

GD2 CAR T cells with inducible IL-18 in refractory solid tumors

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Article

Keywords: GD2, CAR T cells, phase I clinical study, inducible IL-18, solid tumors, neuroblastoma, bone sarcomas

Posted Date: October 7th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11094009/v1>

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Additional Declarations: **Yes** there is potential Competing Interest. Melanie Jungblut, Andreas Bosio, Martin Helm and Werner Mueller are employees of Miltenyi Biotec B. V. & Co. Katja Petry is an employee of Miltenyi Biomedicine GmbH.

Abstract

We report the first clinical evaluation of IL-18-engineered CAR T-cell therapy in patients with solid tumors. In an academic phase I dose-finding study, 23 patients with advanced solid tumors received GD2IL18CART, a GD2-specific CAR T-cell product with antigen-inducible IL-18 secretion. Treatment at the recommended dose was associated with clinically significant but manageable inflammatory toxicity, whereas dose escalation resulted in fatal hyperinflammation in one patient. GD2IL18CART cells expanded *in vivo* in all patients, accompanied by increases in serum IL-18, IFN- γ and IL-18BP. Objective responses occurred in four of eleven patients with neuroblastoma, including complete remissions, but not in patients with other tumor types. These findings demonstrate the feasibility of IL-18-engineered CAR T-cell therapy for solid tumors and provide evidence of antitumor activity in neuroblastoma. Exploratory tissue analyses identify features of the tumor microenvironment that may constrain therapeutic activity and provide targets for further improvement.

Introduction

Cellular therapy with chimeric antigen receptor (CAR)-redirected T cells can induce durable remissions in refractory B cell malignancies¹⁻³. Clinical responses have also been reported in patients with solid tumors⁴⁻⁸, but overall antitumor activity remains limited. Engineering CAR T cells to co-express activating cytokines is a strategy to enhance and sustain their activity within the immunosuppressive tumor microenvironment (TME)^{9,10}. To provide local pro-inflammatory support, we selected the IL-1 family cytokine IL-18, which enhances cytotoxic T-cell and NK-cell function, promotes IFN- γ production, and supports inflammatory remodeling of the TME¹¹. Physiologically, IL-18 activity is tightly regulated by the soluble decoy receptor IL-18 Binding Protein (IL-18BP), which limits excessive IFN- γ driven inflammation¹². In preclinical models, we and others have shown that IL-18 secretion by CAR T cells enhances proliferation, reduces exhaustion, activates bystander immune cells, and promotes a pro-inflammatory TME¹³⁻¹⁷. The first clinical evaluation of an IL-18-enhanced CAR T-cell product, a CD19-specific CAR with constitutive IL-18 co-expression, showed encouraging activity in patients with refractory lymphoma at remarkably low cell doses¹⁸.

We previously developed GD2IL18CART, a GD2-specific autologous CAR T-cell product with activation-inducible IL-18 secretion designed to restrict cytokine release to sites of CAR engagement¹⁵. GD2 is a cell-surface ganglioside highly expressed in neuroblastoma and several other solid malignancies¹⁹⁻²³. We conducted a phase I study (EU CT 2022-501725-21-00) of GD2IL18CART and report safety, biological activity, and preliminary antitumor activity after completion of dose escalation. To our knowledge, this is the first reported clinical experience with an IL-18-enhanced CAR T-cell product in patients with solid tumors.

Results

Patient and product characteristics

Between May 2024 and April 2026, 34 pediatric (0-17 years) and adult patients with GD2-positive malignancies were screened for enrollment. Nine were excluded because rapidly progressive disease was

judged by the investigators to preclude completion of the 28-day safety and 3-month efficacy assessments, and two because of insufficient cardiac function (Fig. 1A). Twenty-three patients (14 pediatric, 9 adult) underwent leukapheresis and received a single infusion of autologous GD2IL18CART cell product following standard lymphodepleting chemotherapy (Fig. 1A, B). No bridging therapy was administered between leukapheresis and CAR T-cell infusion. All patients had highly advanced, metastatic disease refractory to standard therapies, and many had also received off-label or investigational treatments^{6,24-27} (Table 1, Extended Data Table 1). All patients with neuroblastoma had previously received multiple courses of GD2-directed antibody therapy. Tumor GD2 expression, assessed by immunofluorescence staining and semiquantitative intensity scoring²⁸, was high (3+) in 12 of 14 pediatric patients and 5 of 9 adult patients and moderate (predominantly 2+) in the remaining patients (Table 1, Extended Data Table 1, Extended Data Fig. 1).

GD2IL18CART products were successfully manufactured from fresh apheresis material for all patients at either of two academic GMP facilities and met all in-process and release specifications. Final products contained a median of 5.2×10^9 T cells (range 4.3 to 6.0×10^9), with a median viability of 95.7% (range 92.8 to 97.5%) and median CAR expression of 46.1% (range 29.0 to 69.0%) (Extended Data Fig. 2). All products were infused fresh on day 14 after leukapheresis, following 3 days of fludarabine/cyclophosphamide lymphodepletion (Fig. 1B).

Safety

All 23 patients completed the 28-day dose-limiting toxicity (DLT) observation period. Pediatric dose escalation started at DL1 (1×10^5 CAR-positive T cells/kg) and proceeded to DL2 (5×10^5 /kg) after no DLTs occurred in the first three patients. The single patient treated at DL2 (P_04) developed fatal dose-limiting hyperinflammation (see below). The trial was temporarily suspended and, following protocol amendment, pediatric dose escalation resumed from DL1 using smaller dose increments. Ultimately, seven pediatric patients received DL1, six DL1.25 (2×10^5 /kg), one DL2, and nine adult patients DL1 (Fig. 1A).

Cytokine release syndrome (CRS) was reported in 22 of 23 patients and was grade 1 or 2 in 20 (Fig. 2A, Extended Data Fig. 3). Grade 3 CRS was reported in two patients. One event was associated with a pulmonary infection in a pediatric patient with neuroblastoma (P_11) (Extended Data Fig. 3). The second was reported on day 39 in an adult patient with Ewing sarcoma (A_03), after CAR T-cell contraction and in the context of neutropenic *E. coli* sepsis and fulminant disease progression. Given the late onset and concurrent documented infection, this event was considered unlikely to be CAR T-cell-mediated. Excluding this event from analysis of CRS onset, CRS developed between days 4 and 15 (median, day 8.5), without differences between pediatric and adult patients or between dose levels.

At DL1 and DL1.25, immune effector cell-associated neurotoxicity syndrome (ICANS) occurred in three adult patients and was limited to grade 1 events, manifesting as mild tremor with shaky handwriting (Fig. 2A, Extended Data Fig. 3). Symptoms resolved within 2 to 3 days with corticosteroid treatment (Fig. 2B).

A characteristic toxicity of GD2IL18CART therapy was a delayed second wave of systemic inflammation. Immune effector cell-associated hemophagocytic lymphohistiocytosis-like syndrome (IEC-HS)²⁹ occurred in 4 of 7 pediatric patients treated at DL1, including three grade 2 events and one grade 3 event, and in 7 of 9 adult patients, all grade 1 or 2 (Fig. 2A, Extended Data Fig. 3). IEC-HS developed between days 6 and 23 (median, day 12.5), thus within the 28-day DLT observation period. At DL1.25, only one of six pediatric patients fulfilled the diagnostic criteria of IEC-HS²⁹. However, four additional patients developed individual clinical or laboratory features of secondary inflammation, including recurrent fever, fluid retention, hypofibrinogenemia and hyperferritinemia, without meeting formal IEC-HS criteria (Extended Data Table 2). Another frequent adverse event was mild to moderate bone pain, often localized to sites of osseous disease and occurring across age groups, dose levels and diagnoses (Extended Data Table 2).

Anti-inflammatory treatment was initiated early according to protocol (Fig. 2B). All patients with CRS received at least one dose of tocilizumab. Secondary inflammatory events were managed initially with anakinra, which was administered to 7 of 14 pediatric and 6 of 9 adult patients. Short courses of dexamethasone (≤ 7 days) were administered to 9 of 13 pediatric patients treated at DL1 or DL1.25. Corticosteroids were also administered to 8 of 9 adult patients, including treatment for > 7 days in 5 patients. Apart from patient P_04, one additional patient (A_03) received ruxolitinib for grade 2 IEC-HS with elevated liver enzymes (Fig. 2B, Extended Data Fig. 3). The more frequent use of corticosteroids and greater need for prolonged anti-inflammatory treatment in adults contributed to the decision not to escalate dosing beyond DL1 in this population.

Patient P_04 developed dose-limiting hyperinflammation with multiorgan failure and brainstem edema and died on day 38 despite intensive anti-inflammatory treatment with tocilizumab, anakinra, ruxolitinib, high-dose corticosteroids and etoposide (Fig. 2B, Extended Data Fig. 3). Emapalumab, an IFN-γ blocking antibody with reported activity in CAR T-cell-associated hyperinflammation^{30,31}, was not available for off-label use in Germany.

In addition to the fatal inflammatory event at DL2, two DLTs occurred, both at DL1 and both due to persistent profound neutropenia in patients with severely impaired bone marrow reserve after intensive prior therapy (P_06, A_03). In both patients, neutropenia resolved after stem cell boosts. Few additional clinically significant hematologic adverse events were reported (Extended Data Table 2). As expected following lymphodepleting chemotherapy, transient cytopenias were observed. According to the protocol, however, abnormal laboratory values were recorded as adverse events only when considered clinically significant by the investigator, typically when therapeutic intervention was required. Moreover, cytopenias occurring as a feature of IEC-HS were captured as part of the syndrome and were not required to be reported separately as hematologic adverse events. Consequently, the adverse event data may underrepresent cytopenias not considered clinically significant and IEC-HS-associated cytopenias and do not permit systematic assessment of all treatment-associated cytopenias.

