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Analysis of IL-6 serum levels and CAR-T cell specific digital PCR in the context of cytokine release syndrome (CRS)

Brief Communication

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Highlights

- The increase of serum IL-6 levels in parallel to the clinical manifestation of CRS following CAR-T cell therapy can contribute to the interpretation of clinical symptoms.
- Serum concentration of CAR-T cells are measurable by CAR-T cell specific sequences by digital droplet PCR (ddPCR) in the peripheral blood of CAR-T cell recipients.
- Further studies should explore a possible correlation of the kinetics of the CAR-T cell concentration in the peripheral blood of the recipients with the risk for CRS/CRES development.

Abstract

CAR-T cell therapies are more and more frequently applied for relapsed B-cell lymphomas and acute lymphoblastic leukemia. Considering the frequency of cytokine release and CAR-T-cell related encephalopathy syndrome (CRS/CRES) following CAR-T administration, strategies enabling timely prediction of impending CRS/CRES are a clinical need. We evaluated the dynamics of serum IL-6 levels and CAR-T transgene copy numbers by digital droplet PCR (ddPCR) in the peripheral blood of eleven consecutive patients with aggressive B-cell malignancies. Four of eleven patients developed CRS, and three patients had CRES (33%), with two of them with previous CRS. IL-6 levels raised on the day of clinical manifestation of CRS. All CRS patients showed increased IL-6 peak levels (median IL-6 peak 606 in CRS patients vs. 22 pg/ml in non-CRS; $p=0.0061$). Different patterns emerged from the dynamics of CAR-T/ μg genomic DNA: “rapid increase and rapid decrease with complete disappearance”, “rapid increase and slow decrease with higher persistence”, “rapid increase and rapid decrease with lower persistence”, and “slow increase and rapid decrease with almost disappearance”. Patients with the pattern “rapid increase and slow decrease with higher persistence” of CAR-T/ μg genomic DNA concentration seemed at higher risk to develop CRS/CRES. Thus, dynamics of CAR-T transgene copy numbers merit further evaluation for a possible association with manifestation of CRS. Increased IL-6 serum levels at CRS manifestation may contribute to the interpretation of symptoms.

Introduction

Following the Swissmedic approval of Kymriah[®] (tisagenlecleucel; Novartis) 2018 for chimeric antigen receptor (CAR)-T cell therapy for adults with relapsed or refractory (r/r) B-lineage ALL and high-grade B-cell lymphomas after failure of ≥ 2 therapy lines, followed by the approval of Yescarta[®] (axicabtagen ciloleucel, Kite/Gilead) by Swissmedic in 2019, a growing number of Swiss centers have established CAR-T therapy. In addition, CAR-T therapy is currently explored for other hematologic malignancies, e.g. multiple myeloma.¹

CAR-T therapies can be associated with relevant risks. Particularly within the first 10 days following CAR-T infusion, but also later, patients should be monitored for symptoms characteristic for cytokine release syndrome (CRS) and CAR-T-cell related encephalopathy syndrome (CRES). Pathogenesis of CRS was found to be mediated by a hyperactivated immune response with an overproduction of inflammatory cytokines (e.g. IL-1 β , IL-6, IL-12, IL-15, TNF- α) and by vascular endothelial dysfunction. Increased permeability of the blood-brain barrier resulting from endothelial dysfunction with a transit of cytokines such as IL-6, TNF- α , or IFN- γ in the cerebrospinal fluid has been suggested to cause CRES.² Therapeutic intervention of CRS may comprise antipyretics, tocilizumab targeting the IL-6 receptor (IL-6R), or steroids. CRES was reported to develop in up to 50% of CAR-T recipients, with grade ≥ 3 in up to a half of them.³⁻⁷ The statistical risk of CRS was higher in ALL patients with significant disease burden.^{8, 9} An individual risk stratification for developing CRS and timely consideration of therapeutic intervention would facilitate clinical management of CAR-T recipients in both in- and outpatient settings.

