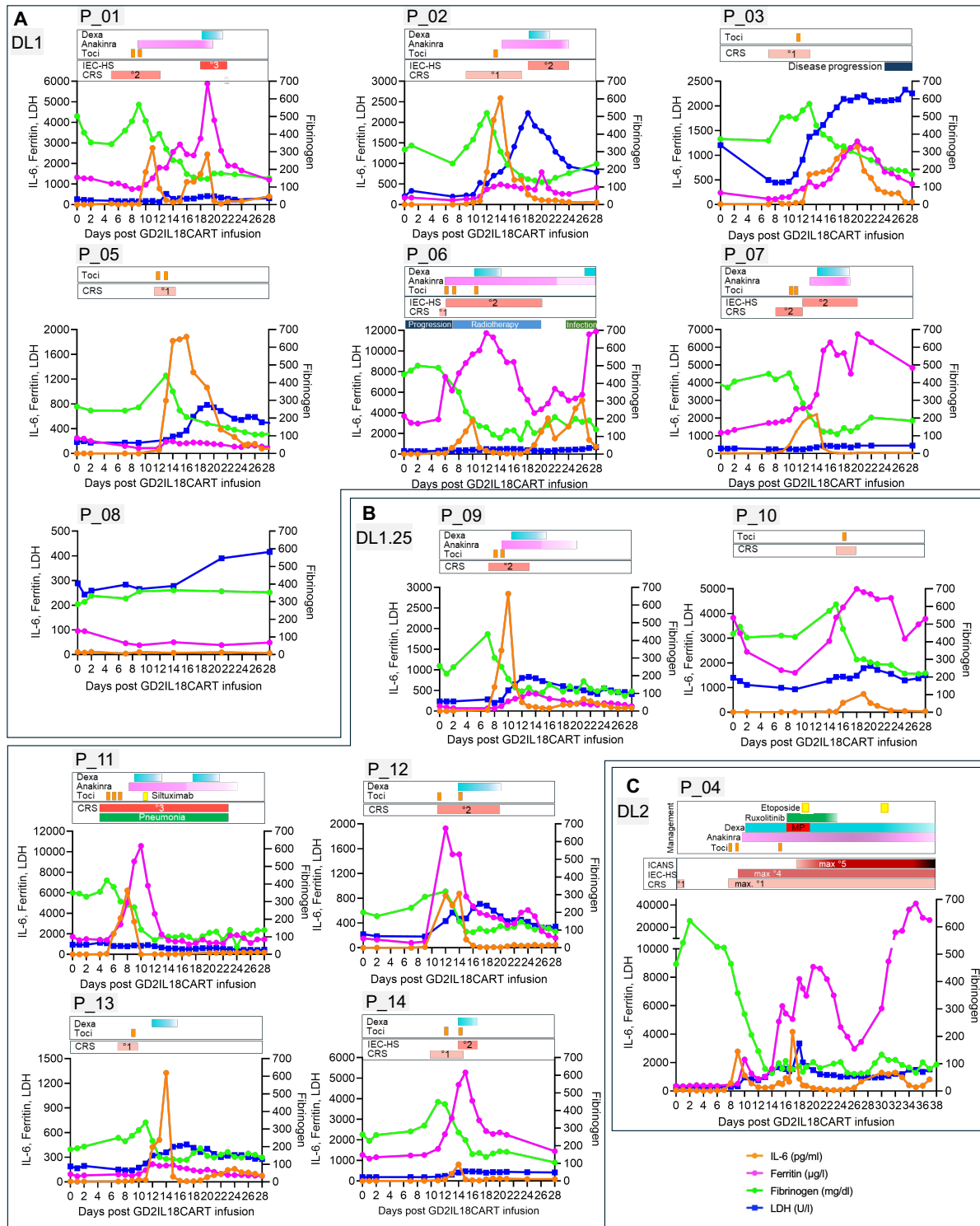
Patient	Disease	Time of biopsy	Origin of biopsy
P_01	NBL	First of four relapses prior to GD2IL18CART	Lymph node metastasis
P_02	NBL	Most recent relapse	Bone metastasis
P_03	NBL	Most recent relapse	Bone metastasis
P_04	EwS	Most recent relapse	Bone metastasis
P_05	NBL	Most recent relapse	Bone metastasis
P_06	NBL	Earlier relapse	Bone metastasis
P_07	OS	Most recent relapse	Bone metastasis
P_08	NBL	Most recent relapse	Bone and soft tissue metastasis
P_09	OS	Most recent relapse	Bone metastasis
P_10	NBL	Second of three relapses prior to GD2IL18CART. GD2-positive status of the most recent relapse after first GD2-CART therapy was confirmed by bone marrow flow cytometry.	Bone metastasis
P_11	NBL	Most recent relapse	Abdominal metastasis
P_12	NBL	Earlier relapse	Brain metastasis
P_13	NBL	First diagnosis	Abdominal primary tumor
P_14	NBL	First diagnosis	Abdominal primary tumor
A_01	EwS	Earlier relapse	Soft tissue metastasis
A_02	EwS	Earlier relapse	Pulmonary metastasis
A_03	EwS	First diagnosis	Pelvic bone tumor
A_04	BC	Most recent relapse	Skin metastasis
A_05	EwS	First diagnosis	Bone metastasis
A_06	BC	Earlier relapse	Liver metastasis
A_07	EwS	First diagnosis	Vertebral bone tumor
A_08	OS	Most recent relapse	Lung metastasis
A_09	EwS	Most recent relapse	Pulmonary metastasis

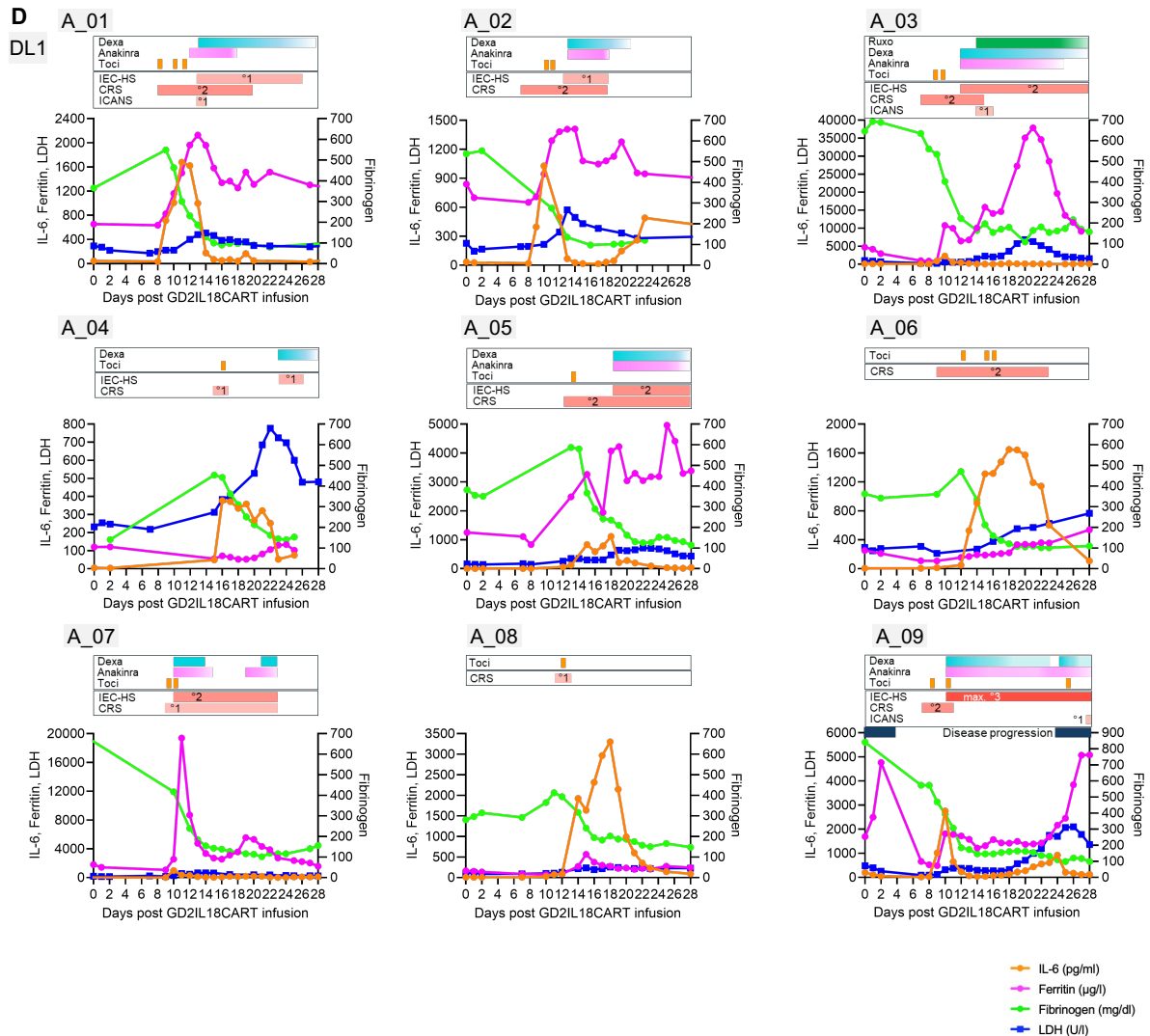
NBL, neuroblastoma; EwS, Ewing sarcoma; OS, osteosarcoma; BC, breast cancer.



Extended Data Figure 2. Product characteristics

(A, B) Cellular composition of the apheresis products **(A)** and GD2IL18CART products **(B)** determined by immunophenotyping for all patients. Cell populations include neutrophils (NEU, CD3-CD14-CD19-CD56+CD16+SSC^{high}), eosinophils (EOS, CD3-CD14-CD19-CD56-CD16-SSC^{high}); monocytes (MO, CD3-CD14+), B cells (BC, CD3-CD19+), natural killer (NK) cells (CD3-CD14-CD19-CD56+CD16+SSC^{low}), natural killer T cells (NKT, CD3+CD56+CD16+), CD8+ T cells (CD3+CD56-CD16-CD4-CD8+), CD4+ T cells (CD3+CD56-CD16-CD4+CD8-) and double positive (DP, CD4+/CD8+) T cells. **(C)** Release parameters of final GD2IL18CART products for all patients.





Extended Data Figure 3. Clinical course, inflammatory toxicities and management after GD2IL18CART infusion.