Following joint review with the DMC, the investigators decided not to pursue further dose escalation despite the absence of formal DLTs at DL1.25, given the risk of life-threatening inflammatory toxicity. Consequently, the recommended dose was established at DL1.25 for pediatric patients and DL1 for adults.

Clinical responses and outcome

Tumor response was assessed 12 weeks after GD2IL18CART infusion (Fig. 3A). Among 11 heavily pretreated pediatric patients with neuroblastoma, 4 (36%) achieved an objective response according to the International Neuroblastoma Response Criteria (INRC)³², including 2 complete metabolic responses with normalization of baseline SIOPEN (International Society of Pediatric Oncology Europe Neuroblastoma group) skeletal scores of 29 and 1, respectively³³ (Fig. 3B). Both complete responses were maintained at month 6, but reassessment at month 12 revealed asymptomatic osseous relapse in both patients. Two additional patients (P_05, P_12) achieved partial responses. In P_05, only a single weakly metabolically active bone lesion remained, which showed concurrent regression on magnetic resonance tomography (MRI) and was not a site of subsequent relapse (Fig. 3B). At month 6, this patient developed progressive disease at four other osseous sites. Three additional patients had stable disease at week 12, including two with reductions in SIOPEN skeletal scores from 22 to 20 (P_10) and from 31 to 27 (P_13), corresponding to an overall disease control rate of 64%. One patient had progressive disease at week 12, and three had rapidly progressive disease before formal response assessment.

No objective responses were observed in patients with Ewing sarcoma, osteosarcoma, or breast cancer. One of three patients with osteosarcoma had stable disease at week 12. The pediatric patient with Ewing sarcoma had stable disease at the last assessment before death from inflammatory toxicity, whereas all six adult patients had progressive disease. In one of these patients, transient regression of pleuropulmonary disease was followed by progression with loss of GD2 uptake in experimental whole-body [⁶⁴Cu]GD2 PET/CT scans, consistent with GD2-negative escape (Extended Data Fig. 4). Experimental [⁶⁴Cu]GD2 PET/CT scans were performed outside the clinical study, independently of the study protocol and at the discretion of the treating physicians. Re-biopsies from two other patients with progressive Ewing sarcoma demonstrated persistent GD2 expression (Extended Data Fig. 4).

Among patients with neuroblastoma with available post-treatment assessments, no GD2-negative escape was identified. All four post-treatment biopsies remained GD2-positive, and recurrent or progressive disease assessed by experimental [⁶⁴Cu]GD2 PET/CT in three patients was also GD2-positive (Extended Data Fig. 4). Post-treatment GD2 expression was not assessed in osteosarcoma, and serial assessment in one patient with breast cancer showed reduced but persistent GD2 expression (Extended Data Fig. 4).

GD2IL18CART *in vivo* expansion and persistence

GD2IL18CART cells expanded in peripheral blood to levels detectable by flow cytometry in every treated patient, peaking between days 12 and 28 (median, day 14) (Fig. 4A). Peak CAR T-cell counts ranged from 11/μl to 4,212/μl in patients treated at DL1 or DL1.25 and reached 3,950/μl in P_04 before treatment with etoposide (Fig. 4B). Dose escalation was associated with higher peak CAR T-cell expansion in the pediatric cohort (n=14, Jonckheere-Terpstra test, p=0.0224) and in the overall study population including adults, who were all treated at DL1 (p=0.0160). Areas under the curve (AUCs) did not differ significantly between dose levels (Fig. 4C).

Assessment of long-term CAR T-cell persistence was limited in many patients because early disease progression shortened follow-up. Ten patients lost detectable CAR T cells during follow-up, including all four responders, in whom circulating CAR T cells became undetectable between months 3 and 6. In the remaining 13 patients, GD2IL18CART cells were still detectable by flow cytometry at their last available peripheral blood assessment, performed between days 37 and 126. The longest observed CAR T-cell persistence was in two patients treated at DL1.25, with cells detectable through weeks 17 and 18, respectively.

No treatment-emergent human anti-chimeric antibody (HACA) responses were detected. A single pretreatment-positive sample from a patient without prior GD2-targeted therapy was considered likely to reflect nonspecific assay reactivity (Extended Data Fig. 5).

Among circulating CAR-positive T cells, the median proportion with a central memory phenotype was 8.9% on day 14, 8.1% on day 21 and 4.9% on day 28 time points (Extended Data Fig. 6). No differences in CAR T-cell expansion or phenotype were observed between responders and nonresponders. Vector integration site analysis on days 14 and 28 demonstrated a polyclonal repertoire in all 23 patients, with fewer clones detected as CAR T-cell numbers declined after peak expansion (Extended Data Fig. 7).

***In vivo* cytokine secretion**

GD2IL18CART is engineered to secrete IL-18 upon CAR engagement¹⁵. Following CAR T-cell infusion, serum concentrations of total IL-18 increased above baseline in all patients, peaking between days 12 and 28 (median, day 14) before returning to baseline (Fig. 4D). At DL1 and DL1.25, median peak IL-18 was 1,225 pg/ml (range, 676 to 5,040), corresponding to a median 3.6-fold increase over baseline (range, 1.2 to 10.7-fold; Wilcoxon signed-rank test, $p < 0.0001$). IFN- γ peaked in parallel with IL-18 (median, day 14) at a median of 289 pg/ml (range, 47 to 1,956), followed by the IFN- γ inducible chemokine CXCL9 (median, day 16) (Fig. 4D). Consistent with the physiological regulation of IL-18, serum concentrations of the high-affinity soluble decoy receptor IL-18BP increased in all patients and peaked later (median, day 19), reaching a median 6.2-fold increase above baseline (range, 1.4 to 15.2-fold; Wilcoxon signed-rank test, $p < 0.0001$) (Fig. 4D).

Additional cytokine analyses and longitudinal CAR T-cell and cytokine kinetics in representative patients are shown in Extended Data Fig. 8.

The cytokine profile of P_04 at DL2 differed markedly from that of all other patients. Peak IL-18 (5,481 pg/ml) was only modestly higher than the maximum at lower dose levels and remained below the previously proposed threshold for IL-18 dominant cytokine storm syndrome³⁴. In contrast, IFN- γ and CXCL9 reached 30,129 pg/ml and 178,879 pg/ml, respectively (Fig. 4D, Extended Data Fig. 8). Despite marked and sustained IL-18BP induction, IFN- γ remained excessive. In cerebrospinal fluid, IFN- γ exceeded serum concentrations, whereas IL-18 remained low (235 and 342 pg/ml) at both measured time points (Extended Data Fig. 8). Thus, adaptive IL-18BP upregulation appeared intact in P_04 but was insufficient to prevent the exceptionally high IFN- γ concentrations associated with the fatal hyperinflammatory syndrome. Clinical diagnostic testing for primary HLH and related hereditary hyperinflammatory disorders by exome-based sequencing with copy number variation analysis identified no pathogenic or likely pathogenic variants.

Pre- and post-treatment tumor tissue analysis

Pre-treatment tumor tissue from four patients with neuroblastoma, including two responders (P_01 and P_05) and two nonresponders (P_03 and P_06), was profiled by multiplex spatial imaging (MICS)^{35,36}. Responder samples showed more pronounced lymphocyte infiltration than nonresponder samples, including tertiary lymphoid structures in P_01 (Fig. 5, Extended Data Fig. 9). The myeloid compartment showed features consistent with an immunosuppressive profile across samples. Post-treatment analysis of a rapidly progressive soft-tissue lesion from P_03, obtained on day 31 while circulating CAR T cells remained detectable, showed reduced immune infiltration after treatment and an increase in the tumor-cell fraction to 90%, with depletion of B and T cells and a shift toward M2-like macrophages (Fig. 5, Extended Data Fig. 9). Although limited by the small number of available samples, these exploratory analyses suggest that limited lymphocyte infiltration and an immunosuppressive TME may represent barriers to treatment activity.

Discussion

In this first-in-human study of an IL-18-enhanced CAR T-cell product in solid tumors, GD2IL18CART administered as a single infusion at comparatively low cell doses showed robust *in vivo* expansion in every patient and objective responses in patients with highly refractory neuroblastoma. Treatment at the recommended doses was associated with clinically significant but manageable inflammatory toxicity, with a characteristic delayed phase that frequently met criteria for IEC-HS. IEC-HS has been reported with other CAR T-cell products^{7,37} and was not observed in initial clinical studies of IL-18-enhanced CAR T cells in hematologic malignancies^{18,38}, indicating that IEC-HS is not specific to IL-18-secreting CAR T-cell products. At the lower dose levels, IEC-HS was predominantly grade 1 or 2 and responded to anti-inflammatory treatment, whereas the single patient treated at DL2 developed fatal hyperinflammation. Cytokine kinetics after GD2IL18CART administration demonstrated engagement of the physiological IL-18 regulatory axis, with IL-18 induction followed by IFN- γ and subsequent IL-18BP upregulation. In P_04, systemic IL-18 was not exceptional, whereas IFN- γ concentrations in serum and cerebrospinal fluid were excessive despite strong IL-18BP induction. The mechanism underlying this exceptional response remains unclear and may involve patient-specific factors.

A key biological finding was the robust expansion of GD2IL18CART in every patient despite cell doses substantially lower than those used with other CAR T-cell products active in solid tumors^{4,6,10}. Peak circulating cell numbers reached or exceeded those reported with CD19-specific CAR T cells^{39,40}, consistent with clinical experience with constitutively IL-18-secreting CAR T cells in hematologic

malignancies¹⁸. This is notable because CAR T cells in solid tumors must traffic to and engage antigen within the tumor tissue before activation and expansion. The magnitude of expansion achieved at these low doses is consistent with a role for activation-inducible IL-18 secretion in enhancing CAR T-cell expansion in patients with solid tumors.