Material and Methods

We here aimed at improving risk prediction for developing CRS in CAR-T cell recipients by establishing a panel of laboratory assessments. We evaluated whether introducing routine laboratory parameters might facilitate timely identification of CRS and trigger therapeutic interventions. We established a digital droplet PCR (ddPCR) assay for CAR-T cell specific TCR measurement from peripheral blood (PB) and introduced daily IL-6 assessments.

Patients

All initial 11 patients receiving CAR-T therapy since 01/2019 at Bern University Hospital were included (**Supplemental Tables S1-2**). Cut-off for inclusion was August 27, 2019. All patients gave written informed consent, and the study was approved by the local ethics committee of Bern, Switzerland (#2018-00628).

Lymphocyte apheresis, lymphocyte depletion chemotherapy, and CAR-T infusion

We performed lymphocyte collections on the Spectra Optia (Terumo BCT[®]) device, using the Continuous Mononuclear Cell Collection (CMNC) procedure. We analyzed peripheral

CD3+ cell counts of blood and lymphocyte products by multiparameter flow cytometry (BD FACSCanto II). Lymphocyte depleting chemotherapy comprised 3 days of 300 mg/m² cyclophosphamide and 30mg/m² fludarabine.

Ten patients received tisagenlecleucel (Kymriah[®]; patients #1-7, #9-11) and one patient (#8) received the JCAR017 CAR-T investigational product (Celgene; JCAR017-BCM-001 study). The calculated dose of CAR-positive viable T-cells from patient #10 receiving Kymriah[®] composed 0.5×10⁸ (below the required minimum of 0.6×10⁸); considered as out of specification procedure; **Supplemental Table S3**).

Establishment of ddPCR for CAR-T cell quantification

We designed a specific ddPCR assay to quantify sequences of the intracellular domain of the CAR-T constructs using serial recipient's peripheral blood samples. We extracted genomic DNA using the QIAamp DNA mini kit (Qiagen, Rotkreuz, Switzerland). CAR-T cell specific copy numbers were assessed with the QX200 droplet digital PCR (ddPCR) system (Bio-Rad, Cressier, Switzerland). 250 ng of DNA were digested with 3U HaeIII (New England Biolabs, Ipswich, MA, USA) for 15 min at room temperature prior to processing for ddPCR. The ddPCR reaction was set up using ddPCR Supermix for Probes (no dUTP) (Bio-Rad, Cressier, Switzerland) according to the manufacturer's instructions with final concentrations for primers of 700 nM and probes of 200 nM. RPP30 (ribonuclease P protein subunit 30) was used for reference.¹⁰ After droplet generation on the AutoDG automated droplet generator (Bio-Rad, Cressier, Switzerland), PCR was performed with 40 cycles for 30 sec at 94°C for denaturation followed by 1 min at 55°C for annealing and extension.

We calculated copy numbers per µg of DNA using the instrument output of copies per µL for each assay (CAR-T and RPP30). It is assumed that one copy of RPP30 represents one copy of a haploid genome. Given that one haploid genome equals 3.3 pg DNA, the concentration of the DNA in the analyzed sample was calculated as follows: DNA concentration (µg/µL) = RPP30 concentration from the droplet reader (copies/µL) * 3.3 / 1*10⁶.¹¹ Subsequently, the CAR-T concentration (copies/µL) can be divided using the calculated DNA concentration to obtain the copies of CAR-T/µg of analyzed DNA: CAR-T concentration (copies/µg) = CAR-T concentration from the droplet reader (copies/µL) / RPP30 DNA concentration (µg/µL). We designed primers and probes for the CAR-T transgenes to target the intracellular junction sequence between the effector (4-1BB) and costimulatory (CD3ζ) domains, similar to Milone et al.¹²