Clinical courses, key laboratory parameters, inflammatory toxicities and anti-inflammatory interventions from GD2IL18CART infusion (day 0) through day 28 are shown for all patients, **(A)** pediatric patients treated at DL1 (1×10^5 CAR+ T cells/kg). **(B)** Pediatric patients treated at DL1.25 (2×10^5 CAR+ T cells/kg). **(C)** Single pediatric patient treated at DL2 (5×10^5 CAR+ T cells/kg). **(D)** Adult patients treated at DL1. Maximum toxicity grades are indicated. Individual patients with notable clinical events are described below.

P_03: In this patient with neuroblastoma, a large soft tissue mass in the right humerus rapidly progressed with associated pain from day 24 after GD2IL18CART infusion, despite continued detection of CAR T cells in peripheral blood. Local radiotherapy was administered to a total dose of 36 Gy. The patient died from progressive disease on day 78.

P_04: The single patient treated at this dose was an 8-year-old boy with chemotherapy-refractory, progressive multifocal soft tissue and bone relapse of Ewing sarcoma, including a skull metastasis with extradural contact to the medial temporal lobe. Following fever on day 1 (grade 1 CRS) and a second grade 1 CRS episode on day 8, IEC-HS emerged and progressed despite treatment with tocilizumab, anakinra, corticosteroids and ruxolitinib. Emapalumab was not available for off-label use in Germany. Until day 16, the patient remained fully responsive and clinically active without neurological symptoms. On day 17, he developed a seizure. Brain imaging showed focal brainstem edema, followed by dysphagia requiring ICU transfer and intubation (ICANS grade 4). Etoposide (50 mg/m^2) was administered as third-line anti-inflammatory therapy. Two days later, the patient became unresponsive and remained

comatose. Although follow-up MRI scans showed gradual resolution of brainstem edema without parenchymal damage, irreversible brain injury was documented on the final MRI performed on day 37, and the patient died on day 38. Because direct dural involvement by the skull metastasis could not be excluded as a contributing factor to neurological hyperinflammatory manifestations, the protocol was amended to exclude patients with metastases in direct contact with the dura.

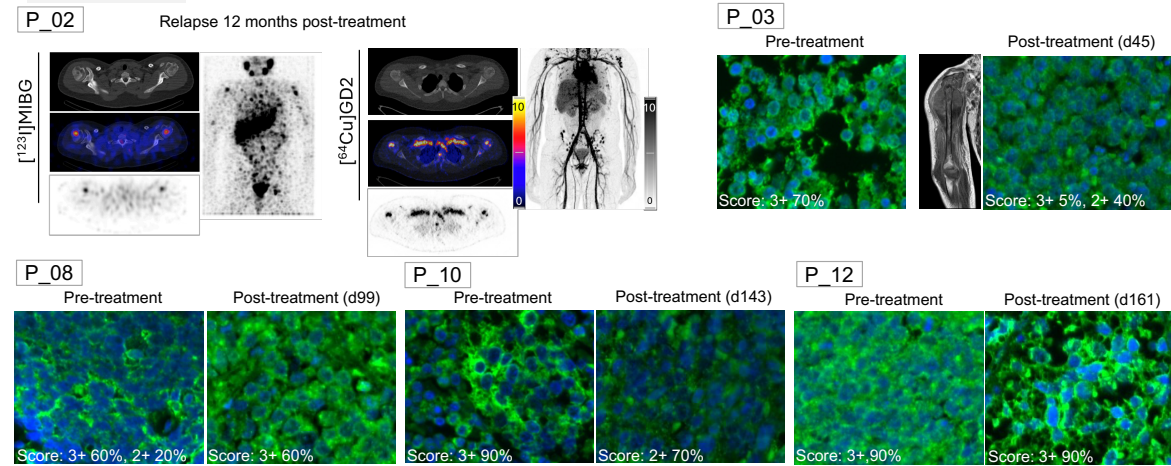
P_06. At enrollment, the patient had highly refractory, slowly progressive multifocal neuroblastoma, including metabolically active cervical and axillary lymph node metastases. Rapid tumor growth during the first days after GD2IL18CART infusion led investigators to initiate radiotherapy on day 4 (total dose 24 Gy in 12 fractions of 2 Gy), to prevent compression of cervical vessels and ulcerative growth. On day 24, the patient developed new persistent fever with capillary leak syndrome after resolution of CAR T-cell-associated inflammatory toxicities. Chest CT revealed bilateral pulmonary infection with radiographic features suggestive of aspergillosis. The episode was successfully managed with antifungal therapy and a short second course of dexamethasone. After consultation with the DMC, the late inflammatory episode was considered more consistent with an infectious trigger than with GD2IL18CART-related toxicity. The patient remained neutropenic and subsequently received a hematopoietic stem cell boost from the donor of a prior haploidentical stem cell transplantation. Persistent grade 4 hematotoxicity without resolution by day 28 fulfilled the protocol definition of DLT. The patient died from progressive disease on day 104.

P_11: In this patient with refractory neuroblastoma, CRS onset on day 5 coincided with bilateral pneumonia, which responded to broad-spectrum antimicrobial therapy. To limit inflammatory escalation in this setting of a concomitant infectious trigger, the patient received tocilizumab, one dose of siltuximab, anakinra and a dexamethasone pulse. The patient recovered within days and subsequently developed a manageable episode of capillary leak syndrome at the peak of CAR T-cell expansion, without progression to IEC-HS, together with radiographic worsening of bilateral pneumonia. A second steroid pulse and intensified antimicrobial treatment resulted in rapid clinical recovery and resolution of pneumonia. The patient died from progressive disease on day 109.

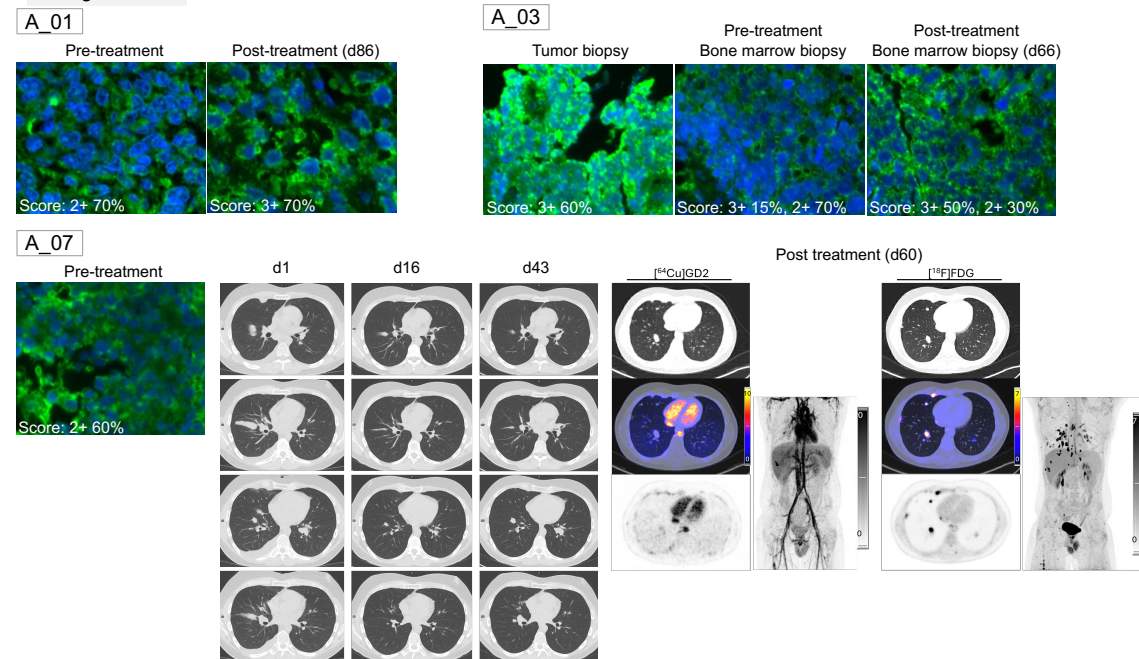
A_03: This patient with disseminated Ewing sarcoma had multiple disease manifestations involving bone, bone marrow, visceral organs and lymph nodes at the time of infusion. Although inflammatory toxicity remained limited to grade 2 or lower after infusion, persistent fever and rising liver enzymes despite treatment with anakinra and corticosteroids prompted the addition of ruxolitinib. Failure of hematological recovery by day 28 occurred in the context of both bone marrow disease progression and ongoing inflammation and fulfilled the protocol definition of DLT. The patient recovered from neutropenia after a stem cell boost but subsequently died from progressive disease before day 56.

CRS, cytokine release syndrome; DL, dose level; DLT, dose-limiting toxicity; DMC, data monitoring committee; IEC-HS, immune effector cell-associated hemophagocytic lymphohistiocytosis-like syndrome; IL-6, interleukin-6; LDH, lactate dehydrogenase. DL1, 1×10^5 CAR+ cells/kg, DL2, 5×10^5 CAR+ cells/kg, DL1.25, 2×10^5 CAR+ cells/kg.

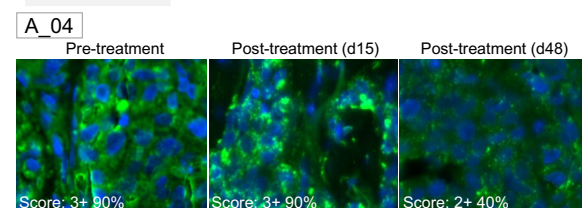
A Neuroblastoma



B Ewing sarcoma



C Breast cancer



Extended Data Figure 4. GD2 expression in tumor tissues before and after GD2IL18CART infusion. GD2 expression was determined by immunofluorescence staining in FFPE tumor biopsies obtained before treatment and at indicated post-treatment time points in selected patients with (A) neuroblastoma, (B) Ewing sarcoma and (C) breast cancer. Staining and semiquantitative scoring were performed as described in Extended Data Figure 1 and previously²⁸. For P_02 and A_07, noninvasive *in vivo* GD2 imaging using [64Cu]Cu-NOTA-ch14.18/CHO-PET/CT was performed outside the clinical study. Images are shown in comparison with [123I]-mIBG scintigraphy (P_02) and [18F]FDG-PET/CT (A_07), respectively. Serial chest CT images in A_07 demonstrate a transient partial response of pleuropulmonary Ewing sarcoma manifestations on day 16, followed by recurrence on day 43.