Robust systemic expansion translated into antitumor activity but not sustained long-term tumor control. GD2IL18CART induced objective responses in 4 of 11 patients with highly refractory neuroblastoma (36%) and disease control in 7 (64%). Both complete responses were maintained at month 6, although osseous relapse was detected at month 12, and no objective responses occurred in patients with Ewing sarcoma, osteosarcoma, or breast cancer. The most favorable response rates and long-term outcomes reported to date in neuroblastoma were achieved with a third-generation CAR T-cell product administered at high cell doses^{4,6}. Direct comparison is limited by differences in patient populations and treatment. Our cohort consisted exclusively of heavily pretreated patients, all with prior GD2-directed immunotherapy and most with high tumor burden, and the study did not allow repeat infusions. These findings indicate that robust systemic expansion alone is insufficient for sustained disease control and that, despite the immune-stimulatory effects expected from IL-18 secretion, important barriers to CAR T-cell efficacy persist in solid tumors.

Longitudinal phenotypic and functional characterization of persisting CAR T cells in our study was limited, precluding assessment of whether terminal T-cell differentiation, exhaustion or other mechanisms of impaired CAR T-cell fitness compromised sustained antitumor activity. More comprehensive longitudinal immune profiling will therefore be incorporated into subsequent studies evaluating strategies to support GD2IL18CART activity within the TME.

Among patients with available post-treatment assessments, GD2-negative escape was not identified in neuroblastoma, whereas GD2-negative escape occurred in one patient with Ewing sarcoma. This is consistent with the greater heterogeneity of GD2 expression in non-neuroblastoma tumors. Whole-body antibody imaging, as recently established for GD2^{41,42}, may provide a more comprehensive assessment of antigen distribution than individual biopsies and help define the contribution of antigen escape to treatment failure.

Exploratory spatial profiling further suggested greater baseline lymphocyte infiltration in responder samples, and paired analysis of a rapidly progressing neuroblastoma lesion showed an immunosuppressive microenvironment with reduced lymphocyte infiltration after treatment. Although based on few samples, these observations suggest that the TME may limit treatment efficacy and provide a rationale for strategies to improve tumor infiltration, bystander-cell recruitment, and local immune activation.

This study is limited by its small and heterogeneous population, which precludes conclusions regarding disease- or age-specific patterns of activity and toxicity. Tissue analyses were exploratory and restricted to a small number of samples, and the study lacked a comparator CAR T-cell product without inducible IL-18 secretion, precluding direct attribution of the observed expansion or toxicity profile to IL-18.

In conclusion, GD2IL18CART demonstrated robust in vivo expansion at low administered cell doses and antitumor activity in refractory neuroblastoma. The fatal hyperinflammatory event at higher dosing identifies an important safety constraint, whereas the lack of sustained tumor control despite universal expansion highlights the need to overcome tumor-intrinsic and microenvironmental resistance.

Methods

Study design and patients

The study is a multicenter phase I safety, dose-finding and feasibility trial conducted in Germany and approved by the Paul-Ehrlich-Institut and the responsible ethics committees on March 19, 2024 (EU CT 2022-501725-21-00). The study enrolls patients with GD2-positive relapsed and/or refractory (r/r) neuroblastoma, Ewing sarcoma, osteosarcoma or breast cancer with measurable disease after failure of all standard therapies. The dose-finding phase has been completed, whereas dose-extension cohorts are ongoing. The primary objective of the dose-finding phase was to characterize the incidence and severity of adverse events and determine a recommended dose using a Bayesian Optimal Interval (BOIN) design. Dose escalation was conducted independently in pediatric and adult cohorts, starting at 1×10^5 CAR-positive T cells per kilogram. Inclusion and exclusion criteria are given in Extended Data Table 3, and major protocol changes during study conduct are listed in Extended Data Table 4.

CAR T-cell product and manufacture

GD2IL18CART is a fresh autologous CD4/CD8-enriched T-cell product genetically modified with a self-inactivating (SIN) lentiviral vector encoding a GD2-specific CAR containing 4-1BB and CD3z signaling domains and NFAT-driven inducible IL-18 in a single construct^{15,43}. The GD2 binding domain was originally derived from the 14.G2a hybridoma antibody using a phage-display approach⁴⁴. The single-vector architecture has been previously described³. GD2IL18CART products are manufactured in a decentralized manner at two academic point-of-care manufacturing centers at University Hospital Erlangen and University Hospital Muenster, respectively using a clinical-scale GMP vector batch from Miltenyi Biotec and a 12-day semiautomatic process on the CliniMACS Prodigy (Miltenyi Biotec, Bergisch Gladbach, Germany), starting from fresh leukapheresis material. Manufacturing has been described in detail previously¹⁵. Products were transported to the trial sites and administered fresh within 24 hours of release without cryopreservation.

Treatment and assessments

Patients received lymphodepletive chemotherapy with fludarabine (40 mg/m^2 per day on three consecutive days) and cyclophosphamide (600 mg/m^2 per day on two consecutive days) followed by a single fresh GD2IL18CART infusion. Patients were monitored for dose-limiting toxicities (DLTs) for 28 days. Adverse events were graded according to CTCAE version 5.0, CRS and ICANS according to ASTCT consensus criteria⁴⁵, and IEC-HS according to Hines et al.²⁹. According to the protocol, abnormal laboratory values

were recorded as adverse events when considered clinically significant by the investigator. Tumor response was assessed at week 12 and subsequently at protocol-defined time points using disease-specific response criteria. The clinical trial protocol, including the statistical analysis plan, is provided with the manuscript.

GD2 expression in tumor tissues

GD2 expression was assessed by immunofluorescence staining, as previously published in detail²⁸. Briefly, formalin-fixed paraffin-embedded (FFPE) tissues were deparaffinized with xylene for 30 minutes (2 × 15 minutes) and rehydrated in decreasing concentrations of ethanol (99%, 96%, 70%). Antigen retrieval was performed in a steamer with Tris/EDTA buffer (pH 8.5) for 2.5 minutes, followed by cooling to room temperature (RT) for 20 minutes in the same buffer. Endogenous peroxidases were blocked with 3% H₂O₂ diluted in PBS for 15 minutes. For protein blocking, the slides were blocked with normal goat serum for 1 hour at RT. After washing with PBS, slides were incubated overnight at 4°C with the unconjugated primary anti-GD2 antibody (clone 14.G2a) diluted 1:50 in PBS supplemented with 10% (v/v) goat serum. Five 5-minute washes in Tris-buffered saline supplemented with 0.1% (v/v) Tween 20 (TBST) were conducted after each incubation. Then the slides were incubated with HRP-conjugated secondary antibody (Polymer HRP Ms+Rb) for 10 minutes at RT, followed by staining with the tyramide signal amplification method (TSA) using the Opal fluorophore 520 (1:100) for 10 minutes at RT. The slides were counterstained with DAPI and enclosed with Prolong Diamond Antifade solution. Imaging was performed with the Vectra 3.0 system (PerkinElmer, Rodgau, Germany). GD2 staining intensity and distribution were assessed by direct microscopic examination. Semiquantitative scoring was performed independently by two experienced operators, including a pathologist. A sample was considered GD2-positive per study protocol when ≥50% of tissue expressed GD2 at high (3+) or intermediate (2+) intensity.

Quantification of circulating CAR T cells by flow cytometry and immunophenotyping

CAR T-cell quantification was performed centrally at the University Hospital of Erlangen. EDTA-anticoagulated peripheral blood was collected on the indicated days post-CAR T-cell infusion. Red blood cell lysis was performed according to the manufacturer (Miltenyi Biotec) and cells were stained with anti-human CD3 (clone SK7), CD4 (RPA-T4), CD8 (RPA-T8), anti-CD45 (HI30), CD45RA (HI100), CCR7 (G042H7), and GD2 CAR 14G2a Idiotypic Antibody (REA1351). Flow cytometry data were acquired on an LSRFortessa (BD Biosciences) and data were analyzed using Kaluza software v2.1 (Beckman Coulter, Krefeld, Germany).

Vector copy number analysis

For the GD2IL18CART cell products, the mean vector copy number per cell (VCN) was determined by Minerva. At Hannover Medical School, the VCN determination for the patient samples included in the Insertion Site Analysis was performed by droplet digital PCR (ddPCR) with a TaqMan approach on a QX200TM system (Bio-Rad GmbH, Dreieich, Germany). The number of viral sequences was normalized to a genomic reference sequence. Viral sequences were quantified by targeting WPRE element, whereas primers targeting the PTBP2 gene were used for normalization of genomic DNA (gDNA)⁴⁶.

Insertion Site Analysis

Insertion site analysis (ISA) was performed using the INSPIRED (INtegration Site Pipeline for palRed-End readDs) workflow, as described by Sherman and colleagues⁴⁷. In brief, genomic DNA was fragmented by sonication using a Covaris 220 sonicator (Covaris, Woburn, MA, USA). For samples with very low gDNA concentrations (<95 ng), whole-genome amplification was performed prior to sonication with the REPLI-g Mini kit (Qiagen). After sonication, samples were purified with AMPure Bead (Beckman and Coulter, Krefeld, Germany), end-repaired with the NEBNext Ultra End II Repair/dA-Tailing Module (New England Biolabs (NEB), Frankfurt, Germany) and subjected to linker ligation using the NEBNext Ultra II Ligation Module (NEB). A unique linker was used for each sample to prevent cross-contamination between samples during batch processing. Primers specific for the SIN long-terminal repeat (LTR) region of the lentivirus, combined with linker-specific primers, were used to amplify vector genome junctions. The amplification from the SIN-LTR into the vector (instead of the genome) was controlled and largely prohibited using blocking oligonucleotides. In a second nested PCR, LTR-specific index primers, together with linker-specific primers, were used to attach next-generation sequencing (NGS) adapters for Illumina paired-end sequencing. The PCR products were visualized on 2% agarose gels, mixed in equal ratios, and AMPure purified to retrieve final NGS libraries. Libraries were sequenced on Illumina flow cells with 15 million clusters. Bioinformatic steps were generally performed as described by Berry and colleagues⁴⁸. Demultiplexed sequences in FASTQ format were quality filtered, aligned and annotated to the human genome (hg38). The plasmid vector sequences served as a reference for LTR regions and vector trimming. Figures and tables were generated with customized post-processing steps in R and Excel. The integration sites were analyzed for their diversity/polyclonality, distance to genomic features, clonal imbalance (prominent clones contributing > 10% to the overall sequencing pool after VCN normalization), and proximity to known proto-oncogenes.