The sequences for the tisagenlecleucel (Kymriah) CAR-T assay were 5'-GAAGATGGCTGTAGCTGCC-3' for the forward primer annealing in the 4-1BB domain, 5'-GCTCCTGCTGAACTTCACTC-3' for the reverse primer annealing in the CD3ζ domain, and FAM- GAAGAAGAAGAAGGAGGATGTGAACTG-BHQ1 for the probe also annealing in the

4-1BB domain. The reverse primer was 5'-GCTTCTGCTGAACTTCACCC-3' for the JCAR017 assay. As sequences are identical for the respective sequences of 4-1BB, forward primer and probe remained the same. The assay for RPP30 was described by Härmälä et al.⁹

ddPCR has several advantages compared to standard real-time PCR such as higher sensitivity, absolute quantification without external calibration curves, improved precision especially in low target concentrations, high tolerance to inhibitors and less variation in PCR efficiency, to mention only the most important points.

IL-6 assessment

We determined IL-6 serum levels on a Cobas 8000 automated analyzer. According to the manufacturer, the upper limit of the 95% reference range in apparently healthy adults is <7 pg/mL, between-day variation of the IL-6 assay was 1.8%. CRP was determined on a Cobas 8000 automated analyzer (Roche, Rotkreuz, Switzerland). The consensus reference range of the CRP assay was <5mg/L,¹³ between-day variation of the CRP assay was 1.1%.

Results

Patients' characteristics

Eleven patients were included (median, 68 years; 25-74 years; 7/11 females). One patient had B-ALL and ten patients had aggressive B-cell lymphomas being DLBCL (2), secondary DLBCL (5), follicular lymphoma (2), and T-cell/histiocyte-rich large B-cell lymphoma (1).

Supplemental Table S1 summarizes clinical characteristics and therapies (median three preceding lines). Ten of 11 patients (#2-11) underwent CAR-T cell therapy with a significant lymphoma load (partial remission, stable or progressive disease), whereas the single patient with a history of B-lineage ALL (#1) was in complete remission (**Table 1B**). The assessment of CRS followed recent proposals.¹⁴⁻¹⁶

Frequency of CRS/CRES

Supplemental Table S2 summarizes the development of CRS and CRES. Four patients (#2-5) developed CRS grade 1 (1) and 2 (3), and three patients (#4, 5, 8) experienced CRES (grade 1, 2, 3; one each). Whereas CRS occurred on days +2 (1), +3 (1) and +4 (2) after CAR-T infusion, CRES was documented slightly later at days +6, +7 and + 12 for each patient. Following initial recognition of developing CRS, tocilizumab was administered immediately and resulted in a complete recovery within the next 48 hours. Yet, patient #5, suffering from moderate CRS on day +2, developed severe CRES at day +6 and required steroids and ICU monitoring. In two other CRES patients, one patient received steroids whereas the second patient, also following CRS, had supportive treatment only. At the time of tocilizumab administration, patients showed no clinical and microbiologic evidence of

infectious disease. The patient achieving MRD-negativity before CAR-T infusion (#1) developed neither CRS nor CRES. All lymphoma patients had CAR-T cell therapy with a significant lymphoma load.

Dynamics of IL-6 serum levels

We analyzed correlation of IL-6 serum levels with the manifestation of CRS and CRES (**Table 1A**). Patients #2-5, who developed CRS grade 1-2 (patients #4 and 5 presented at the same time with CRES) had IL-6 peak serum levels between 232 and 3,573 pg/mL. One patient (#8) showed CRES only and had an IL-6 peak level of 22 pg/mL. In contrast, patients #1, 6, 7, 9-11 without CRS/CRES had low peak IL-6 levels varying between 9 and 54 pg/mL.