A

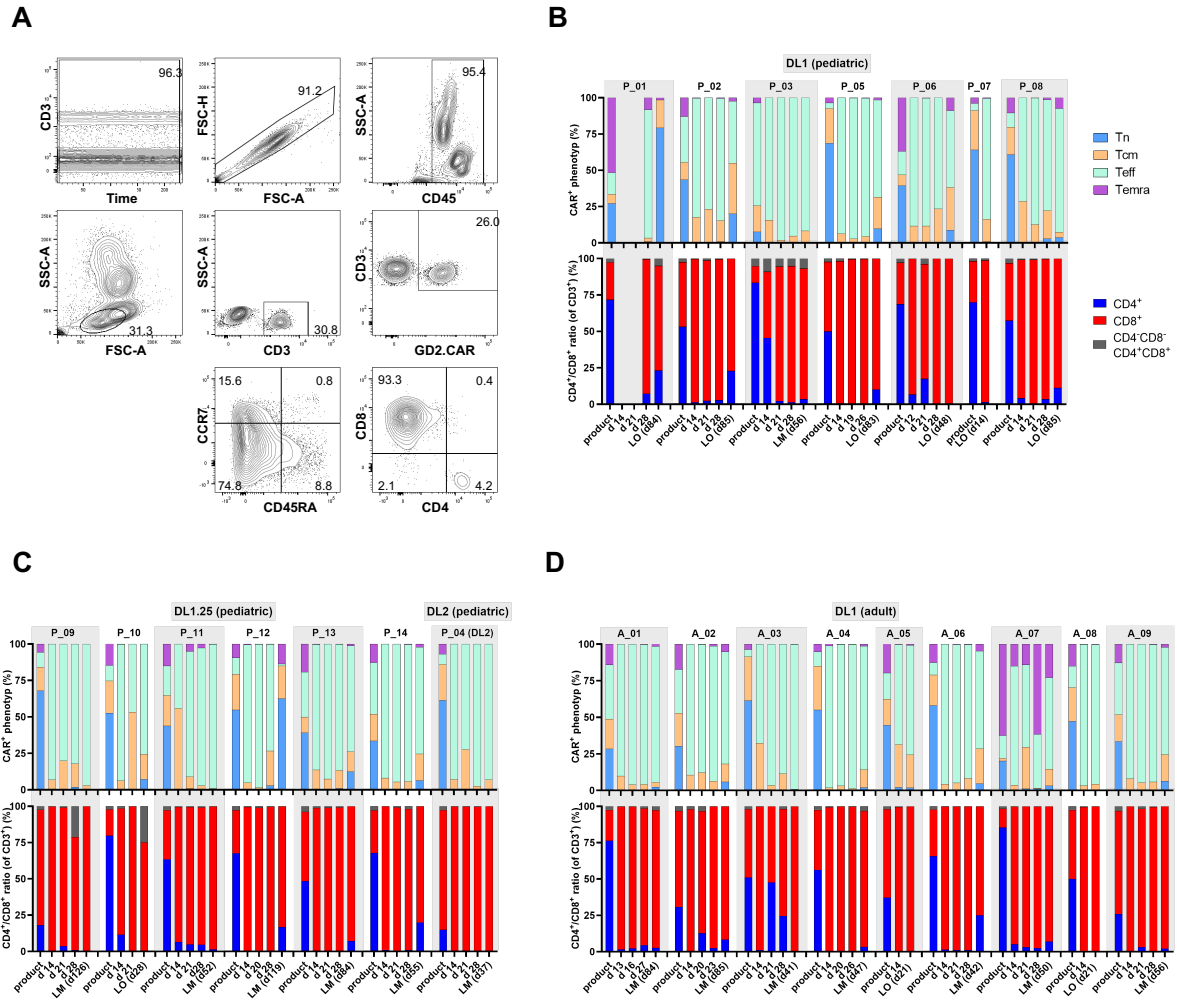
Dose level	Patient	Day	HACA µg/ml	Dose level	Patient	Day	HACA µg/ml
DL1	P_01	-6	0.23	DL1.25	P_09	-5	<0
		28	0.40			28	<0
		364	0.41			126	<0
	P_02	-5	<0		P_10	-5	<0
		28	<0			28	<0
		371	0.177			93	<0
	P_03	-5	<0		P_11	-5	0.21
		28	<0			28	<0
		56	<0			52	<0
	P_05	-5	<0		P_12	-5	<0
		26	<0			28	<0
		181	<0			77	<0
	P_06	-5	0.86		P_13	-5	<0
		28	1.10			28	<0
		48	1.41			56	<0
	P_07	-5	0.28		P_14	-5	0.08
		28	<0			28	<0
		294	<0			55	<0
DL2	P_08	-5	0.22	DL2	P_04	-6	<0
		28	0.28			28	<0
		121	0.48				

B

Dose level	Patient	Day	HACA µg/ml
DL1	A_01	-5	<0
		27	<0
		84	<0
	A_02	-5	<0
		23	<0
		85	<0
	A_03	-5	0.43
		28	0.52
		52	0.29
	A_04	-5	<0
		26	<0
	A_05	-5	<0
		28	<0
		85	<0
	A_06	-5	<0
		28	<0
	A_07	-5	<0
		28	0.03
	A_08	-5	4.93
		28	5.79
		57	5.88
	A_09	-6	<0
		28	<0

Extended Data Figure 5. Human anti-chimeric antibody (HACA) analysis.

Serum samples collected from all patients were analyzed at baseline (-5 or day -6, start of lymphodepletive therapy), on day 28 (+/- 2 days) and at last serum assessment with a validated ELISA allowing for the detection of anti-14.18 antibodies. **(A)** Pediatric patients. **(B)** Adult patients. The lower limits of detection (LOD) of all five experiments performed ranged between 0.00 and 1.98 µg/ml (median 0.44, SD 0.91), and lower limits of quantification (LLOQ) between 0.21 and 2.34 (median 1.21, SD 0.95). Values above LOD/LLOQ are marked in yellow (A_08). The isolated positive result in patient A_08 (osteosarcoma, non-responder) is unlikely to represent treatment-induced HACA, as reactivity was already present at baseline before exposure to GD2IL18CART or previous GD2-directed antibody therapy. Orange, neuroblastoma; turquoise, osteosarcoma; purple, Ewing sarcoma; green, breast cancer.

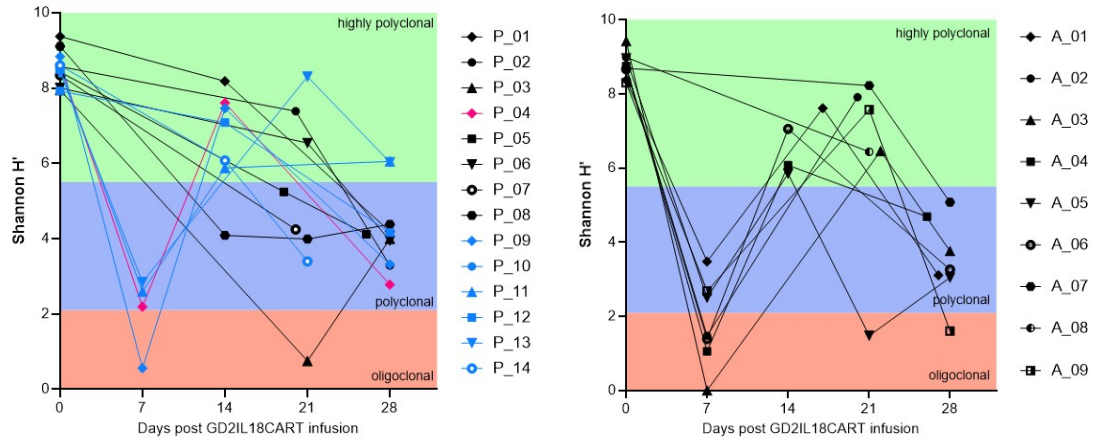


Extended Data Figure 6. CAR T-cell phenotypes in the cell product and in peripheral blood during *in vivo* expansion by flow cytometry.

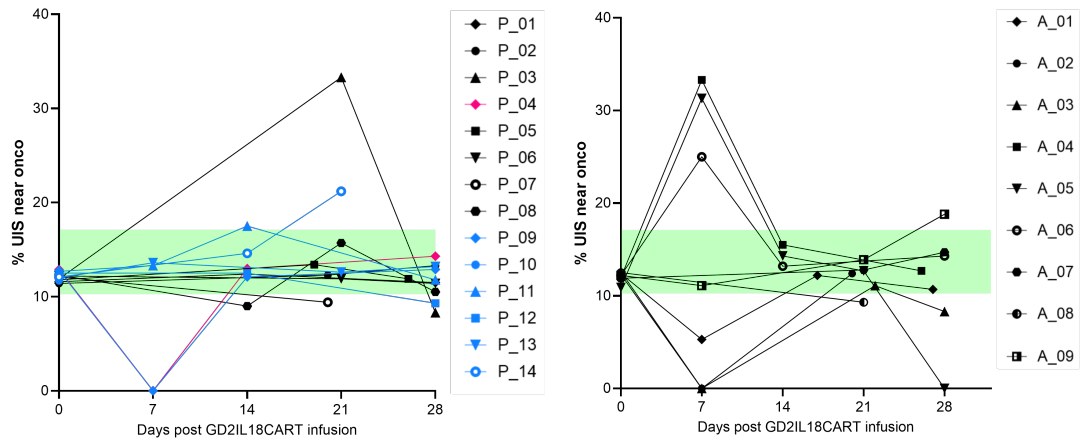
Time parameter was used to monitor instrument stability, doublets were excluded by FSC-H/FSC-A, lymphocytes were determined by CD45⁺/SSC-A, T cells were gated by CD3⁺. T cells were further subdivided into CAR-positive T cells (black) and CAR-negative T cells (grey). Populations of CAR-positive and CAR-negative T cells were subdivided into CD4⁺ and CD8⁺ T cells. T-cell differentiation of CAR-positive and CAR-negative T cells was determined by CCR7/CD45RA staining.

(A) Gating strategy. Proportions of CAR T cells with the indicated phenotypes in the peripheral blood of patients post CAR T cell infusion. **(B-D)** CAR T-cell analyses from **(B)** pediatric patients treated at DL1, **(C)** DL1.25 or DL2 or **(D)** adult patients treated at DL1. Tn, naïve T cells; Tcm, central memory T cells; Teff, effector/effector memory T cells; Temra, effector memory cells re-expressing CD45RA. LO, last observation; LM, last measurement.