Human anti-chimeric antibody analysis

Human anti-chimeric antibody (HACA) responses were assessed by sandwich ELISA using the previously described anti-idiotypic antibody ganglidiomab directed against the GD2-specific CAR binding domain as a standard for quantification⁴⁹. Serum samples obtained before and at two time points after GD2IL18CART infusion were incubated on microtiter plates coated with 14.G2a for capture, followed by detection of bound human immunoglobulins using biotinylated 14.G2a and NeutrAvidin-conjugated horseradish peroxidase. Color development was measured spectrophotometrically according to standard ELISA procedure.

Cytokine analysis

Quantification of peripheral blood cytokines was performed in a centralized manner at University of Muenster using ProteinSimple's Immunoassay Platform for Protein Quantification (Ella™ Automated ELISA) and SimplePlex Ella Cartridges (Bio-Techne, SPCKC-PS), loaded with IL-18BP, CXCL10/IP-10, IFN-γ 3rd Gen, IL-18/IL-1F4, IL-2Ra/CD25 and MIG/CXCL9 assays for use with human plasma/serum. Following the manufacturer's recommendations, serum samples were stored at -80°C, thawed on ice, then centrifuged at 10,000 x g for 4 minutes to remove particulates. Subsequently, 50 µl of serum diluted in manufacturer's

sample diluent at either 1:2 or 1:50 dilution was loaded onto the cartridge along with assay-specific wash buffer and recombinant protein controls. Cartridges were processed and analyzed using the Ella system.

Experimental [⁶⁴Cu]Cu-NOTA-ch14.18/CHO ([⁶⁴Cu]GD2) PET/CT

Whole-body [⁶⁴Cu]GD2 PET/CT imaging was not part of the clinical trial protocol and was performed outside the study at the discretion of the treating physicians. Imaging methodology has been described previously⁴². Retrospective analysis of the data was performed under a separate local ethics approval (no. 2026-0577-BO_retro) and with separate informed consent.

Multicolor spatial imaging

FFPE tissue blocks were prepared from archived tissue blocks according to standard immunohistochemistry protocols. Three µm thick sections were prepared from pre- and post-treatment samples using a microtome and mounted onto Epredia™ SuperFrost Plus™ Adhesion slides (FisherScientific) according to the positioning template of the MACSwell Two Imaging Frames (Milenyi Biotec) to indicate the optimal position of the sections on the slide. Subsequently, slides were incubated overnight at 56°C. For deparaffinization, slides were treated for 20 min with xylene and then rehydrated through graded concentrations of ethanol ending in distilled water (dH₂O) (100%, 95%, 80%, 70%, 50% ethanol, dH₂O for 1 min each). Antigen retrieval was carried out in Tris-EDTA citrate (TEC) buffer (pH9) for 20 min at 98°C in an Epredia Lab Vision PT-Module (Thermo Fisher). After washing with MACSima Running Buffer (Milenyi Biotec), slides were mounted into MACSwell™ Two Imaging Frames (Milenyi Biotec). MACSima™ Imaging Cyclic Staining (MICS) technology was applied as previously described^{35,36}. In brief, the MACSima Imaging platform (Milenyi Biotec) allows fully automated liquid handling in combination with widefield microscopy to enable cyclic imaging with a multitude of antibodies targeting selected markers of interest. All samples were processed using the same MACSima instrument. The procedure is based on cycles of staining, imaging, and erasure using either optical bleaching or fluorescent dye release for REAlease antibodies with the REAlease® Release Reagent (Milenyi Biotec). Antibodies used were conjugated to FITC, PE or APC and are listed below. Regions of interest (ROI) were selected based on GD2 and DAPI pre-staining, avoiding areas of necrosis before loading the samples onto the instrument. In brief, 10 µg/ml of purified Mouse Anti-Human Disialoganglioside GD2 antibody (BD) was applied onto the sections and incubated overnight at 4-8°C, followed by a secondary staining for 1 h at RT using the Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 647 (Thermo Fisher Scientific). Raw data from each ROI was processed using the integrated preprocessing in MACSiQ View version 1.3.2 or 1.3.3, choosing the result with the best stitching and registration for each ROI (1.3.2 for pre-treatment ROI 1, 1.3.3 for all other ROIs). Exposure selection was set to "high" for each ROI. For bioinformatics analysis, cell segmentation was performed in MACSiQ View 1.3.2 as described previously^{35,36}. For each ROI, regions that showed strong artifacts from the analysis were excluded by drawing region exclusion gates in the MACSiQ View Software; these were later included in the "Unassigned" category. Biomarker features and image exclusion membership were exported as CSV files, and all subsequent analyses were done in Python 3.11, using scanpy, numpy, pandas, seaborn, and matplotlib. There, average cell intensity was normalized using a log10 transform, and cells were annotated using

Leiden clustering using the markers as described in the antibody table. First, cells were clustered into general cell compartments including markers as indicated in the table below, followed by a second round of clustering within each compartment to assign cell types. Each cluster was assigned a cell type based on its expression profile using violin plots (Extended Data Fig. 9B), and clusters with similar profiles were merged. Cells showing positive signal for multiple markers were assigned to the most likely compartment, with preference given to lymphoid or myeloid assignments over assignment to the tumor compartment when overlapping marker expression was observed. Cells showing no specific staining were labeled "Unassigned".

Antigen	Clone	Vendor	Catalog number	Dilution Factor/ concentration	Conjugate	Rationale
a-smooth Actin	REAL650	Miltenyi Biotec	130-123-370	100	FITC	Stroma compartment, blood vessels
BCL2	REA872	Miltenyi Biotec	130-114-339	50	APC	Tumor compartment
CD11b	REA1321	Miltenyi Biotec	130-128-773	25	PE	Myeloid compartment
CD11c	REAL1327	Miltenyi Biotec	130-133-325	50	PE	Myeloid compartment, macrophages, DCs
CD123	REA918	Miltenyi Biotec	130-115-265	25	APC	Myeloid cells
CD14	REA1314	Miltenyi Biotec	130-128-771	50	PE	Monocytes
CD15	VIMC6	Miltenyi Biotec	130-113-482	50	APC	Neutrophils
CD16	REA1324	Miltenyi Biotec	130-128-774	25	PE	Monocytes
CD163	REA1309	Miltenyi Biotec	130-127-908	50	PE	Myeloid compartment, M2 macrophages
CD171	REAL1365	Miltenyi Biotec	130-132-024	50	APC	Tumor compartment
CD1c	REAL1005	Miltenyi Biotec	130-127-024	50	FITC	Dendritic cells
CD20	REAL1069	Miltenyi Biotec	130-135-440	50	PE	Lymphoid compartment, B cells (N-Terminus)
CD20	REA1087	Miltenyi Biotec	130-118-292	50	FITC	Lymphoid compartment, B cells (C-Terminus)
CD3	REA1151	Miltenyi Biotec	130-120-267	50	FITC	Lymphoid compartment, T cells

CD31	REA1312	Miltenyi Biotec	130-128-769	25	PE	Stroma compartment, endothelial cells
CD38	REAL719	Miltenyi Biotec	130-126-438	25	PE	B cells
CD4	REA1307	Miltenyi Biotec	130-127-906	50	PE	Lymphoid compartment, CD4 T cells
CD45	2B11& PD7/26	Novus Biologicals	NBP2-34528PE	10 µg/ml	PE	Lymphoid compartment
CD57	REA769	Miltenyi Biotec	130-111-810	50	PE	NK cells
CD66b	REA306	Miltenyi Biotec	130-122-922	60	PE	Neutrophils
CD68	REA1306	Miltenyi Biotec	130-128-345	100	PE	Myeloid compartment, macrophages
CD79a	REA1168	Miltenyi Biotec	130-120-939	50	PE	B cells
CD8a	REA1024	Miltenyi Biotec	130-117-202	50	APC	Lymphoid compartment, CD8 T cells
Chromogranin A	REAL1033	Miltenyi Biotec	130-127-827	50	APC	Tumor compartment
HLA-DR	REAL550	Miltenyi Biotec	130-123-076	50	FITC	Immune cells
iNOS	4 E5	Novus Biologicals	NBP2-22119PE	2 µg/ml	PE	Myeloid compartment, M1-macrophages
Ki67	REAL1047	Miltenyi Biotec	130-127-837	50	FITC	Proliferation
Podoplanin	REA446	Miltenyi Biotec	130-117-799	50	PE	Stroma compartment, lymphatic vessels
S100A9	REAL1441	Miltenyi Biotec	130-133-107	25	PE	Myeloid compartment, neutrophils
Vimentin	REAL1008	Miltenyi Biotec	130-127-018	50	APC	Fibroblasts

Declarations

Acknowledgements

We thank the chair of the DMC (Paul-Gerhardt Schlegel) and all further members (John Haanen, Verena Hoffmann, Michael Lauseker, Barbara Savoldo, Alfred Zippelius) for their dedicated support and guidance throughout the trial. We further thank Winfried Krüger and Philip Hardtstock and their teams for clinical vector production, Silvia Rüberg, Fabio El Yassouri, Sonja Lange, Anijutta Appelshoffer, Svetlana Khorkova, Julia Femel, Fabian Staubach for support in multicolor spatial imaging analysis, Sandy Reiß, Gianni D'Apollonio, Victoria Schoppel, Stephanie Moi, Sara Gamali, Stefanie Schaffer, Heidi Spreitzer, Lena Tarantik, Alena Hammerschmitt, Nicole Bialas, Nina Köhl, Maike Stahlhut, Maike Hagedorn and Lena Rehberg for excellent technical assistance, Florentine Schonath and Maria Xydia for their contributions to immune monitoring analyses, Ursula Bonsmann for regulatory support, Christine Ernst, Ana Ferreira, Luisa Arold, Jacqueline Braun, Soraya Kharboutli, Zofia Wotschofsky and Jens Habekost for patient documentation and study nurse activities, Holger Lode and Nicolai Siebert for providing the anti-idiotypic antibody ganglidiomab, Judith Horvath for expert support with clinical diagnostics, Marc Saake and Johannes Schwenck for additional support with the radiology and nuclear medicine imaging and Birger Brefeld for legal advice. We thank the referring physicians and the patients and their families.

