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Zamtocabtagene Autoleucel in Relapsed/refractory B-NHL: 5-year Follow Up of a CD20/19 tandem CAR T Cell Phase 1 Trial

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Abstract:
Emerging long-term data indicates relapse rates of over 50% after CD19 redirected chimeric antigen receptor (CAR) T cell therapy in relapsed or refractory (r/r) B-cell non-Hodgkin lymphoma (B-NHL). To reduce selective pressure on the CD19 antigen we conducted a first-in-human phase I clinical trial of zamtocabtagene autoleucel (zamto-cel) - a non-cryopreserved tandem CD20-CD19-directed CAR-T cell therapy. Two predefined dose levels (DL1=1x106 and DL2=2.5x106 CAR+ T cells/kg body) were applied. The primary endpoint (EP) was the maximum tolerated dose (MTD). Secondary EPs included adverse events (AEs), best overall response (BOR) and biomarker assessments. A total of 12 patients, 6 per dose level were treated. No DLT and no cytokine release syndrome (CRS) or immune effector cell-associated neurotoxicity syndrome (ICANS) grade ≥3 were observed. Thus, MTD was not reached. The BOR by investigator assessment was 75% with 5/12 patients (42%) achieving complete remission (CR) until month 12 with no relapse in clinical evaluation up to 5 years after infusion. CR was associated with higher mean Cmax and detection of zamto-cel beyond month 6. Additional product characterization revealed increased expression of CD27 and CD127 along with increased expansion of CAR+ TCM cells in patients with CR, thus facilitating persistence and improved outcomes in r/r B-NHL treated with zamto-cel. Based on the promising risk-to-benefit ratio, evaluation of zamto-cel at DL2 is ongoing in pivotal Phase II clinical trials for patients with r/r aggressive B-NHL. This trial was registered at www.clinicaltrials.gov as #NCT03870945.

Conflict of interest: COI declared - see note

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Zamtocabtagene Autoleucel in Relapsed/refractory B-NHL: 5-year Follow Up of a CD20/19 tandem CAR T Cell Phase 1 Trial

Short Title: 5-Year Follow Up of Zamtocabtagene autoleucel

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Key words: Zamto-cel, B-NHL, tandem CAR T cell, CAR T cell persistence

Data sharing statement: For original data, please contact Joana.Costa@miltenyi.com

Abstract

Emerging long-term data indicates relapse rates of over 50% after CD19 redirected chimeric antigen receptor (CAR) T cell therapy in relapsed or refractory (r/r) B-cell non-Hodgkin lymphoma (B-NHL). To reduce selective pressure on the CD19 antigen we conducted a first-in-human phase I clinical trial of zamtocabtagene autoleucel (zamto-cel) - a non-cryopreserved tandem CD20-CD19-directed CAR-T cell therapy. Two predefined dose levels (DL1=$1x10^6$ and DL2=$2.5x10^6$ CAR^+ T cells/kg body) were applied. The primary endpoint (EP) was the maximum tolerated dose (MTD). Secondary EPs included adverse events (AEs), best overall response (BOR) and biomarker assessments. A total of 12 patients, 6 per dose level were treated. No DLT and no cytokine release syndrome (CRS) or immune effector cell-associated neurotoxicity syndrome (ICANS) grade ≥3 were observed. Thus, MTD was not reached. The BOR by investigator assessment was 75% with 5/12 patients (42%) achieving complete remission (CR) until month 12 with no relapse in clinical evaluation up to 5 years after infusion. CR was associated with higher mean C_{max} and detection of zamto-cel beyond month 6. Additional product characterization revealed increased expression of CD27 and CD127 along with increased expansion of CAR^+ T_{CM} cells in patients with CR, thus facilitating persistence and improved outcomes in r/r B-NHL treated with zamto-cel. Based on the promising risk-to-benefit ratio, evaluation of zamto-cel at DL2 is ongoing in pivotal Phase II clinical trials for patients with r/r aggressive B-NHL. This trial was registered at www.clinicaltrials.gov as #NCT03870945.