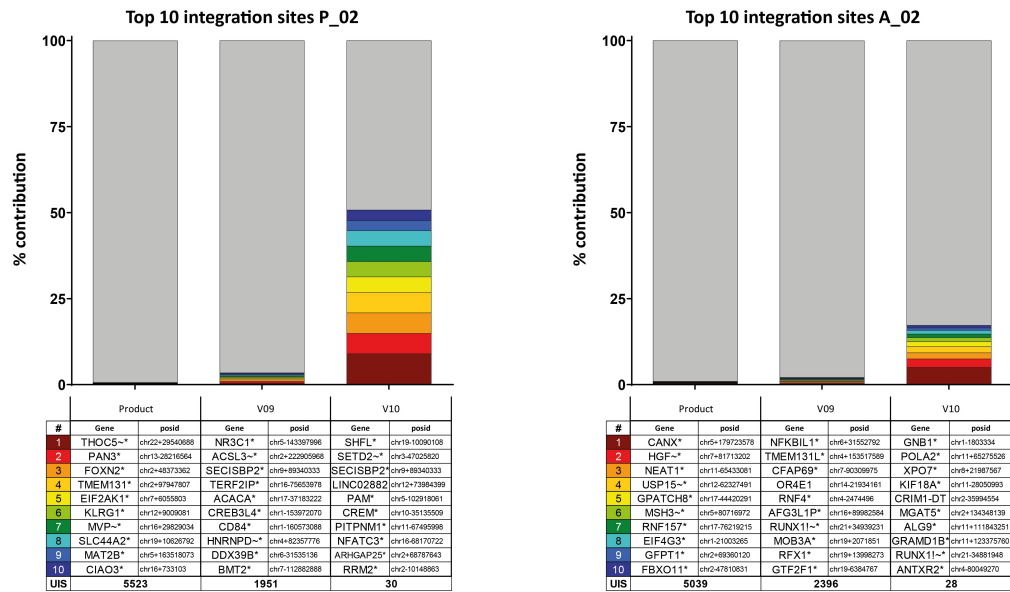
A



B

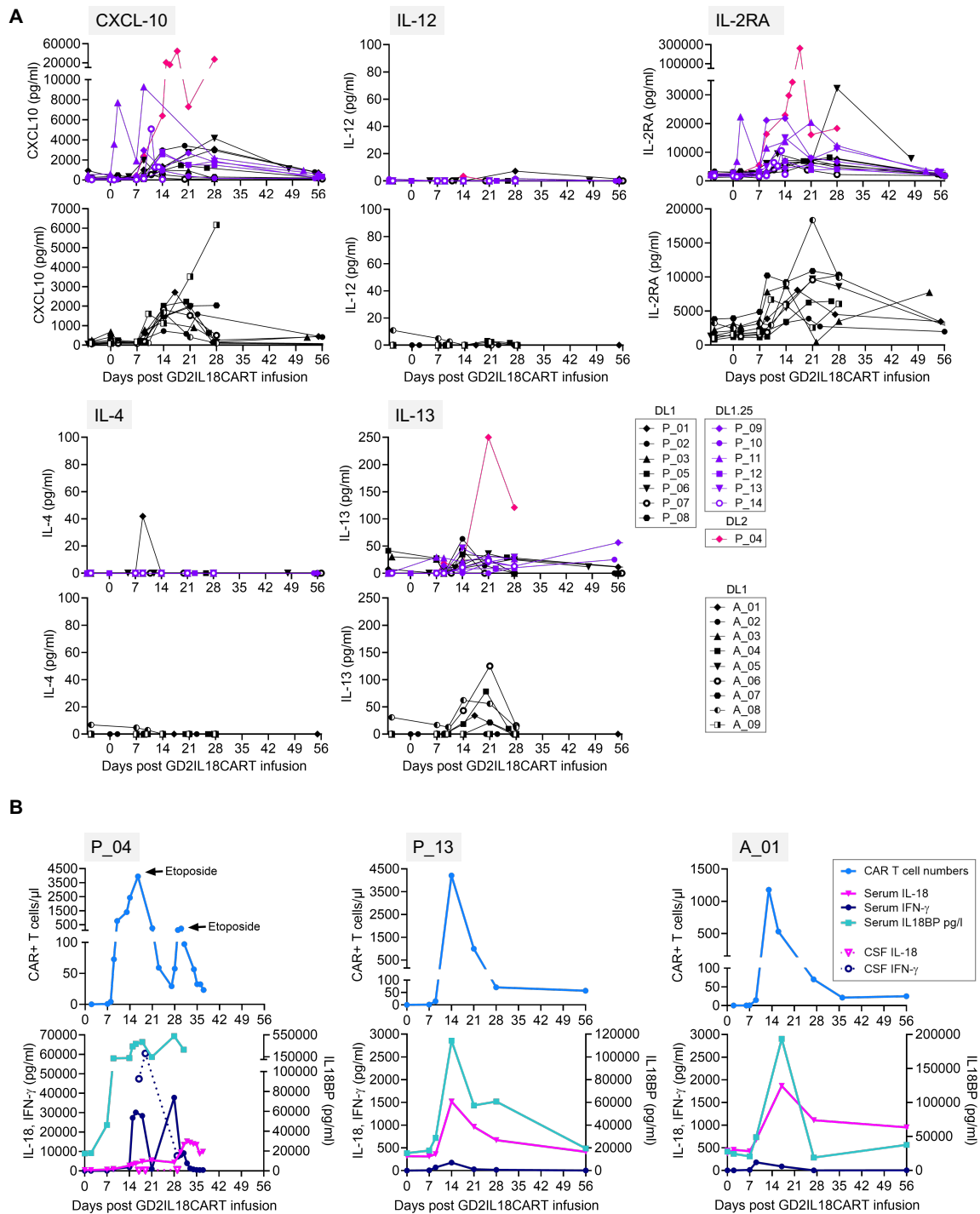


C



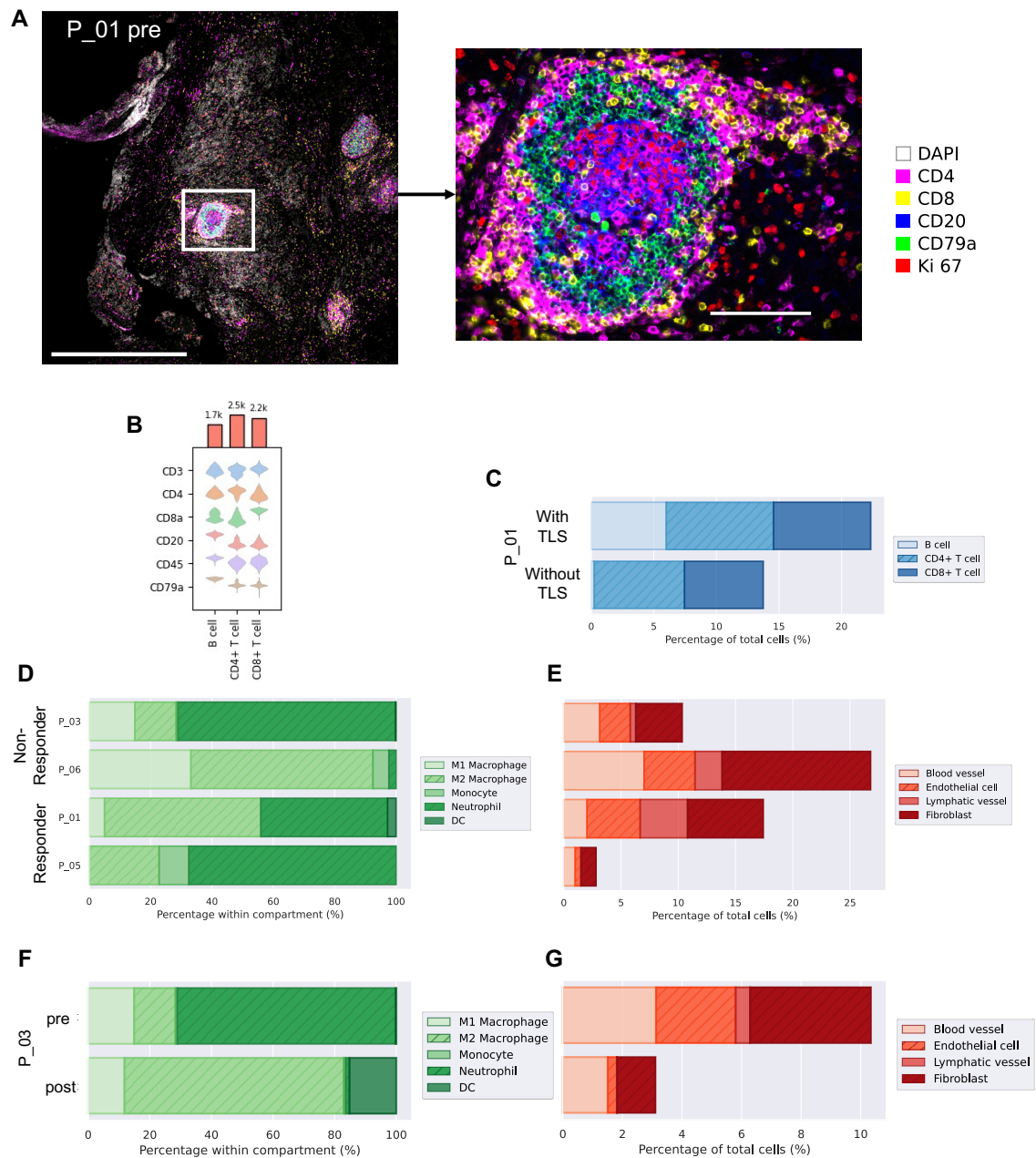
Extended Data Figure 7. Integration Site Analysis (ISA) of individual patient samples.

To understand the integration site profile of the lentiviral GD2IL18 vector in T cells of patients, extracted gDNA from the drug products and from peripheral blood at indicated visits (V09, day 14 and V10, day 28) was analyzed using the INSPIRED (Integration Site Pipeline for palRed-End readDs) workflow^{47,48}. Analyses in pediatric patients were limited by restrictions to blood sampling for complementary research. The diversity and clonality of samples can be described by the number of unique integration sites (UIS) and their contribution to the overall pool of integrations (illustrated by the Shannon diversity index and Gini coefficient). **(A)** Shannon Diversity Index. Shannon values of $2.1 < x \leq 5.5$ show a polyclonal and Shannon values > 5.5 a highly polyclonal situation. All patient samples showed a polyclonal to highly polyclonal integration site pattern. **(B)** Gini coefficient. The Gini index runs from 0 to 1. A low Gini index suggests a polyclonal situation with even sequence abundance distribution. **(C)** Unique Integration Sites (UIS). The graph shows the number of detected unique integrations within the analyzed sample. **(D)** UIS near oncogenes. UIS within 100 kb of oncogenes in indicated time points. The green-colored area indicates the confidence intervals of the random controls including the expected mean percentage (16%) of hits close to (proto)-oncogenes according to the size of the dataset. The percentages of hits near oncogenes in patient samples were statistically not different from matched random control datasets. **(E)** Stacked bar graphs of the top 10 contributing integrations at each measured time point over time. The colored areas represent the top 10 contributing integrations at any time point. The grey area represents all other integrations. The x-axis gives the time point of sampling in days after treatment. * integration within a transcription unit; ~ integration within 50 kb of proto-oncogene. Overall, all analyzed patient samples showed high Shannon and low Gini indices, reflecting a polyclonal to highly polyclonal and typical lentiviral insertion site pattern with no dominant clones and no integrations close to high-risk (proto)-oncogenes.