Funding statement

This work was funded by the German Federal Ministry of Education and Research (BMBF, grant 01EN2007), by Deutsche Krebshilfe (Priority Program Translational Oncology grant 111975), by a grant from the Deutsche Forschungsgemeinschaft (CRC1450) and by Kinderkrebshilfe Muenster e.V.. The development of [⁶⁴Cu]GD2 was supported by the Deutsche Forschungsgemeinschaft under Germany's Excellence Strategy – EXC 2180–390900677: Cluster of Excellence iFIT “Image-Guided 970 and Functionally Instructed Tumor Therapies”.

References

1. Maude, S.L., *et al.* Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia. *N Engl J Med* **378**, 439–448 (2018).
2. Neelapu, S.S., *et al.* Five-year follow-up of ZUMA-1 supports the curative potential of axicabtagene ciloleucel in refractory large B-cell lymphoma. *Blood* **141**, 2307–2315 (2023).
3. Martin, T., *et al.* Ciltacabtagene Autoleucel, an Anti-B-cell Maturation Antigen Chimeric Antigen Receptor T-Cell Therapy, for Relapsed/Refractory Multiple Myeloma: CARTITUDE-1 2-Year Follow-Up. *J Clin Oncol* **41**, 1265–1274 (2023).
4. Del Bufalo, F., *et al.* GD2-CART01 for Relapsed or Refractory High-Risk Neuroblastoma. *N Engl J Med* **388**, 1284–1295 (2023).
5. Hegde, M., *et al.* Autologous HER2-specific CAR T cells after lymphodepletion for advanced sarcoma: a phase 1 trial. *Nat Cancer* **5**, 880–894 (2024).
6. Locatelli, F., *et al.* GD2-targeting CAR T cells in high-risk neuroblastoma: a phase 1/2 trial. *Nat Med* **31**, 3689–3699 (2025).

7. Mackensen, A., et al. CLDN6-specific CAR-T cells plus amplifying RNA vaccine in relapsed or refractory solid tumors: the phase 1 BNT211-01 trial. *Nat Med* **29**, 2844–2853 (2023).
8. Qi, C., et al. Claudin18.2-specific CAR T cells in gastrointestinal cancers: phase 1 trial final results. *Nat Med* **30**, 2224–2234 (2024).
9. Chmielewski, M., Kopecky, C., Hombach, A.A. & Abken, H. IL-12 release by engineered T cells expressing chimeric antigen receptors can effectively Muster an antigen-independent macrophage response on tumor cells that have shut down tumor antigen expression. *Cancer Res* **71**, 5697–5706 (2011).
10. Steffin, D., et al. Interleukin-15-armoured GPC3 CAR T cells for patients with solid cancers. *Nature* **637**, 940–946 (2025).
11. Kaplanski, G. Interleukin-18: Biological properties and role in disease pathogenesis. *Immunol Rev* **281**, 138–153 (2018).
12. Aizawa, Y., et al. Cloning and expression of interleukin-18 binding protein. *FEBS letters* **445**, 338–342 (1999).
13. Avanzi, M.P., et al. Engineered Tumor-Targeted T Cells Mediate Enhanced Anti-Tumor Efficacy Both Directly and through Activation of the Endogenous Immune System. *Cell Rep* **23**, 2130–2141 (2018).
14. Chmielewski, M. & Abken, H. CAR T Cells Releasing IL-18 Convert to T-Bet(high) FoxO1(low) Effectors that Exhibit Augmented Activity against Advanced Solid Tumors. *Cell Rep* **21**, 3205–3219 (2017).
15. Fischer-Riepe, L., et al. Preclinical Development of CAR T Cells with Antigen-Inducible IL18 Enforcement to Treat GD2-Positive Solid Cancers. *Clin Cancer Res* **30**, 3564–3577 (2024).
16. Hu, B., et al. Augmentation of Antitumor Immunity by Human and Mouse CAR T Cells Secreting IL-18. *Cell Rep* **20**, 3025–3033 (2017).
17. Jaspers, J.E., et al. IL-18-secreting CAR T cells targeting DLL3 are highly effective in small cell lung cancer models. *J Clin Invest* **133**(2023).
18. Svoboda, J., et al. Enhanced CAR T-Cell Therapy for Lymphoma after Previous Failure. *N Engl J Med* **392**, 1824–1835 (2025).
19. Schulz, G., et al. Detection of ganglioside GD2 in tumor tissues and sera of neuroblastoma patients. *Cancer Res.* **44**, 5914–5920 (1984).
20. Kailayangiri, S., et al. The ganglioside antigen G(D2) is surface-expressed in Ewing sarcoma and allows for MHC-independent immune targeting. *Br J Cancer* **106**, 1123–1133 (2012).
21. Roth, M., et al. Ganglioside GD2 as a therapeutic target for antibody-mediated therapy in patients with osteosarcoma. *Cancer* **120**, 548–554 (2014).
22. Erber, R., et al. Variable Expression of the Disialoganglioside GD2 in Breast Cancer Molecular Subtypes. *Cancers* **13**(2021).
23. Reppel, L., et al. Targeting disialoganglioside GD2 with chimeric antigen receptor-redirected T cells in lung cancer. *J Immunother Cancer* **10**(2022).
24. Cheung, I.Y., et al. Survival Impact of Anti-GD2 Antibody Response in a Phase II Ganglioside Vaccine Trial Among Patients With High-Risk Neuroblastoma With Prior Disease Progression. *J Clin Oncol* **39**,

215–226 (2021).

25. 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).
26. Lewis, E.C., et al. A subset analysis of a phase II trial evaluating the use of DFMO as maintenance therapy for high-risk neuroblastoma. *Int J Cancer* **147**, 3152–3159 (2020).
27. Sundquist, F., et al. A Phase II Trial of a Personalized, Dose-Intense Administration Schedule of (177)Lutetium-DOTATATE in Children With Primary Refractory or Relapsed High-Risk Neuroblastoma-LuDO-N. *Front Pediatr* **10**, 836230 (2022).
28. Kailayangiri, S., et al. Protocol for assessing GD2 on formalin-fixed paraffin-embedded tissue sections using immunofluorescence staining. *STAR Protoc* **5**, 103199 (2024).
29. Hines, M.R., et al. Immune Effector Cell-Associated Hemophagocytic Lymphohistiocytosis-Like Syndrome. *Transplant Cell Ther* **29**, 438 e431–438 e416 (2023).
30. Rainone, M., et al. Interferon-gamma blockade in CAR T-cell therapy-associated macrophage activation syndrome/hemophagocytic lymphohistiocytosis. *Blood Adv* **7**, 533–536 (2023).
31. Schuelke, M.R., et al. Emapalumab for the treatment of refractory cytokine release syndrome in pediatric patients. *Blood Adv* **7**, 5603–5607 (2023).
32. Park, J.R., et al. Revisions to the International Neuroblastoma Response Criteria: A Consensus Statement From the National Cancer Institute Clinical Trials Planning Meeting. *J Clin Oncol* **35**, 2580–2587 (2017).
33. Lewington, V., et al. (123)I-mIBG scintigraphy in neuroblastoma: development of a SIOPEN semi-quantitative reporting method by an international panel. *Eur J Nucl Med Mol Imaging* **44**, 234–241 (2017).
34. Kaneko, S., et al. Serum Cytokine Profiling Differentiates Underlying Diseases in Cytokine Storm Syndrome. *Arthritis Rheumatol* **78**, 463–474 (2026).
35. Kinkhabwala, A., et al. MACSima imaging cyclic staining (MICS) technology reveals combinatorial target pairs for CAR T cell treatment of solid tumors. *Sci Rep* **12**, 1911 (2022).
36. Stahl, D., et al. CSF1R(+) myeloid-monocytic cells drive CAR-T cell resistance in aggressive B cell lymphoma. *Cancer Cell* **43**, 1476–1494 e1410 (2025).
37. Shah, N.N., et al. CD4/CD8 T-Cell Selection Affects Chimeric Antigen Receptor (CAR) T-Cell Potency and Toxicity: Updated Results From a Phase I Anti-CD22 CAR T-Cell Trial. *J Clin Oncol* **38**, 1938–1950 (2020).
38. Geyer, M.B., et al. CD371-targeted CAR T cells secreting interleukin-18 exhibit robust expansion and clear refractory acute myeloid leukemia. *Blood* **146**, 3163–3174 (2025).
39. Wang, M., et al. KTE-X19 CAR T-Cell Therapy in Relapsed or Refractory Mantle-Cell Lymphoma. *N Engl J Med* **382**, 1331–1342 (2020).

40. Gardner, R.A., et al. Intent-to-treat leukemia remission by CD19 CAR T cells of defined formulation and dose in children and young adults. *Blood* **129**, 3322–3331 (2017).

41. Trautwein, N.F., et al. First-in-Humans PET/MRI of In Vivo GD2 Expression in Osteosarcoma. *J Nucl Med* **64**, 337–338 (2023).

42. Trautwein, N.F., et al. A novel approach to guide GD2-targeted therapy in pediatric tumors by PET and [(64)Cu]Cu-NOTA-ch14.18/CHO. *Theranostics* **14**, 1212–1223 (2024).