At the day before clinical manifestation of CRS (#2-5), the IL-6 range was low (12-15 pg/mL), and it increased to 24-409 pg/mL 24 hours later in 3 of 4 cases (#2, 3, 5) when CRS was diagnosed. At the day after emergence of CRS and administration of tocilizumab, the IL-6 levels ranged between 15 and 1,002 pg/mL. In one patient with CRS (#4), the IL-6 level remained only slightly increased on these days with 13 pg/mL and 11 pg/mL, respectively. In the single patient with CRES only (#8), the IL-6 level was low, with 8 and 4 pg/mL at the day before and at the day of diagnosis of CRES, respectively. **Figure 1A** shows the dynamics of the serum IL-6 concentrations following CAR-T infusion in the patients with CRS, **Figure 1B** in the patients without CRS.

The clinical occurrence of CRS was accompanied by significantly increasing IL-6 serum levels. 24 hours before, however, IL-6 serum levels were only moderately elevated (if at all) in patients with subsequent CRS. Furthermore, patients with CRS showed higher peak serum IL-6 levels after CAR-T infusion as compared to the patients without CRS (median IL-6 peak 606.0 vs. 22.0 pg/ml, $p=0.0061$; Mann-Whitney-U test; **Figure 1C**). As only one patient was developing CRES alone (in parallel to an only moderate increase of IL-6 level), definite conclusions on IL-6 serum levels for isolated CRES manifestation are not possible from our cohort.

Dynamics of CAR-T/ μ g genomic DNA concentration in the peripheral blood

We determined the dynamics of copies CAR-T/ μ g genomic DNA in the peripheral blood by evaluating the velocity of increase and the type of decrease and persistence of copies after CAR-T cell administration (**Table 1B**). Description of increase: "Rapid increase" was defined as an increase of more than 1,000 CAR-T copies/microgram DNA within the first two weeks following CAR-T cell infusion, whereas values below that level were assigned to "slow increase". Description of subsequent decrease: Slower decrease of the CAR-T copies/microgram DNA to a level above 600 CAR-T copies/microgram DNA within the first month was called "slow decrease with higher persistence" and a decrease to a level of 100-

600 copies “rapid decrease with lower persistence”. Based on that, we found four different patterns in the dynamics of CAR-T/ μ g genomic DNA copies after CAR-T cell administration:

Pattern 1, “rapid increase and rapid decrease with complete disappearance”: In the patient with ALL (#1) with MRD negativity at the time of CAR-T infusion, we identified a short rapid peak from 0 copies/ μ g genomic DNA at day 9 to 15'330 copies/ μ g genomic DNA at day 14 in the peripheral blood. This was followed by a sharp decrease starting on day 16 following CAR-T infusion. ddPCR became negative at day 23 and remained negative in all subsequent blood (and bone marrow) assessments during follow-up since then (**Figure 2A**). This patient developed neither CRS nor CRES.

Pattern 2, “rapid increase and slow decrease with higher persistence”: In patients #3-6 and #8, we overserved a rapid increase up to a peak range of 4'520-139'656 copies CAR-T/ μ g genomic DNA at days 7-11 followed by a slow decrease in the next three weeks (range, 786-5'954 copies/ μ g DNA at day 30; available for patients #3-6) (**Figure 2B**). Four of these five patients developed CRS +/- CRES (patients #3-5, #8). Only patient #6 had neither CRS nor CRES (**Supplemental Table S2**).

Pattern 3, “rapid increase and rapid decrease with lower persistence”: In patients #2, 7, 9, and 11, we observed a rapid increase with a maximal range of 1'925-8'339 copies CAR-T/ μ g genomic DNA at days 9-14 and, again, a rapid decrease to a range of 99-575 copies/ μ g genomic DNA at day 30 after CAR-T infusion (**Figure 2C**). Three of these 4 patients (#7, 9, 11) developed no evidence of CRS/CRES (**Supplemental Table S2**).