Key Points

- The tandem CD20/CD19 CAR T product zamto-cel is well tolerated in a phase 1 trial of relapsed/refractory B-NHL patients
- Long-term responses up to 5 years were accompanied by expression of CD27 and CD127 along with increased expansion of CAR+ T_{CM} cells

Introduction

Chimeric antigen receptor (CAR) T cell therapy directed against CD19 has heralded a new era in the treatment of relapsed/refractory B-cell non-Hodgkin lymphoma (r/r B-NHL)[1,2]. Long-term follow up of the pivotal ZUMA-1 trial has shown that only 31% of patients remain in remission after 5 years[3]. Resistance against anti-CD19 CAR T cells has been attributed to several factors: Limited CAR T cell activity due to T cell exhaustion[4], accompanied by poor persistence[5] as well as tumor-driven mechanisms such as an immunosuppressive microenvironment [6-9] and downregulation or even loss of the target antigen[10,11]. Targeting a single antigen leads to selective pressure and thus facilitates antigen loss[12]. Therefore, generating multispecific CAR T cells provides an exciting avenue to bolster efficacy. Successfully engineering multispecific CARs with equivalent potency across targets is challenging[13,14]. The tandem CAR targeting CD20 and CD19 (pLTG1497) has previously shown robust anti-lymphoma activity[15] and mitigated downregulation of B-cell antigens[16]. Clinical evidence suggests that dual targeting of CD20 and CD19 is safe because off-tumor toxicity for both antigens is limited to B-cells[17,18] with manageable long-term effects of prolonged B-cell aplasia[19].

It is well established that a less-differentiated cell state yields enhanced engraftment of adoptive T cell therapies[20,21]. In CAR T cell products the proportion of memory T cells influences their clinical efficacy[22-24]. CD27 is known as a co-stimulator of T cell expansion, however it does not induce effector cell differentiation. Thus, CD27 takes a central role in T cell memory formation and persistence[25,26]. Tumor-specific T cell persistence can be further enhanced by the cytokine IL-7[27] and genetically engineered T cell therapies either secreting IL-7 or overexpressing IL-7 receptor (IL7R, CD127) have been shown to result in improved anti-tumor reactivity[28,29].

We conducted a phase I clinical study of autologous pLTG1497-transduced CAR T cells (Zamtocabtagene autoleucel; zamto-cel) manufactured in a 12-day process without cryopreservation on the CliniMACS Prodigy. This manufacturing approach was two days shorter than previous processes and avoided the need for cryopreservation [15]. Here, we report safety, preliminary efficacy evaluated with a follow-up of 5 years, and in-depth product characterization along with in vivo profiling of zamto-cel. Importantly, complete response (CR) in patients was not associated with dose level, but in vivo expansion and persistence of zamto-cel. Subsequent profiling of pre-infusion drug product (DP) from patients with CR not only revealed higher levels of central memory ($CD62L^+/CD45RO^+$) CAR T cells, but increased levels of CD27 and CD127/IL7R facilitating memory formation and enhanced zamto-cel persistence.

Patients and Methods

Study design and patient population

We conducted a prospective, multi-center, open label phase I first-in-human clinical trial to evaluate feasibility, dosage, efficacy, safety, and toxicity of treatment with ex vivo expanded autologous T cells genetically modified to express a CD20 and CD19 tandem CAR (zamto-cel)[16] in subjects with r/r B-NHL (**Supplementary Figure 1**). Two predefined dose levels (DL) were investigated in two cohorts utilizing a 6+3 trial design. Cohort 1 (DL1) was treated with $1x10^6$ CAR^+ T cells/kg bodyweight (BW) and cohort 2 (DL2) with $2.5x10^6$ CAR^+ T cells/kg BW.

Key eligibility criteria included histologically confirmed B-NHL, measurable disease per PET-CT as per Lugano classification[30] and Eastern Cooperative Oncology Group (ECOG) 0-2. Specifically, in DL1, patients with r/r $CD20^+$ and $CD19^+$ B-NHL and no available approved standard therapy were included, while DL2 was restricted to patients with DLBCL, relapsed or refractory after only one treatment line, who were not eligible for high-dose chemotherapy (HDC) and autologous stem cell transplantation (ASCT). A positivity for CD19 and CD20 (by flow cytometry or immunohistochemistry) was not required anymore. Detailed inclusion and exclusion criteria are provided in the **Supplementary Information S1**.