43. Zimmermann, K., et al. Design and Characterization of an "All-in-One" Lentiviral Vector System Combining Constitutive Anti-GD2 CAR Expression and Inducible Cytokines. *Cancers* **12**, 375 (2020).

44. Rossig, C., Bollard, C.M., Nuchtern, J.G., Merchant, D.A. & Brenner, M.K. Targeting of GD2-positive tumor cells by human T lymphocytes engineered to express chimeric T-cell receptor genes. *Int J Cancer* **94**, 228–236 (2001).

45. Lee, D.W., et al. ASTCT Consensus Grading for Cytokine Release Syndrome and Neurologic Toxicity Associated with Immune Effector Cells. *Biol Blood Marrow Transplant* **25**, 625–638 (2019).

46. Heinz, N., et al. Retroviral and transposon-based tet-regulated all-in-one vectors with reduced background expression and improved dynamic range. *Hum Gene Ther* **22**, 166–176 (2011).

47. Sherman, E., et al. INSPIRED: A Pipeline for Quantitative Analysis of Sites of New DNA Integration in Cellular Genomes. *Mol Ther Methods Clin Dev* **4**, 39–49 (2017).

48. Berry, C.C., et al. INSPIRED: Quantification and Visualization Tools for Analyzing Integration Site Distributions. *Mol Ther Methods Clin Dev* **4**, 17–26 (2017).

49. Siebert, N., et al. Validated detection of anti-GD2 antibody ch14.18/CHO in serum of neuroblastoma patients using anti-idiotypic antibody ganglidiomab. *J Immunol Methods* **398-399**, 51–59 (2013).

Table

Table 1. Patient characteristics

	Pediatric patients (n=14)	Adult patients (n=9)	All patients (n=23)
Sex, n			
Male	11	6	17
Female	3	3	6
Age, median (range) in yrs	8.8 (4.2-17.5)	32 (22-57)	12.7 (4.2-57)
Cancer Type			
Neuroblastoma	11	n.a.	11
Ewing sarcoma	1	6	7
Osteosarcoma	2	1	3
Breast cancer	n.a.	2	2
Sites of disease at screening, n			
Bone	14	4	18
Bone marrow*	4	1	5
Soft tissues	5	1	6
Lymph nodes	3	3	6
Lung/pleural	1	7	8
Liver	-	1	1
Skin	-	1	1
SIOPEN score** (median, range)	3.5 (1-31)	n.a.	
Previous lines of therapy (median, range)	3 (2-6)	4 (3-8)	3 (2-8)
History of (n)			
- GD2 antibody therapy (NBL)	11/11	n.a.	
- GD2 CAR T cell therapy (NBL)	1/11	n.a.	
- haploidentical transplant (NBL)	6/11	n.a.	
- high-dose therapy with aHSCT (NBL, EwS)	11/12	3/6	
GD2 status (predominant intensity), n			
3+	12	5	17

2+

2

4

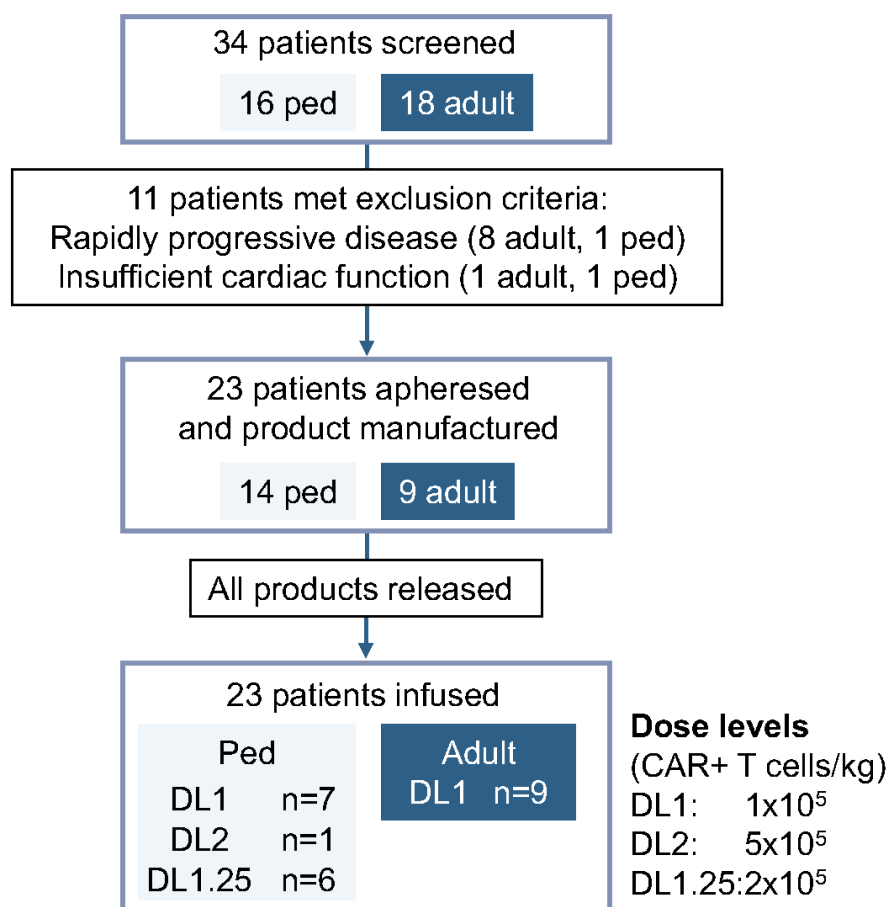
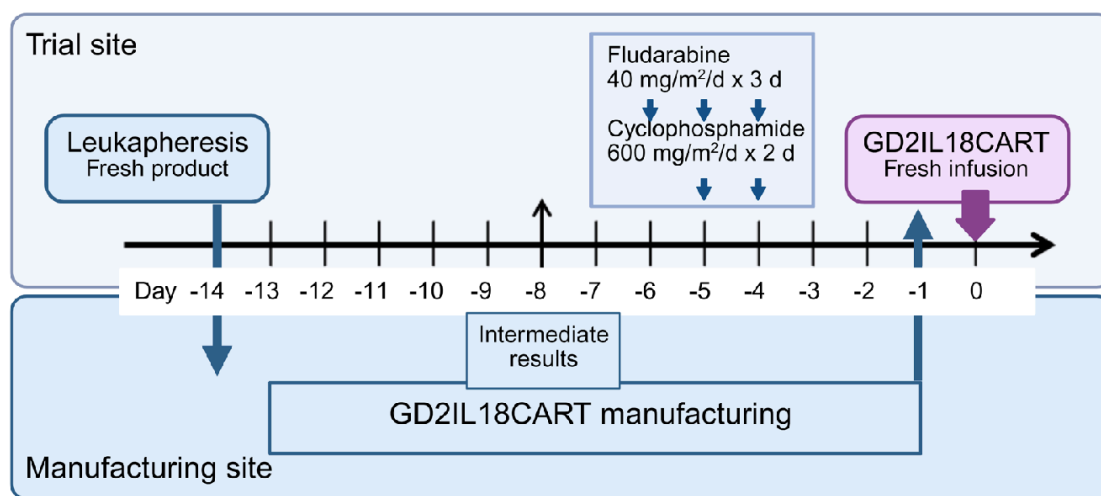
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*as assessed by bone marrow cytomorphology and/or flow cytometry.

**in the 8 NBL patients with bone/bone marrow disease assessed by whole-body [¹²³I]metaiodobenzylguanidine (mIBG) scintigraphy, for which SIOPEN scoring is formally defined. In additional 3 NBL patients evaluated by whole-body 2-deoxy-2-[¹⁸F]fluoro-D-glucose (FDG) positron emission tomography/computed tomography (PET/CT) (n=2) or PET/CT using the somatostatin receptor tracer [¹⁸F]SiFAlin-TATE (n=1), analogous lesion distribution and intensity scoring yielded values of >19, 8, and 8, respectively.

NBL, neuroblastoma; EwS, Ewing sarcoma; aHSCT, autologous hematopoietic stem cell transplantation

Figures

A**B****Figure 1****Trial status and intervention scheme.**

(A) Patient flow diagram showing screening, enrollment and dose assignment in pediatric and adult dose-finding cohorts. The initial dose-escalation scheme was amended after fatal dose-limiting toxicity occurred in patient P_04 treated at DL2 to allow more gradual escalation with intermediate dose levels (DL1.25, 2 x

10⁵ CAR-positive T cells/kg and DL1.5, 3 x 10⁵ CAR-positive T cells/kg). No patients received DL1.5. **(B)** Fresh leukapheresis products were shipped from trial sites to either of two academic manufacturing centers for semi-automated manufacture of GD2IL18CART products¹⁵. Initiation of lymphodepleting chemotherapy was guided by intermediate results from a quality control performed on day 6. Fresh products were delivered to trial sites and administered within 24 hours.