Pattern 4, “slow increase and rapid decrease with almost disappearance”: Finally, patient #10 demonstrated very slow increase of copies CAR-T/ μ g genomic DNA with a lower peak (377 copies/ μ g genomic DNA) on day 20 followed by a rapid decrease and near disappearance (10 copies/ μ g genomic DNA). Notably, this patient developed progression in the fourth week post CAR-T infusion and succumbed to progressive disease (PD) on day 30 (**Figure 2D**).

Figure 2E illustrates the dynamics of the CAR-T/ μ g genomic DNA load in the peripheral blood in all 11 patients. In 3 of 4 patients, CAR-T/ μ g genomic DNA load was measured within 48 hours preceding CRS (**Table 1B**). Notably, all of them demonstrated a rapid increase of CAR-T/ μ g genomic DNA at the time when CRS was documented: 16 vs 241 median CAR-T/ μ g genomic DNA two days before and on the day of CRS, respectively.

Discussion

CAR-T treatment represents an innovative and highly promising immuno-cellular autologous approach for previously incurable patients with relapsed/refractory (r/r) CD19+ B-cell malignancies. Recent studies of CAR-T in hematological malignancies with Kymriah[®]

resulting in a CR rate of >60% in r/r B-ALL,¹⁷ a CR rate of 40% in r/r DLBCL¹⁸, or with Yescarta[®] with a CR rate of 52% in r/r large B-cell lymphomas,⁷ have triggered the approval of CAR-T treatment in these diseases. Acknowledging the potential benefits of CAR-T, clinicians face novel challenges considering collecting, manufacturing, and shipping of CAR-T cells, but also severe and less familiar side effects like CRS and CRES. Biomarkers and diagnostic algorithms, defining early recognition and prevention of CRS/CRES, are lacking so far.

The incidence of CRS compromised 44% (n=4/11) in our patient cohort and was slightly lower than the documented range of 50%-100% according to the literature.^{7, 17, 18} The manifestation of CRS/CRES in our patients occurred between days +2-4 and +6-12 after application of the CAR-T cells, respectively, and was comparable to previous reports.^{7, 18} Similarly, the occurrence of CRES shortly after CRS^{19, 20} was confirmed in our patient group as two thirds of our patients with CRES had preceding CRS.

Remarkably, we found the serum concentrations of IL-6 not to be predictive for the development of CRS/CRES in our cohort, as they did not differ significantly in the days before CRS compared to patients without CRS/CRE. However, an increase of IL-6 levels at clinical presentation of CRS provided valuable information for interpreting symptoms and triggering tocilizumab treatment.

In fact, two patients (#2, 5) showed doubling of IL-6 serum concentration and the third patient (#3) demonstrated a 27-fold increase compared to the level measured on the day preceding CRS. Although the remaining fourth patient with CRS lacked any dynamics of IL-6 on the day of CRS, all of them showed considerably increased levels in the subsequent follow-up (median IL-6 peak 606.0 pg/mL in CRS patients vs. 22.0 pg/mL in non-CRS patients, p=0.0061). In sharp contrast, patients without CRS showed no increase of IL-6 levels during follow-up. Thus, IL-6 levels significantly correlated with the onset of CRS. Along with our observation, Teachey et al. investigated the dynamics of IL-6 in 51 B-ALL patients undergoing CAR-T treatment. They reported that the one-month-peak IL-6 values were remarkably increased in B-ALL patients with severe CRS versus non-severe CRS in the first month following CAR-T therapy. In fact, the median IL-6 peak concentration was 8,309 pg/mL in patients with severe CRS (grade 4-5) as compared to 122 pg/mL in patients without CRS or with mild CRS only (grade 1-3) according to Lee et al.,²¹ while also being not predictive for the development of CRS before its clinical manifestation.⁹

Considering the dynamics of CAR-T/ μ g genomic DNA, all patients in our study demonstrated an expected initial expansion of CAR-T transgene copy number followed by subsequent reduction. We recognized four patterns of CAR-T copy number dynamics descriptively termed “rapid increase and rapid decrease with complete disappearance”, “rapid increase and slow decrease with higher persistence”, “rapid increase and rapid decrease with lower

persistence” and “slow increase and rapid decrease with almost disappearance”. Patients assigned to the category “rapid increase and slow decrease with higher persistence” seemed to have a higher risk for the development of CRS/CRES.