Extended Data Figure 8. Inflammatory cytokine secretion patterns after GD2IL18CART
(A) Serum concentrations of CXCL-10, IL-12, IL2RA, IL-4 and IL-13 in serum of pediatric (upper panels) and adult (lower panels) patients by Ella™ Automated ELISA.

(B) Longitudinal serum concentrations of total IL-18 (IL-18/IL-18BP), IL-18BP, IFN- γ and CXCL9 during CAR T-cell expansion and contraction in four patients. Cerebrospinal fluid (CSF) concentrations are shown for P_04. P_02 is a neuroblastoma patient with mild inflammatory toxicity and a complete response to GD2IL18CART at DL1. P_04 represents the pediatric Ewing sarcoma patient treated at DL2 who developed fatal hyperinflammation. P_13 represents a pediatric neuroblastoma patient with mild toxicity (maximum grade 1 CRS) and stable disease, and A_01 an adult Ewing sarcoma patient with moderate, manageable toxicity, including CRS grade 2, ICANS grade 1 and IEC-HS grade 1, and progressive disease.



Extended Data Figure 9. Extended data set for multicolor spatial imaging analysis

(A) MICS images of pre-treatment tumor tissue of responding patient P_01. Markers are depicted in colors as indicated. DAPI is omitted in the magnified image for clarity. White rectangle indicates a tertiary lymphoid structure at higher magnification. Scale bars represent 1 mm (B) and 100 μ m (D). **(B)** Representative violin plot used for cell type annotation, showing marker expression levels of lymphoid subsets after subclustering and overall cell numbers in the pre-treatment sample from patient P_01. **(C)** Percentages of total cells assigned to the lymphocyte compartment with and without inclusion of tertiary lymphoid structures (TLS) in P_01. **(D)** Percentages of myeloid subsets within the myeloid compartment for pre-treatment samples of responding patients P_01 and P_05 and non-responding patients P_03 and P_06. **(E)** Percentages of total cells for stroma subsets for pre-treatment samples of responding patients P_01 and P_05 and non-responding patients P_03 and P_06. **(F)** Percentages of myeloid subsets within the myeloid compartment of pre- and post-treatment samples of patient P_03. **(G)** Percentages of total cells for stroma subsets for pre- and post-therapeutic tumor tissue of P_03.

Extended Data Table 1. Detailed patient characteristics at enrollment

	Disease	Sex	Age (yrs at enrollment)	First line therapy	Number of previous treatment lines	Previous relapse therapies	Sites of disease	SIOPEN score	GD2 status
Pediatric patients									
P_01	NBL	f	17	3xN5/N6, surgery, TreoMel/aHSCT, radio, 13-cis RA	4	Surgery, radio, dinu, RIST ¹ , IT	Bone	29	3+ 50%
P_02	NBL	m	12	3xN5/N6, surgery, BuMel/aHSCT, radio, dinu	2	RIT, radio, haploHSCT + dinu	Bone, BM	1	3 + 70%
P_03	NBL	f	4	2xN4, 3xN5/N6, surgery, BuMel/aHSCT, radio, 3xdinu	2	ITD, radio, haploHSCT + dinu, ALK inhibitor	Bone, soft tissue bulk, LNs	2	3 + 70%
P_04	EwS	m	8	iEUROEwing (VDC/IE), surgery, VC/IE	2	HD-Ifo, IT	Multifocal bone, BM, soft tissues	n.a.	3+ 20%, 2+ 50%
P_05	NBL (MYCN)	m	9	3xN5/N6, N8, surgery, MIBG, BuMel/aHSCT, radio, dinu, DFMO ²	2	ITD, MIBG, haploHSCT + dinu, GD2/GD3 vaccine ³	Bone, BM	3	3 + 80%
P_06	NBL	f	8	N3x5/N6, surgery, radio, 4xN7*	6	ToTem, BuMel/aHSCT, radio, 13-cis RA, dinu, MIBG, haploHSCT + dinu, ToCycD, Lu-177 DOTATATE ⁴	Bone, cervical and axillary LNs, soft tissues	4	3+ 20%, 2+ 40%
P_07	OS	m	9	MAP, surgery	3	CE, cabo	Multifocal bone	n.a.	3+ 70%
P_08	NBL (MYCN)	m	8	6xN5/N6, surgery, BuMel/aHSCT, radio, dinu	3	ITD, MIBG, haploHSCT + dinu, ToCyc, radio	Bone	1	3+ 60%, 2+ 20%
P_09	OS	m	9	MAP, resection of primary and of skip metastasis, IfoEto	3	cabo, surgery, GemDoc, IfoEto	Multifocal bone, pleura, lungs, mediastinal LN	n.a.	3+ 60%

P_10	NBL	m	13	3xN5/N6, surgery, BuMel/aHSCT, radio, dinu	3	IT, ITD, haploHSCT + dinu, radio, ALLO GD2-CART01 ⁵	Bone	22	3+ 90%
P_11	NBL	m	11	3xN5/N6, surgery, BuMel/aHSCT, dinu, radio	3	Surgery, radio, IT, ITD	Bone (disseminated), pararenal bulk	n.a. (FDG PET) analogous bone score >19 [#]	3+ 90%
P_12	NBL	m	6	Rapid-COJEC, surgery, BuMel/aHSCT, radio, 5xdinu + isotretinoin	2	Surgeries, B-IT, radio	Multifocal bone, soft tissues	n.a. (18F-SiFAlin-TATE-PET), analogous bone score 8 [#]	3+ 90%
P_13	NBL	m	7	3xN5/N6, surgery, BuMel/aHSCT, dinu, radio	2	ITD	Multifocal bone, bone marrow	31	3+ 70%
P_14	NBL	m	4	Rapid-COJEC, surgery, BuMel/aHSCT	2	ITD	Multifocal bone	n.a. (FDG PET), analogous bone score 8 [#]	3+ 90%

Adult patients

A_01	EwS	m	26	EWING-2008 (VIDE, surgery, VAC/VAI)	4	VIT, HD-Ifo, surgery, HITOC, radio, cabo	Pleural, pulmonary, mediastinal soft tissues/LN	n.a.	2+ 70%
A_02	EwS	m	32	EWING-2008 (VIDE, radio, VAC), TreoMel/aHSCT	8	Multiple surgeries, IT, HD-Ifo, cabo, radio, Tem/olaparib	Pulmonary, mediastinal LN	n.a.	2+ 80%
A_03	EwS	m	24	EWING-2008 (VIDE, radio, VAI)	4	HD-Ifo, ToCyc, TreoMel/ aHSCT, radio, cabo	Multifocal bone, BM, pulmonary	n.a.	3+ 60%
A_04	BC	f	33	Paclitaxel/carbo, Epirubicin, Cyc plus pembro	4	Capecitabine, SG, PLD, CDK4/6 inhibitor	Skin, lung, LN	n.a.	3+ 90%
A_05	EwS	m	35	iEURO-Ewing (VDC/IE, VC/IE), radio	3	IT, TreoMel/aHSCT, ToCyc, GemDoce	Multifocal bone	n.a.	3+ 70%
A_06	BC	f	57	TAC, radio	5	CDK4/6 anti-hormone, bevacizumab plus	Bone, Liver	n.a.	3+ 25% 2+ 40%

						paclitaxel, surgery, SG, PLD, gemcitabine			
A_07	EwS	m	28	iEURO-Ewing (VDC/IE, VC/IE), radio	3	HD-Ifo, cabo	Lung, pleural, bone	n.a.	2+ 60%
A_08	OS	m	19	MAP, resection of primary	4	Surgery, Ifo/Eto, GemDoc	Lung, pleural	n.a.	3+ 60%
A_09	EwS	f	22	EWING-2008, radio	6	IT, CE, radio, CycVino, tumor vaccination, cabo, ToCyc	Lung, pleural	n.a.	3+ 70%

13-cis RA, 13-cis retinoic acid; aHSCT, autologous hematopoietic stem cell transplantation; BC, breast cancer; B-IT, bevacizumab, irinotecan, temozolomide; BuMel, busulfan/melphalan high-dose chemotherapy; cabo, cabozantinib; Carbo, carboplatin; CE, carboplatin, etoposide; Cyc, cyclophosphamide; CycVino, cyclophosphamide, vinorelbine; dinu, dinutuximab-beta; DFMO, difluoromethylornithine; EwS, Ewing sarcoma; GemDoc, gemcitabine, docetaxel; haploHSCT, haploidentical hematopoietic stem cell transplantation; HD-Ifo, high-dose ifosfamide; IfoEto, ifosfamide, etoposide; HITOC, hyperthermic intrathoracic chemotherapy (cisplatin, doxorubicin); HR, high risk; IfoEto, ifosfamide, etoposide; ITD, irinotecan, temozolomide, dinutuximab-beta; LN, lymph nodes; MAP, high-dose methotrexate, adriamycin, cisplatin; MIBG, iodine-131 metaiodobenzylguanidine therapy; MYCN, MYCN-amplified; N4, doxorubicin, vincristine, cyclophosphamide; N5, cisplatin, etoposide, vindesine; N6, vincristine, dacarbazine, ifosfamide, doxorubicin; N7, cyclophosphamide; N8, topotecan, cyclophosphamide, etoposide; OS, osteosarcoma; PLD, pegylated liposomal doxorubicin; RIT, rapamycin, irinotecan, temozolomide; RIST, rapamycin, irinotecan, dasatinib, temozolomide; SG, Sacituzumab govitecan; SIOOPEN score, semi-quantitative evaluation of skeletal meta-iodobenzylguanidine (mIBG) uptake on a 0–6 scale in 12 anatomical regions⁶; TAC, taxole, adriamycin, cyclophosphamide; Tem, temozolomide; ToTem, topotecan, temozolomide; ToCyc(D), topotecan, cyclophosphamide(dinu); TreoMel, treosulfan/melphalan high-dose therapy. VAC, vincristin, actinomycin-D, cyclophosphamide; VAI, vincristin, actinomycin-D, ifosfamide; VC/IE, vincristin, cyclophosphamide, ifosfamide, etoposide; VDC/IE, vincristin, doxorubicin, cyclophosphamide, ifosfamide, etoposide. VIDE, vincristin, ifosfamide, doxorubicin, etoposide; VIT, vincristin/irinotecan/temozolomide.