Informed consent was obtained from each patient before any study related procedure. The study was carried out in accordance with the Declaration of Helsinki, International Council of Harmonization Good Clinical Practice guidelines and applicable regulatory requirements and was approved by the responsible independent ethics committees. Adverse events (AE) including neurotoxicity reporting was classified according to CTCAE Version 5[31]. CRS was graded using criteria from Lee et al.[32] and treated according to institutional guidelines.

Endpoints and statistical analysis

The primary endpoints considered were the determination of the maximum tolerated dose (MTD) as well as safety and toxicity assessment of zamto-cel per adverse events (AE), reported according to CTCAE Version 5[31]. MTD was defined as the dose level at which < 33% of subjects experienced dose-limiting toxicity (DLT) until day 28 after infusion. Further information on endpoints and statistical analyses is included in **Supplementary Information S2.**

Results

Manufacturing of zamto-cel and lymphodepletion

Zamto-cel – a centrally manufactured product - was successfully produced for all 12 patients. The planned dose of the non-cryopreserved product was produced on the fully automated CliniMACS® Prodigy System with a production time of 12 days and

administered to all patients intravenously. Median time from leukapheresis to re-infusion was 14 days (range 13 - 14 days).

Lymphodepleting chemotherapy was administered intravenously from day -5 to day -3 prior to zamto-cel infusion and consisted of cyclophosphamide 300 mg/m^2 body surface area (BSA) and fludarabine 30 mg/m^2 BSA from day -5 to day -3 at DL1. To align the lymphodepletion regimen with the US protocol (NCT 04792489) cyclophosphamide dose was adjusted to 500 mg/m^2 BSA per day for DL2. Further information on manufacturing, lymphodepletion chemotherapy as well as laboratory measurements can be found in **Supplemental Information S2**.

Patient characteristics

Between February and December 2019, 12 patients (6 at DL1 and 6 at DL2) were enrolled at three trial sites in Germany and all underwent leukapheresis. Median age was 72 years (range 20-78), ten patients >65 years and eight >70 years. Histologies included DLBCL (n=8), transformed follicular lymphoma (n=2), primary mediastinal B-cell lymphoma (n=1) and mantle cell lymphoma (n=1). All subjects (12/12; 100%) presented with CD20$^+$ histology and 10/10 (100.0%) with CD19$^+$ histology at screening (2 samples in DL2 were not analyzed for CD19). Nine (75.0%) subjects had an advanced Ann Arbor stage of III/IV, six (50%) patients had refractory disease at study entry and IPI was ≥3 in seven (58.3%) patients (**Table 1, Supplementary Table 1**).

The median follow-up time among patients was 24.6 months (range, 4.6-60.3). Six patients (3 in each DL) finished the active part of the study by completing the 1-year follow-up and continued to be followed in the trial (3 of these 6 patients participated in year 3 visit). Six patients, with documented relapse or refractory disease, finished the trial prematurely due to withdrawal of consent (n=4) or were lost to follow-up (n=1) or died (n=1) (**Supplementary Figure 2**).

Zamto-cel Manufacturing

All leukapheresis products (LPs) showed similar CD3$^+$ cell percentages of 39.9% ± 10.9% and all DPs had a high CD3$^+$ cell purity (99.3% ± 0.7%) and viability (94.0% ± 3.1%) (**Supplementary Figure 3a+b**). Patients who achieved a CR had higher amounts of B cells in the LP than non-CR patients (6.0 ± 3.9 vs 0.7 ± 1.3) (**Supplementary Figure 3c**). Mean transduction frequency was 18.0% (21.0% ± 4.8% in CR vs 15.9% ± 6.7% in non-CR; p=0.19) (**Supplementary Figure 3d)**. While we observed a CD4:CD8 ratio of 1.5 ± 0.9 in the LP, we found a mean CD4:CD8 ratio of 4.5 in CAR$^+$ cells (6.0 ± 4.5 in CR vs 3.5 ± 2.1 in non-CR; p=0.19) (**Supplementary Figure 3e**). After CD4 and CD8 selection and addition of cytokines, DPs showed a consistent T cell expansion of 10.5 ± 3.5-fold in CD3$^+$ cells, 9.5 ± 3.2-fold in CAR$^+$CD4$^+$ cells and 12.0 ± 4.5-fold in CAR+CD8+ cells, with no difference between CR and non-CR patients (**Supplementary Figure 3f+g**).