Certainly, activation and expansion of CAR-T products following receptor-antigen interaction with their target cells principally can contribute to the manifestation of CRS and CRES. Increasing concentrations of IFN- γ and TNF- α due to the CAR-T cells and tumor lysis lead to upregulation of monocyte and macrophage levels. The latter produce high concentrations of inflammatory mediators, particularly of IL-6, and enforce the clinical presentations of CRS.^{9,22} Other than that, the literature shows clearly that patients with a higher tumor burden are more prone for the occurrence of CRS due to a supra-physiological immune reaction accompanying CAR-T cell application.²³⁻²⁵

Given the numerous clinical and CAR-T cell associated factors that may impact on the risk and development of CRS, one may only speculate whether an early increase and a higher persistence of CAR-T transgene copy numbers in the follow-up may be predictive for an increased risk of CRS. However, definite conclusions are not possible at this time, and prospective, adequately powered studies are necessary to identify a possible association in larger cohorts. At present, molecular assays for CAR-T cell measurement have to be developed in the individual centers for the different CAR-T products and are not yet commercially available.

Beyond that, the identification of the described CAR-T cell copy concentration patterns suggests to investigate whether the patterns “rapid increase and slow decrease with higher persistence (of the CAR-T cells)” and “rapid increase and rapid decrease with lower persistence” may be associated to better response to CAR-T cell therapy, whereas the patterns “rapid increase and rapid decrease with complete disappearance (of the CAR-T cells)” and “slow increase and rapid decrease with almost disappearance” may be associated to inferior efficacy of CAR-T cell treatment. However, these are preliminary observations, as the size of the subgroups was limited and the follow-up too short to allow definite correlations of CAR-T cell copy patterns with efficacy and response of lymphomas to CAR-T cell therapy. Moreover, the dynamics of CAR-T cell copy numbers in the recipients’ peripheral blood reflect only incomplete insights into the expansion and persistence of CAR-T cells following CAR-T cell infusion, whereas other compartments such as lymph nodes, spleen, bone marrow and, eventually, other organs are not considered in this concept.

Increase of serum IL-6 concentrations was observed simultaneously to the clinical manifestation of CRS and was facilitating the interpretation of symptoms and the indication to therapeutic intervention, e.g. by tocilizumab.

The investigation of larger cohorts will allow more insights in possible parameters predicting the development of CRS/CRES following CAR-T cell therapy. A better understanding of the dynamics of CRS and CRES and potential laboratory biomarkers will allow boosting the implementation of CAR-T cell therapy for cancer patients with a poor prognosis. We will continue this diagnostic program in subsequent CAR-T patients to optimize in- and outpatient management of CRS and CRES.

AUTHORSHIP STATEMENT

- (1) All authors have contributed significantly to the research described in the paper and have read and approved the final manuscript.
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- (4) All affiliations or financial involvement with any entity with a financial interest in the subject matter are completely disclosed and all financial and material support for this research and work are clearly identified in the manuscript.

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Figure Legends

Figure 1: Dynamics of serum IL-6 concentrations in the peripheral blood in the patients **(A)** developing cytokine release syndrome (CRS) and **(B)** those without. The y-axis illustrates the IL-6 serum concentrations, the x-axis the interval since CAR-T cell infusion. **(C)** Comparison of peak IL-6 serum concentrations in patients developing CRS as compared to those without.