1. Corbacioglu, S., *et al.* Irinotecan and temozolomide in combination with dasatinib and rapamycin versus irinotecan and temozolomide for patients with relapsed or refractory neuroblastoma (RIST-rNB-2011): a multicentre, open-label, randomised, controlled, phase 2 trial. *Lancet Oncol* **25**, 922–932 (2024).
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Extended Data Table 2. Adverse events (AEs). Shown are the maximum grades of related and unrelated AEs reported according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. According to the clinical trial protocol, all abnormal laboratory values which according to the investigator's judgment are clinically significant have to be recorded as AEs. Orange, neuroblastoma; turquoise, osteosarcoma; purple, Ewing sarcoma; green, breast cancer.

		DL1							DL2	DL1.25						DL1									Pat. Affected, N
SOC	Preferred Term	P_01	P_02	P_03	P_05	P_06	P_07	P_08	P_04	P_09	P_10	P_11	P_12	P_13	P_14	A_01	A_02	A_03	A_04	A_05	A_06	A_07	A_08	A_09	Related (unrelated)
Blood and lymphatic system disorders	Anemia	3 (-)	-	-	-	- (3)	3 (-)	-	-	2 (-)	3 (3)	- (3)	- (3)	-	-	- (2)	-	- (3)	- (3)	-	- (3)	-	-	- (2)	4 (9)
	Disseminated intravascular coagulation/coagulopathy	-	-	-	-	-	-	-	-	- (2)	-	2	2	2	-	-	-	-	-	-	-	-	-	-	3 (1)
	Eosinophilia	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	1 (0)
	Febrile neutropenia	3	-	-	-	- (3)	-	-	-	-	-	-	-	- (3)	-	-	-	-	-	- (3)	-	-	-	-	1 (3)
	Neutropenia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	- (4)	- (3)	- (4)	-	-	- (4)	1 (4)
	Thrombocytopenia	-	-	-	-	-	-	-	-	-	-	-	- (3)	-	-	-	-	-	-	-	-	-	-	-	0 (1)
Cardiac disorders	Atrial fibrillation	-	-	-	-	- (1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0 (1)
	Pericardial effusion	2	-	-	-	-	-	-	-	- (3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1 (1)
	Tachycardia	-	-	-	-	-	-	-	-	-	-	-	1	-	-	- (2)	-	-	-	-	-	-	-	- (1)	1 (2)
Ear and labyrinth disorders	Vertigo	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- (1)	-	-	- (1)	-	-	0 (2)
Endocrine disorders	Adrenal insufficiency	-	-	-	-	- (2)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0 (1)
	Hyperthyroidism	-	-	-	-	- (2)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0 (1)
Eye disorders	Dry eye/Eye pain	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- (1)	- (1)	-	-	-	-	-	0 (2)
	Mydriasis	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	1 (0)
	Vision blurred	-	-	-	-	-	-	-	-	-	-	-	- (1)	-	-	-	2	- (1)	-	-	-	-	-	-	1 (2)
Gastrointestinal disorders	Abdominal pain	- (1)	-	- (1)	- (1)	- (2)	- (2)	- (3)	-	1 (2)	-	-	3	-	-	-	-	-	-	-	-	2	-	-	3 (7)
	Aphthous ulcer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- (1)	-	-	0 (1)
	Constipation	-	-	-	-	-	-	-	- (1)	-	-	-	- (2)	-	- (1)	-	- (2)	-	- (2)	-	-	-	- (1)	-	0 (6)
	Diarrhea	- (1)	-	- (1)	- (1)	-	-	-	- (1)	-	-	-	-	-	- (1)	-	-	- (1)	-	- (1)	- (1)	-	-	- (1)	0 (9)
	Dysphagia	-	-	-	-	-	-	-	- (1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0 (1)
	Flatulence	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- (2)	-	-	-	0 (1)
	Gastrointestinal pain	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- (1)	-	-	-	-	0 (1)
	Hemorrhoidal hemorrhage	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- (2)	-	-	-	-	-	-	0 (1)
	Nausea	- (1)	-	- (2)	- (1)	- (1)	- (1)	-	-	-	-	- (1)	- (2)	-	- (2)	-	-	-	- (2)	- (2)	- (2)	-	-	-	0 (11)
	Stomatitis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- (2)	-	-	-	-	-	-	0 (1)
	Vomiting	-	-	- (1)	- (1)	- (2)	-	-	-	- (2)	-	- (1)	-	-	- (2)	-	-	-	-	-	-	-	- (2)	-	0 (7)
	Catheter site pain	-	-	-	-	-	-	-	-	-	-	-	- (1)	- (1)	-	-	-	-	-	-	-	-	-	-	0 (2)

		DL1							DL2	DL1.25						DL1									Pat. Affected, N	
SOC	Preferred Term	P_01	P_02	P_03	P_05	P_06	P_07	P_08	P_04	P_09	P_10	P_11	P_12	P_13	P_14	A_01	A_02	A_03	A_04	A_05	A_06	A_07	A_08	A_09	Related (unrelated)	
General disorders and administration site conditions	Chest pain	-	-	-	-	-	-	-	-	- (2)	-	-	-	- (1)	-	-	-	-	-	-	-	- (1)	-	-	0 (3)	
	Chills	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1 (0)	
	Condition aggravated	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- (2)	-	-	-	0 (1)	
	Disease progression*	-	-	(n/a)	(n/a)	(n/a)	(n/a)	(n/a)	-	(n/a)	(n/a)	(n/a)	(n/a)	-	-	(n/a)	(n/a)	(n/a)	(n/a)	(n/a)	(n/a)	(n/a)	(n/a)	(n/a)	0 (18)	
	Fatigue	-	-	-	-	-	-	-	-	-	-	-	- (1)	-	-	-	2	-	2	-	-	-	-	-	2 (1)	
	Influenza like illness	-	-	-	-	-	-	-	-	-	-	-	- (2)	-	-	-	-	-	2	-	-	-	-	-	1 (1)	
	Localized edema	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- (2)	-	-	-	-	-	-	0 (1)	
	Malaise	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- (1)	-	-	-	-	-	0 (1)	
	Mucosal inflammation	-	-	-	-	- (2)	-	-	-	- (2)	-	-	- (2)	- (2)	-	-	-	-	-	- (1)	-	-	-	-	- (1)	0 (6)
	Edema	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- (1)	-	-	-	0 (1)	
	Pain	-	-	-	2	-	-	-	-	3	- (2)	-	-	- (2)	-	-	-	2	-	-	- (3)	2	3 (3)	-	1	6 (4)
Pyrexia	- (1)	-	-	- (1)	- (1)	- (3)	-	1	-	- (1)	-	- (2)	-	-	-	-	-	- (1)	- (1)	-	-	- (1)	-	- (2)	1 (10)	
Hepatobiliary disorders	Hepatic pain	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	1 (0)	
Immune system disorders	Cytokine release syndrome	2	1	1	1	1	2	-	1	2	1	3	2	1	1	2	2	3	1	1	2	1	1	2	22 (0)	
	Hypersensitivity	-	-	-	-	-	-	-	-	-	- (3)	-	-	- (2)	-	-	-	-	-	-	-	-	- (2)	-	0 (3)	
	Immune effector cell-associated HLH-like syndrome	3	2	-	-	2	2	-	4	-	-	-	-	-	2	1	1	2	2	2	-	2	-	2	13 (0)	
Infections and infestations	Aspergillus infection	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1 (0)	
	Bacteremia	-	-	-	-	-	-	-	-	-	- (2)	-	-	-	-	-	-	-	-	- (3)	-	-	-	-	0 (2)	
	Bacterial infection	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- (3)	-	-	-	-	-	0 (1)	
	Conjunctivitis	-	- (2)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0 (1)	
	Cystitis	-	-	-	-	-	-	-	-	-	-	-	- (3)	-	-	-	-	-	-	-	-	-	-	-	0 (1)	
	Epstein-Barr virus infection reactivation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	1 (0)	
	Escherichia urinary tract infection	-	-	-	-	-	-	-	-	-	-	-	- (1)	-	-	-	-	-	-	-	-	-	-	-	0 (1)	
	Herpes virus infection	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- (2)	0 (1)	
	Infection	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- (2)	-	-	0 (1)	
Klebsiella infection	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- (3)	-	-	-	-	-	0 (1)		

		DL1							DL2	DL1.25							DL1									Pat. Affected, N
SOC	Preferred Term	P_01	P_02	P_03	P_05	P_06	P_07	P_08	P_04	P_09	P_10	P_11	P_12	P_13	P_14	A_01	A_02	A_03	A_04	A_05	A_06	A_07	A_08	A_09	Related (unrelated)	
	Oral candidiasis	-	-	-	-	-	-	-	-	-	-	-	-	- (1)	-	-	-	-	-	-	-	-	-	-	0 (1)	
	Pneumonia	-	-	-	-	- (3)	-	-	-	-	-	-	-	-	-	- (2)	-	-	-	-	-	-	-	-	0 (2)	
	Respiratory tract infection	-	-	- (1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0 (1)	
	Sepsis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- (4)	-	-	-	-	-	-	0 (1)	
	Upper respiratory tract infection	- (1)	-	- (3)	- (2)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0 (3)	
	Urinary tract infection	-	-	-	-	-	-	-	-	-	-	-	- (2)	-	-	-	-	-	- (2)	-	-	-	-	-	-	0 (2)
	Vulvovaginal candidiasis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- (1)	-	- (2)	-	-	-	-	0 (2)
Investigations	Blood fibrinogen decreased	-	-	-	-	- (3)	-	-	-	- (2)	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1 (2)
	Blood potassium decreased	-	-	-	-	-	-	-	-	- (1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0 (1)
	C-reactive protein increased	-	-	-	-	-	-	-	-	- (2)	-	-	-	-	-	-	-	-	-	-	-	- (1)	-	-	0 (2)	
	Gamma-GT increased	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	1 (0)	
	Hepatic enzyme increased	-	-	-	-	-	-	-	-	-	-	-	- (2)	-	-	-	-	-	-	-	-	-	-	3 (2)	1 (2)	
	Immunoglobulins decreased	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- (2)	-	-	0 (1)	
	Klebsiella test positive	-	-	-	-	-	-	-	-	-	-	-	- (1)	-	-	-	-	-	-	-	-	-	-	-	0 (1)	
	Lymphocyte count decreased	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- (3)	-	-	-	-	-	0 (1)	
	Neutrophil count decreased	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	- (3)	-	- (3)	-	-	-	-	-	1 (2)
	Oxygen saturation decreased	-	-	-	-	-	-	-	-	-	-	-	- (1)	-	-	-	-	-	-	-	-	-	-	-	-	0 (1)
	Platelet count decreased	-	-	-	-	- (4)	-	-	-	-	4 (4)	- (4)	-	-	-	-	- (3)	-	4	-	-	-	-	-	-	2 (4)
	Roseolovirus test positive	-	-	-	-	-	-	-	-	-	-	-	- (1)	-	-	-	-	-	-	-	-	-	-	-	-	0 (1)
	Weight decreased	-	-	-	-	-	-	-	-	-	-	-	- (2)	-	-	-	-	-	-	-	-	-	-	-	-	0 (1)
	Weight increased	-	- (1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0 (1)
	White blood cell count decreased	-	-	-	-	- (4)	-	-	-	-	-	-	-	-	-	-	3 (4)	- (3)	-	-	-	-	-	-	-	1 (3)