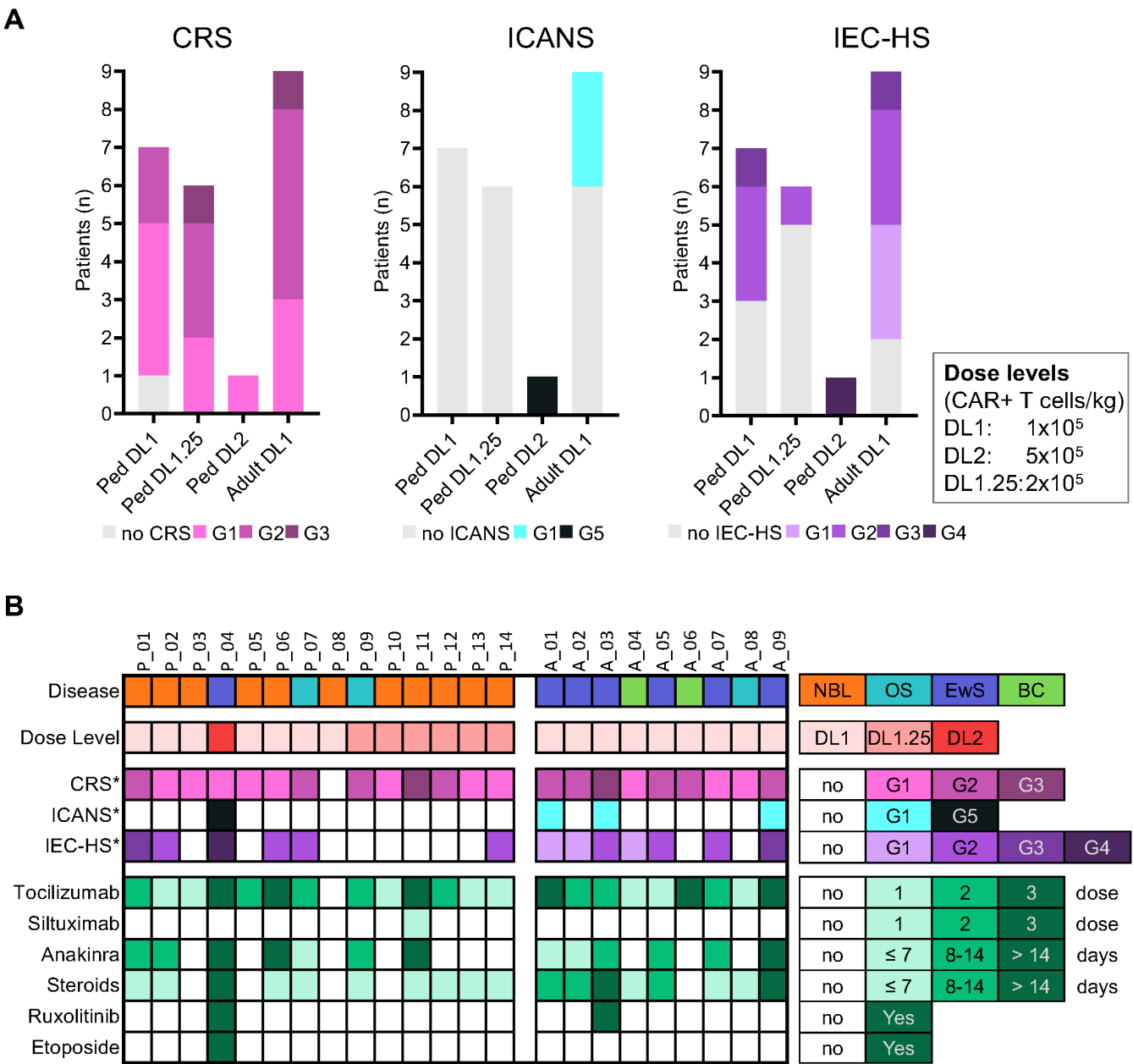


Figure 2

Inflammatory toxicities and management.

(A) Frequency and maximum grades of cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS) and immune effector cell-associated hemophagocytic lymphohistiocytosis-like syndrome (IEC-HS) in pediatric and adult dose-finding cohorts. Dose levels are indicated as DL1 (1×10^5 CAR-positive T cells/kg), DL1.25 (2×10^5 CAR-positive T cells/kg) and DL2 (5×10^5 CAR-positive T cells/kg). The grade 3 CRS event reported in the adult cohort occurred in A_03 on day 39, after CAR T-cell contraction and in the setting of documented neutropenic E. coli sepsis. Its attribution to CAR T-cell-associated CRS was therefore considered unlikely. **(B)** Individual timelines showing onset, course and management of inflammatory toxicities after GD2IL18CART infusion. *Maximum grade.

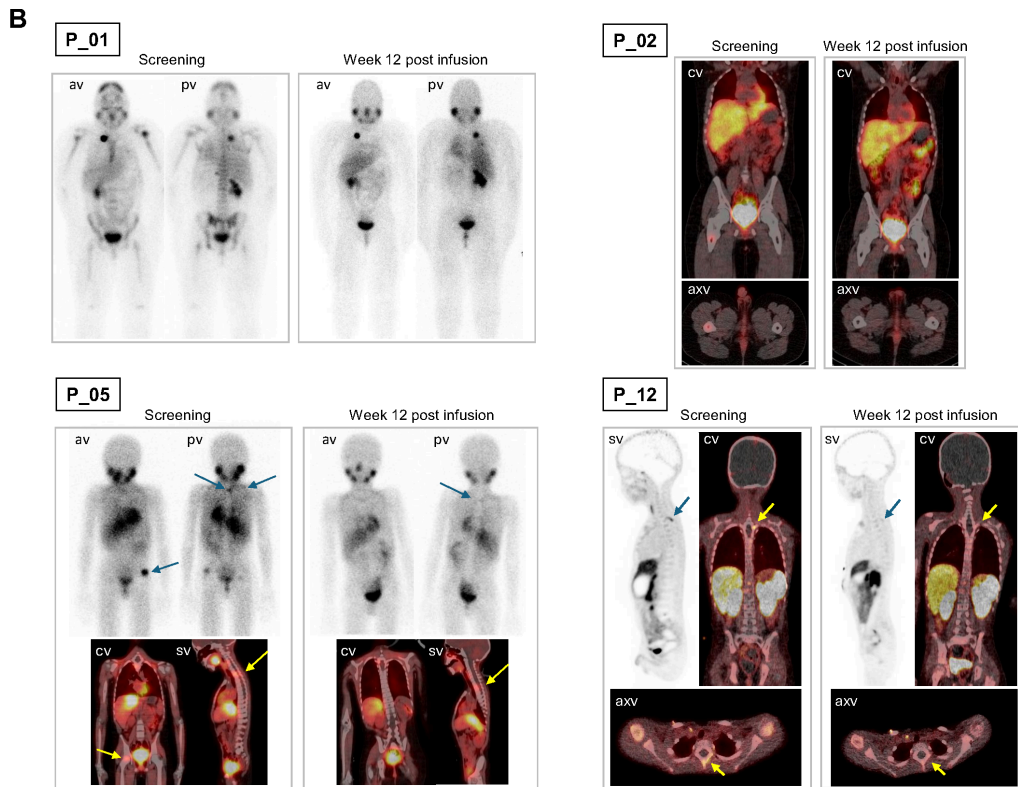
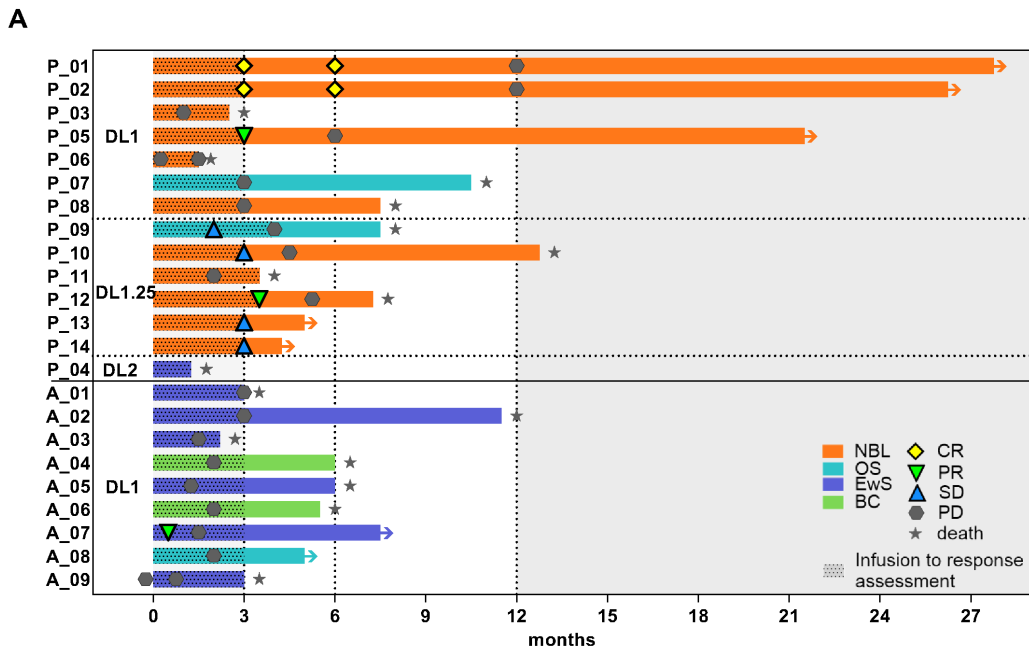


Figure 3

Outcomes after GD2IL18CART therapy.

(A) Swimmer plot showing clinical course, treatment response and follow-up of all 23 patients. Data cut-off was 14 August 2026. **(B)** Representative pre- and post-treatment imaging of responding patients. $[^{123}\text{I}]$ mIBG scintigraphy images in patients P_01 and P_02 (complete responses) and P_05 (partial

response) were acquired 24 h after intravenous injection of [^{123}I]mIBG, and P_12 was assessed by [^{18}F]SiTATE PET/CT. For P_01 and P_05, planar anterior and posterior views are shown, and (additional) SPECT/CT fusion imaging in P_05 (coronal and sagittal view). For P_02, SPECT/CT fusion imaging (coronal and sagittal view) is shown. For P_12, PET data alone (sagittal view) and PET/CT fusion imaging (coronal and axial view) are shown. In P_01, diffuse skeletal [^{123}I]mIBG uptake at screening resolved completely at week 12. Asymmetric uptake in the renal pelvis is due to pre-existing left-sided hydronephrosis. In P_02, the single [^{123}I]mIBG-positive bone lesion (right femur) resolved after treatment. In P_05, multiple skeletal lesions (arrows) regressed, with residual low-level uptake in the second thoracic vertebral body. Only a single weakly metabolically active bone lesion remained (arrow), which showed concurrent regression on magnetic resonance tomography and never became a site of subsequent relapse. In P_12, pre-and post-treatment PET/CT fusion imaging shows resolution of focal bone uptake in T2/T3 vertebral bodies.

av, anterior view; axv, axial view; cv, coronal view; pv, posterior view; sv, sagittal view; sagittal view.