Figure 2: Panels A - D illustrate different patterns of CAR-T/ μ g genomic DNA load in the peripheral blood as illustrated by specific patient examples. **(A)** “rapid increase and rapid decrease with complete disappearance”; **(B)** “rapid increase and slow decrease with higher persistence”; **(C)** “rapid increase and rapid decrease with lower persistence”; **(D)** “slow increase and rapid decrease with almost disappearance”. The concentration of the CAR-T/ μ g genomic DNA load is given on a logarithmic scale on the y-axis. The x-axis depicts the intervals from CAR-T cell infusion. **Panel (E)** illustrates the dynamics of CAR-T/ μ g genomic DNA load in the peripheral blood in all patients.

Table 1A: Levels and dynamics of IL-6 serum concentration.

Values in brackets indicate the day after CAR-T infusion; CRS: cytokine release syndrome; CRES: CAR-T-cell related encephalopathy syndrome.

Patient	CRS yes/no, day #	IL-6 peak concentration, pg/mL	IL-6 concentration on day preceding CRS/CRES manifestation, pg/mL	IL-6 concentration at initiation of CRS/CRES therapy, pg/mL	IL-6 concentration on 2 nd day after CRS/CRES manifestation, pg/mL
1	no	9 (d+35)	-	-	-
2	grade 1 (d+4)	803 (d+5)	14 (d+3)	35 (d+4)	35 (d+5)
3	grade 2 (d+3)	409 (d+4)	15 (d+3)	409 (d+4)	15 (d+5)
4	grade 2 (d+4)	232 (d+39)	13 (d+3)	11 (d+4)	6 (d+5)
5	grade 2 (d+2)	3573 (d+5)	12 (d+1)	24 (d+2)	1002 (d+3)
6	no	10 (d+4)	-	-	-
7	no	15 (d+12)	-	-	-
8	CRES only; grade 2 (d+7)	22 (d+4)	8 (d+6; CRES)	4 (d+7; CRES)	missing
9	no	28 (d+1)	-	-	-
10	no	54 (d+21)	-	-	-
11	no	43 (d+1)	-	-	-

Table 1B: Characteristics and dynamics of CAR-T cell quantification in the peripheral blood by ddPCR.

CTL019: CD19-targeted CAR-T cell (Kymriah, Novartis); JCAR017: CD19-targeted CAR-T cell (Celgene); CR: complete remission; CRS: cytokine release syndrome; CRES: CAR-T-cell related encephalopathy syndrome; PR: partial remission; PD: progressive disease. Values in brackets indicate days after CAR-T infusion; ddPCR, droplet digital PCR

Patient	Remission status at the time of CAR-T cell therapy	CRS yes/no, day #	CTL019/JCAR017 peak concentration, copies per µg DNA	CTL019/JCAR017 concentration 48h before CRS/CRES, copies per µg DNA	CTL019/JCAR017 concentration on day of CRS/CRES therapy, copies per µg DNA	Start of decrease of CAR-T concentration, day No	CTL019/JCAR017 concentration on day +30, copies per µg DNA	CAR-T ddPCR dynamics: description of increase and decrease in the weeks following CAR-T cell administration	Status at last follow-up: (median, weeks, 13; range, 4-32)
1	CR	no	15'330 (d+14)	-	-	d+16	negative	rapid-rapid with complete disappearance	alive in CR
2	PR4	grade 1 (d+4)	2'071 (d+9)	-	97 (d+4)	d+11	99	rapid-rapid with lower persistence	alive in CR
3	PD	grade 2 (d+3)	4'520 (d+10)	5 (d+1)	208 (d+3)	d+13	1'025 (d+21) 567 (d+41)	rapid-slow with higher persistence	alive in CR
4	2 nd relapse	grade 2 (d+4)	46'013 (d+11)	16 (d+2)	NA; 16 on day before (d+2) and 241 on day after (d+4)	d+21	2'090	rapid-slow with higher persistence	death due to PD
5	PD	grade 2 (d+2)	24'270 (d+7)	87 (d+0)	1'118 (d+2)	d+9	5'954	rapid-slow with higher persistence	death due to PD
6	PD	no	9'137 (d+9)	-	1'719 (d+4)	d+11	786	rapid-slow with higher persistence	alive with CR
7	PD	no	5'432 (d+11)	-	397 (d+4)	d+14	340	rapid-rapid with lower persistence	alive with CR
8	PD	CRES only	139'656 (d+7)	1159 (d+4)	139'656 (CRES)/ d+7	d+9	87 (d+93; no measurement since d+14)	rapid-slow with higher persistence	alive with PR
9	PD	no	8'339 (d+14)	-	180 (d+2)	d+18	575	rapid-rapid with lower persistence	alive with PR
10	2 nd relapse	no	377 (d+20)	-	13 (d+7)	d+24	66	slow-rapid with almost disappearance	death due to PD
11	PD	no	1'925 (d+11)	-	79 (d+4)	d+14	210	rapid-rapid with lower persistence	alive with PR