		DL1							DL2	DL1.25						DL1									Pat. Affected, N	
SOC	Preferred Term	P_01	P_02	P_03	P_05	P_06	P_07	P_08	P_04	P_09	P_10	P_11	P_12	P_13	P_14	A_01	A_02	A_03	A_04	A_05	A_06	A_07	A_08	A_09	Related (unrelated)	
Metabolism and nutrition disorders	Electrolyte imbalance	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- (2)	-	-	0 (1)	
	Hyperglycemia	-	-	-	-	- (1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0 (1)	
	Hypocalcemia	-	-	-	-	-	- (3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0 (1)	
	Hypokalemia	-	-	-	-	-	-	-	-	-	-	-	- (1)	-	-	-	-	-	-	-	-	-	- (2)	-	0 (2)	
	Hyponatremia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- (4)	-	-	0 (1)	
	Hypo-phosphatemia	-	-	-	-	-	-	-	-	- (2)	-	- (2)	-	-	-	-	-	-	-	-	-	-	-	-	-	0 (2)
	Metabolic acidosis	-	-	-	-	-	-	-	-	- (2)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0 (1)
Musculoskeletal and connective tissue disorders	Arthralgia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- (2)	-	-	-	-	-	-	-	-	-	0 (1)
	Back pain	-	-	-	-	-	-	-	-	- (1)	-	-	-	- (1)	-	-	-	-	-	-	-	-	-	-	-	0 (2)
	Bone pain	2	2	2	-	2	-	-	-	-	-	-	-	-	2	-	-	- (2)	-	-	-	-	2	-	6 (1)	
	Groin pain	-	-	-	-	-	-	-	-	-	-	-	- (1)	-	-	-	-	-	-	-	-	-	-	-	0 (1)	
	Muscle spasms	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- (1)	-	-	-	-	-	-	0 (1)	
	Musculoskeletal chest pain	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- (2)	-	-	-	-	-	-	0 (1)	
	Pain in extremity	-	-	-	-	-	-	-	-	-	-	-	- (1)	- (1)	-	-	-	- (2)	-	-	-	-	-	-	0 (3)	
Neoplasms benign, malignant and unspecified	Recurrent cancer	- (n/a)	- (n/a)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0 (2)	
	Tumor pain	-	-	- (3)	-	-	2 (3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1 (2)	
Nervous system disorders	Depressed level of consciousness	-	-	-	-	-	-	-	-	-	-	-	- (1)	-	-	-	-	-	-	-	-	-	-	-	0 (1)	
	Headache	1	-	-	-	-	-	-	-	-	-	-	- (1)	-	-	- (1)	-	-	2	-	-	- (2)	-	-	2 (3)	
	Immune effector cell-associated neurotoxicity syndrome	-	-	-	-	-	-	-	5	-	-	-	-	-	-	1	-	1	-	-	-	-	-	1	4 (0)	
	Intracranial hemorrhage	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- (2)	-	-	-	-	-	-	0 (1)	
	Paresthesia	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- (1)	-	-	- (1)	-	1	2 (2)	
	Peripheral sensory neuropathy	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	1 (0)	
	Presyncope	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	1 (0)	
	Polyneuropathy	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- (2)	-	-	-	0 (1)	
	Somnolence	-	-	-	-	-	-	-	-	-	-	-	- (2)	-	-	-	-	-	-	-	-	-	-	-	0 (1)	
	Tremor	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	1 (0)	
Product issues	Device occlusion	-	-	-	-	-	-	-	-	-	-	-	- (2)	-	-	-	-	-	-	-	-	-	-	-	0 (1)	
Psychiatric disorders	Confusional state	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- (1)	-	-	-	-	-	-	0 (1)	
	Hallucination	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- (1)	-	-	0 (1)	

		DL1							DL2	DL1.25						DL1									Pat. Affected, N
SOC	Preferred Term	P_01	P_02	P_03	P_05	P_06	P_07	P_08	P_04	P_09	P_10	P_11	P_12	P_13	P_14	A_01	A_02	A_03	A_04	A_05	A_06	A_07	A_08	A_09	Related (unrelated)
Renal and urinary disorders	Dysuria	-	-	-	-	-	-	-	-	-	-	-	- (1)	-	-	-	-	-	-	-	-	-	-	-	0 (1)
	Pollakiuria	-	-	-	-	-	-	-	-	-	-	-	- (1)	-	-	-	-	-	-	-	-	-	-	-	0 (1)
	Urinary incontinence	-	-	-	-	-	-	-	-	-	-	-	- (1)	-	-	-	-	-	-	-	-	-	-	-	0 (1)
	Urinary retention	-	-	-	-	-	-	-	-	-	-	-	- (2)	-	-	-	- (2)	-	-	-	-	-	-	- (2)	0 (3)
Respiratory, thoracic and mediastinal disorders	Central sleep apnea syndrome	-	-	-	-	-	-	-	-	-	-	-	- (2)	-	-	-	-	-	-	-	-	-	-	-	0 (1)
	Cough	-	-	-	-	-	-	-	-	-	-	-	-	-	- (2)	-	-	-	-	-	-	- (2)	-	-	0 (2)
	Dyspnea	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	- (2)	-	-	-	-	-	-	0 (1)
	Hemothorax	-	-	-	-	-	-	-	-	- (3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0 (1)
	Hypoxia	- (2)	- (2)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0 (1)
	Pleural effusion	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- (2)	- (3)	0 (2)
	Pneumonitis	-	-	-	-	-	-	-	-	-	-	- (3)	-	-	-	-	-	-	-	-	-	-	-	-	0 (1)
	Respiratory failure	-	-	-	-	-	-	-	-	-	-	-	- (2)	-	-	-	-	-	-	-	-	-	-	-	0 (1)
	Dry skin	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1 (0)
Skin and subcutaneous tissue disorders	Erythema	-	-	-	-	-	-	-	-	-	-	-	- (1)	-	-	-	-	-	-	-	-	-	-	-	0 (1)
	Maculopapular rash	-	-	-	-	-	-	-	-	-	-	- (1)	-	-	-	-	-	-	-	-	- (2)	-	-	-	0 (2)
	Night sweats	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- (1)	-	-	1	-	-	1 (1)
	Pruritus	-	-	- (1)	- (1)	-	-	-	-	-	- (1)	-	-	-	- (2)	-	- (2)	-	-	- (1)	-	-	- (1)	-	0 (7)
	Rash	-	-	-	-	-	-	-	-	- (1)	-	-	- (1)	-	-	-	-	1	-	-	-	-	-	1	2 (2)
	Urticaria	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- (1)	-	0 (1)
	Capillary leak syndrome	2	-	-	2	2 (2)	2	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	2	-	6 (1)
Vascular disorders	Hypertension	-	- (3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- (2)	0 (2)
	Hypotension	-	-	-	-	- (3)	-	-	-	-	-	-	-	-	-	-	-	- (1)	-	-	-	-	-	-	0 (2)
	Jugular vein thrombosis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- (2)	-	0 (1)

*Includes also disease progressions reported under the alternative term "Neoplasm progression" in category "Neoplasms benign, malignant and unspecified".
Gamma-GT, gamma-glutamyl transferase.

Extended Data Table 3. Eligibility criteria. CTP V1.9

Criteria for Inclusion	Male or female patients must have a diagnosis of relapsed and/or refractory (r/r) neuroblastoma, Ewing sarcoma, osteosarcoma or advanced breast cancer, judged to be incurable with conventional treatment. • Among patients with advanced breast cancer, - patients with hormone receptor positive disease must have exhausted all endocrine therapy options including a combination with a CDK4/6 inhibitor and chemotherapy must not be a reasonable therapy option, - patients with HER2 positive disease should have received Trastuzumab-Emtansin (T-DM1) and/or T-DXd in second line after a HER2 directed antibody in the (neo)adjuvant setting or in first line therapy, - patients with triple negative disease must have received at least 2 prior lines of chemotherapy including Sacituzumab-Govitecan in the metastatic setting, and patients with combined positive score (CPS) >10 and/or immune cell score (IC) ≥ 1% should have received an immune checkpoint inhibitor previously. • For neuroblastoma, patients with locoregional recurrence must have received surgery and/or radiotherapy followed by relapse chemotherapy. Patients with metastatic recurrence after initial classification as intermediate risk must have received high risk therapy. Patients with recurrence after initial classification as high risk must have received relapse chemotherapy alone or combined with immunotherapy. • Patients with Ewing sarcoma must have received front-line therapy and at least one prior line of relapse chemotherapy according to standard regimens (alkylating agents in combination with topoisomerase inhibitors; irinotecan with temozolomide; gemcitabine and docetaxel; high-dose ifosfamide; carboplatin with etoposide. Patients with an isolated lung relapse must have received pulmonary irradiation either during frontline or relapse therapy. • Patients with osteosarcoma must have received front-line therapy and at least one prior line of relapse chemotherapy (alkylating agents and/or etoposide and/or carboplatin or gemcitabine and docetaxel). Patients with an isolated relapse manifestation (lung metastasis or local relapse) must have unresectable disease. All patients must meet the following disease-specific criteria: - GD2 expression on tumor cells in archival biopsy in the central reference lab. GD2-positive tumor is defined as ≥ 50% of tumor cells expressing ≥ 2+ GD2 using an immunofluorescence staining (IF) assay for specific detection of GD2 expression in formalin-fixed, paraffin-embedded neoplastic tissue or frozen tumor tissue along with a semi- quantitative evaluation. - Evaluable disease based on imRECIST (all disease entities) or in cases of diffuse bone/bone marrow metastasis by whole-body MIBG-scan (neuroblastoma) or whole-body FDG-PET scan (all entities) Age ≥1 and <80 years - Absolute CD3+ T cell count ≥200/μl - ECOG performance score of 0-2 if >16 years old or Lansky performance score of ≥60 if ≤16 years old at screening - No childbearing potential or negative pregnancy test at screening and before chemotherapy in women with childbearing potential - Signed and dated informed consent/assent by patients and/or parents, before conduct of any trial-specific procedure.
Criteria for Exclusion	Patients will be entered into this trial only if they meet none of the following criteria: a. Active solid central nervous system (CNS) metastases or metabolically active metastatic disease in skull with direct contact to dura b. Current autoimmune disease or history of autoimmune disease with potential CNS involvement c. Active clinically significant CNS dysfunction (including but not limited to uncontrolled seizure disorders, cerebrovascular ischemia or hemorrhage, dementia, paralysis) d. History of an additional malignancy other than non-melanoma skin cancer or carcinoma <i>in situ</i> unless disease free for ≥3 years