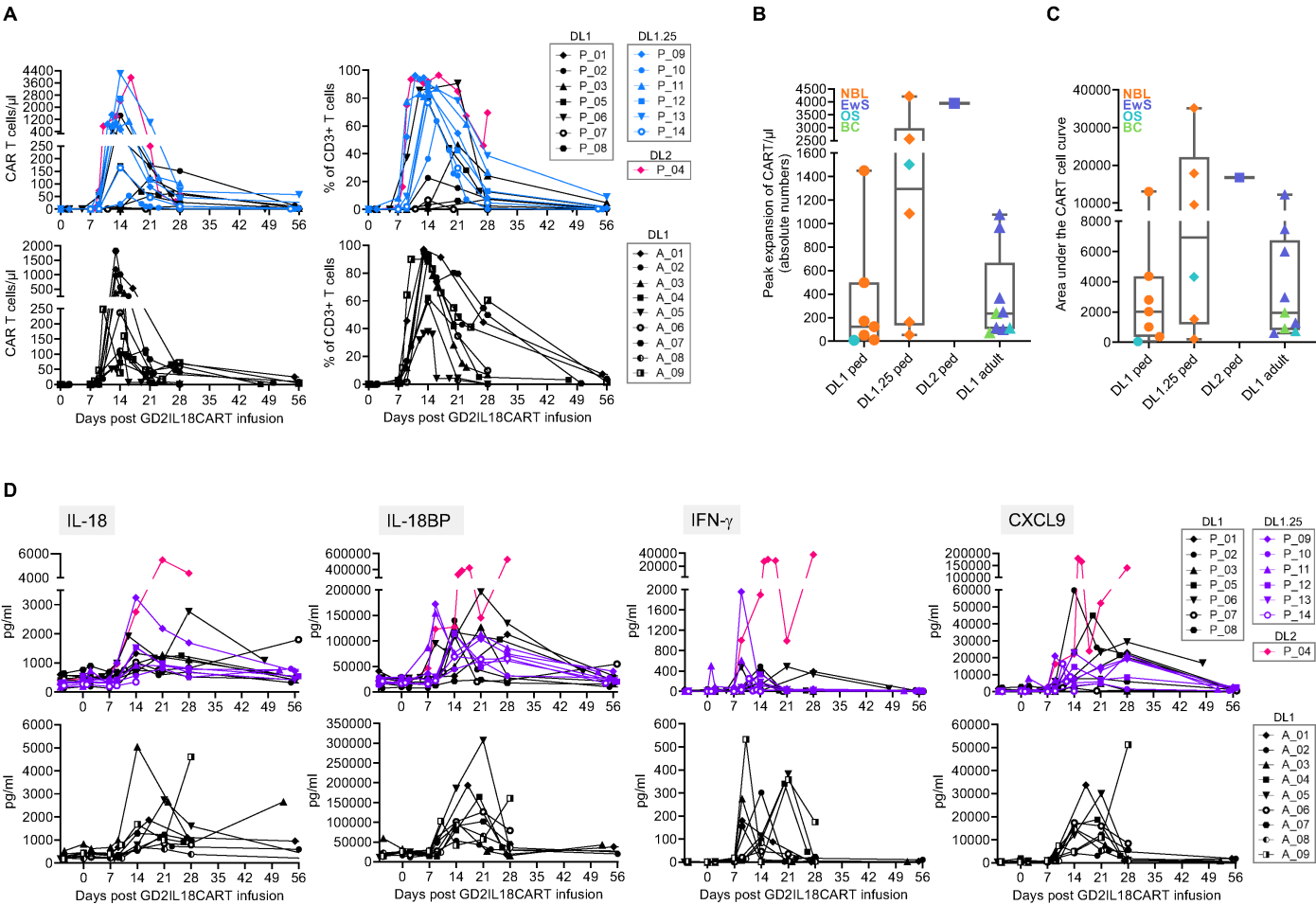


Figure 4

CAR T-cell expansion and cytokine dynamics after GD2IL18CART infusion.

(A) Absolute numbers (left) and proportions (right) of CAR T cells among CD3+ T cells in the peripheral blood of pediatric (upper panel) and adult (lower panel) patients after infusion. CAR T-cell numbers in patient P_01 may underestimate peak expansion, as the original monitoring schedule before protocol amendment did not include assessments between days 14 and 28 when peaks were identified in subsequent patients. **(B)** Peak CAR T-cell expansion. **(C)**Area under the curve (AUC) of CAR T-cell expansion. **(D)** Serum concentrations of IL-18, IL-18BP, IFN-g and CXCL9 in pediatric (upper panel) and adult (lower panel) patients measured by Ella™ Automated ELISA. IL-18 values represent total IL-18, including IL-18 bound to IL-18BP.

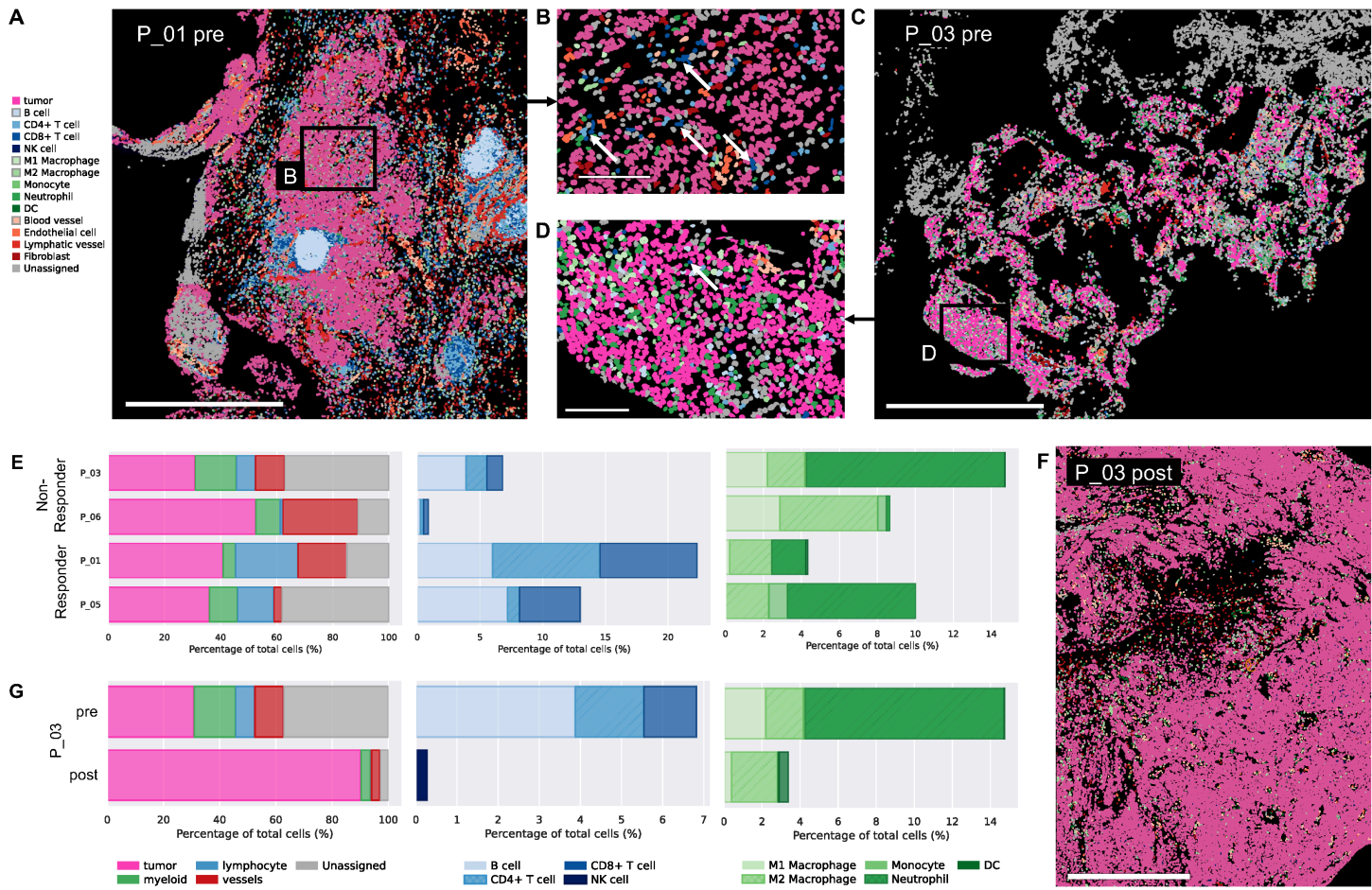


Figure 5

Spatial profiling of pre- and post-treatment tumor biopsies. (A-D) Representative multicolor spatial imaging (MICS) images of segmented pre-treatment tumor tissue from patients with neuroblastoma. Different cell populations are depicted in colors as indicated. Black rectangles in A and C indicate regions shown at higher magnification in B and D. White arrows highlight areas of increased lymphocyte infiltration in P_01 (responder) compared with P_03 (nonresponder). Scale bars, 1 mm (A, C) and 100 μm (B, D). **(E)** Percentages of total cells assigned to major cellular compartments, including lymphocyte and myeloid cell subsets, in pre-treatment tumor tissue from responding patients P_01 and P_05 and non-responding

patients P_03 and P_06. **(F)** MICS image of post-treatment tumor tissue from the non-responding patient P_03 after segmentation. Scale bar, 1 mm **(G)** Percentages of total cells assigned to major cellular compartments, including lymphocyte and myeloid cell subsets, in pre- and post-treatment tumor tissue from P_03.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [ExtendedData.pdf](#)
- [ExtendedDataFigureLegends.docx](#)