Figure 1

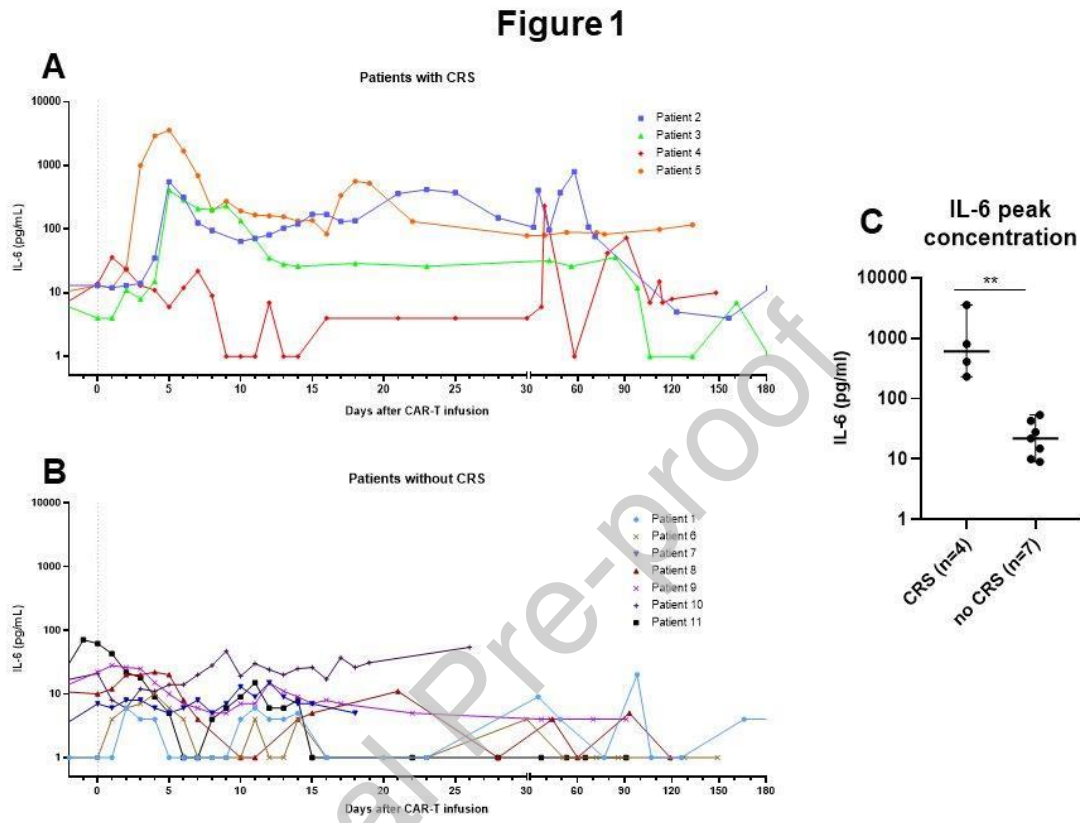


Figure 2

Figure 2