	e. Pulmonary function: Patients with pre-existing severe lung disease or an oxygen requirement of >28% O₂ supplementation or active pulmonary infiltrates on X-ray f. Cardiac function: Pediatric patients: fractional shortening <28% or left ventricular ejection fraction <50% by echocardiography, adult patients: left ventricular ejection fraction <50% by echocardiography g. Renal function: GFR ≤29 mL/min/1.73 m² by CKD-EPI for patients ≥18 yrs or creatinine clearance ≤29 mL/min/1.73 m² by Schwartz formula for patients <18 yrs of age h. Liver function: Patients with a serum bilirubin >3 times upper limit of normal or an AST or ALT >5 times upper limit of normal, unless due to liver infiltration by the underlying malignant disease in the estimation of the Investigator i. Hematological function: Absolute neutrophil count (ANC) < 500/μl or platelet count < 50 × 10³/μl j. Presence of any active infection k. Rapidly progressive disease that in the estimation of the Investigator would compromise ability to complete study therapy l. Pregnant or breast-feeding females m. Concurrent or recent prior therapies: ○ High-dose chemotherapy with autologous stem cell transplantation or requiring autologous stem cell rescue or anticancer biologicals (e.g. mifamurtide, interferons) or immune checkpoint inhibitors or radiation therapy or major surgery within 30 days prior to leukapheresis ○ Neuroblastoma patients who have relapsed after allogeneic or haploidentical stem cell transplantation must be at least 100 days posttransplant, with no evidence of GVHD and not taking immunosuppressive agents for at least 30 days prior to leukapheresis. ○ I¹³¹ MIBG therapy (neuroblastoma) or other targeted radionuclide therapy must be completed at least 3 months prior to apheresis. ○ GD2-specific antibody therapy with dinutuximab beta or naxitamab within 40 days (5 half lives) prior to apheresis. ○ Antiproliferative chemotherapies, epigenetic agents, antibody drug conjugates or antibodies targeting alternative antigens than GD2 within 3 weeks prior to leukapheresis ○ Immunosuppressive agents other than steroids within 2 weeks prior to leukapheresis ○ Short-acting molecularly targeted agents (kinase inhibitors) within 7 days prior to leukapheresis ○ Systemic corticosteroids with the exception of physiologic replacement dosing within 7 days prior to leukapheresis ○ Live vaccines within 30 days prior to leukapheresis n. Hypersensitivity against any drug or its ingredients/impurities that is scheduled or likely to be given during trial participation, e.g. as part of the mandatory preparative chemotherapy, pre-medication for infusion, rescue medication/salvage therapies for treatment related toxicities o. Contraindication of trial related procedures as judged by the Investigator, e.g. tumor biopsy p. Female patients of child-bearing potential not willing to practice a highly effective form of birth control from the time of signing the informed consent form and for 12 months after dosing the IMP q. Male patients of fathering potential not willing to practice a highly effective form of birth control from from the time of signing the informed consent form and for 12 months after dosing the IMP r. Concurrent participation in another interventional trial that could interact with this trial, e.g. CAR T trials s. Cerebral dysfunction, legal incapacity of adult patients; Committal to an institution on judicial or official order
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Extended Data Table 4. Summary of major changes of the clinical trial protocol during study conduct. The clinical trial protocol (CTP) for study EU CT 2022-501725-21-00 underwent several amendments during the dose-finding phase. The study was initially approved by the German competent authority, Paul-Ehrlich-Institut, on 19 March 2024 (CTP version 1.3). Amendment 1 was submitted on 4 July 2024 and approved on 8 August 2024 (CTP Version 1.4). Amendment 2 was submitted on 10 December 2024 (CTP Version 1.5). Revised protocol versions 1.6 and 1.7 were subsequently submitted during the review process, with version 1.7 approved on 13 January 2025. Amendment 5 was submitted on 9 May 2025 (CTP Version 1.8). Revised protocol version 1.9 was subsequently submitted and approved on 27 June 2025. Major changes introduced by these amendments are summarized below.

Number and Date	Major Changes
Amendment 1 (CTP1.4); 04 Jul 2024 Approved 08 Aug 2024	- <i>Correction of inconsistencies in blood sampling:</i> Discrepancies in two tables, "Visit Schedule" (p.19-20) and "Blood per visit (p.94)" were corrected. These corrections do not affect the total blood volumes. - <i>The blood sampling time point at Visit 8 was adjusted:</i> Since samples can not be shipped on Fridays, the blood sample at V8 must be taken on day 9 instead of day 10. All other clinical examinations on Visit 8 will continue to be performed on day 10 (see "Visit Schedule" on p.19-20). - <i>Replacement of a DMC member:</i> Due to time constraints, one DMC member had to resign from the DMC board and was replaced.
Amendment 2 (CTP1.5); 10 Dec 2024 Queries and further amendment (CTP 1.6, CTP 1.7); Approved 13 Jan 2025	<u>CTP Version 1.5</u> - Replacement of the pre-screening procedure by the respective inclusion criterion alone which continues to ensure expert assessment of GD2 status with the validated assay (<i>Kailayangiri S et al.</i>, STAR Protocols, 2024 Jul 22;5(3):103199; <i>Fischer-Riepe L et al.</i>, Clin Cancer Res 2024 Apr 9:OF1-OF14) - <i>Additional visit 9.b:</i> In order not to miss the peak of CAR T cell expansion between day 14 (visit 9) and day 28 (visit 10), an additional visit was added on day 21 (visit 9b). - <i>Addition of two new dose levels:</i> The additional dose levels (DL1.25 and DL1.5) were added to the protocol and DL3 was removed. - <i>Grading of ICANS:</i> To facilitate grading of ICANS in pediatric patients, the ICE and CAPD scores in Table 9 were added to the table legend on p. 61. - <i>Dosage recommendations for seizure prophylaxis with levetiracetam:</i> In order to harmonize seizure prophylaxis at the various locations, age-appropriate dosage recommendations were added on p.62. - <i>DMC members:</i> In order to ensure statistical competence in the DMC at all times, we have added a deputy for the statistician in the DMC. Only one of the two has the right to vote. <u>CTP Version 1.6</u> - <i>Detailed considerations for dose adjustments:</i> The rationale for the dose adjustments made in response to the fatal toxicity observed after the dose increase to DL2 are now described in detail in the amended protocol (1.3.4 Selection, p.41). - <i>Risk assessment adjusted:</i> The risk assessment part of the protocol (1.4.2.4) as rewritten, with consideration of the fatal toxicity that had occurred after dose escalation. - <i>ICANS and IEC-HS sections revised:</i> We further revised the protocol section on ICANS classification and treatment (3.1.3.2) to include more detail and to further encourage the use of prophylactic antiepileptic drugs and early use of steroids, and we expanded on the section on HLH-like toxicities (3.1.3.3) <u>CTP Version 1.7</u> - <i>Hospital stay extended to 14 days:</i> The minimum length of hospital stay required by the protocol was extended to 14 days at the request of the Paul-Ehrlich-Institut.

	- <i>New exclusion criterion:</i> At the request of Paul-Ehrlich-Institut, we added "metabolically active metastatic disease in the skull with direct contact to the dura" as a new exclusion criterion.
Amendment 5 (CTP1.8); 09 May 2025 Queries and further amendment (CTP 1.9); Approved 27 Jun 2025	<u>CTP Version 1.8</u> - <i>Minor adjustment to the definition of dose-limiting toxicities (DLT):</i> Previously, Grade ≥ 4 hematological toxicity was not considered a DLT if it was attributable to bone marrow disease or chemotherapy and resolved within 28 days. This has now been amended to state that Grade ≥ 4 hematological toxicity is also not considered a DLT if it is attributable to radiotherapy and resolves within 28 days. (Synopsis, p.11 and 3.1.2, p.56). - <i>Assessment of disease status using metabolic imaging techniques:</i> For all disease entities, the assessment of disease status at patient enrollment and of response during the course of the study is based on imRECIST, which requires the presence of individual measurable lesions. To account for the often diffuse dissemination of relapsed neuroblastoma in bone and bone marrow, the protocol specified that - at the investigator's discretion - metabolic imaging (MIBG scintigraphy, FDG-PET scans) could be used as an alternative. During the conduct of the study, it became evident that for other disease entities as well, in cases of diffuse bone/bone marrow metastases, the assessment of disease extent and response should be performed using whole-body FDG-PET scans, either as an alternative or as a supplement. The protocol was amended accordingly (pp. 13, 20, 74, 80, 91, 92). - <i>Changes to certain sample collections during study visits:</i> To better assess the course of disease in neuroblastoma patients, an additional measurement of the tumor marker—specifically urinary catecholamine metabolites—has been added at Day 0 (CART administration) (pp. 19, 70, 91). During the course of the study, it became apparent that no CART cells were detectable in the blood of treated patients at the early time point of Visit 6 (Day 2). We have therefore eliminated this sampling from the immune monitoring. It turned out that CART cells were still detectable in the blood of some patients at late time points (week 12, month 4). Consequently, additional blood sampling at these later time points was included into the complementary research program. For pediatric patients, this rescheduling of blood sampling visits results in no change to the total volume of blood collected. For adult patients, the total amount of blood collected over the course of the entire study increases by only 15 ml (pp. 19, 96, 97). <u>CTP Version 1.9</u> - Query from the PEI: The use of scintigraphy or PET scans in patients with advanced disease who do not meet imRECIST criteria is considered acceptable. However, response assessment should be based on established criteria and combined with clinical criteria to ensure correct interpretation. The applicant is requested to specify these criteria in the protocol. Adjustments in protocol V1.9: We have incorporated details regarding the criteria for response assessment via scintigraphy (for neuroblastoma) and PET scans (for bone lesions) - in accordance with international consensus guidelines - into the protocol (Section 6.1, p. 92). In addition to the relevant literature references, further details are provided in additional appendices (Section 12.2, 12.3, and 12.4; p. 127–130).