Safety

No DLT was observed at any dose level. The incidence of adverse events (AE) was assessed as a secondary endpoint. All 12 patients (100.0%) experienced at least one grade ≥ 3 adverse event. The most common treatment-emergent adverse events of any grade were CRS (58.3%), constipation (41.7%), and neutrophil count decrease (33.3%) (**Figure 1a)**. Overall, CRS occurred in 3/6 in DL1 (50.0%) and 4/6 in DL2 (67.0%). Two events of CRS were classified as grade 2, both at DL2, and all others as grade 1. One patient at DL2 received a total of two doses of tocilizumab for grade 2 CRS. The median time to onset of CRS after infusion with zamto-cel was 6 days (range, 0–9 days). All 7/7 patients (100.0%) had a complete resolution of CRS within a median duration of 3 days (range, 2-4 days).

Two events of ICANS of any grade were reported in one patient (8.3%). Both events were of grade 1 and occurred on day 1 to day 3 and on day 8 to day 9.Only the first episode required steroids (10mg of dexamethasone) due to concomitant CRS grade 1 at DL2, and symptoms fully resolved after treatment with dexamethasone. No patient required vasopressors. Neither CRS nor ICANS were associated with ICU admission. One patient required ICU admission on day +75 after intestinal perforation without evidence of recurrent disease. The event was considered related to the study drug most probably due to the intestinal lymphoma responding to zamto-cel.

At screening ten out of twelve patients showed B cell aplasia (one missing value), defined as <0.1 $CD19^{+}CD20^{+}$ cells/µl in the peripheral blood as determined by flow cytometry. By day 14, ten patients showed B cell aplasia (2 missing values). Three out of six patients with analysis at month 6 showed normalized B cell counts. At one year after IMP infusion 3/5 patients with ongoing CR had normal B cell counts. Analysis of IgG levels were available in all patients. Hypogammaglobulinemia of less than 4 g/L was present in 4 patients at least once post infusion (**Supplementary Figure 4**). One participant received intravenous Immunoglobulin preparations. One participant experienced grade 4 neutropenia with a duration of 30 days starting at day+9 after zamto-cel administration. Prolonged neutropenia grade 3 (defined as present at day 28 after zamto-cel administration) was documented for one patient with a duration of 14 days. For both patients, no infections were reported during this period. No further episodes of any hematological toxicities had been reported 90 days after treatment with zamto-cel (post treatment AEs).

Five (41.7%) patients experienced 11 treatment-emergent infections of any grade with a median duration of 13 days (range, 4-107). Two patients experienced 5 infectious events grade ≥ 3. Only one patient suffered from infectious complications considered to be related to the IMP (5 events). This patient was treated at DL1 and experienced a urinary tract infection (grade 3, not considered related to IMP, day 0), and later during the clinical course an abdominal infection (grade 3, day 57, followed by a septic shock (grade 4, day 75) and pneumonia (grade 3, day 75), all of which were considered to be related to the IMP. Another patient at DL1 had a grade 3 catheter-related infection on day 10 not related to the IMP. There were no infectious events grade ≥3 reported at DL2 (**Figure 1a**). Seven patients experienced an

infection during B cell aplasia. However, in all cases B-cell aplasia was already present prior to or at the time of zamto-cel treatment.

One patient at DL1 developed a grade 3 sarcoma 399 days following zamto-cel infusion, which was considered a post treatment AE not related to the IMP. Two cases of death, that were considered unrelated to IMP, occurred on study and were caused by disease progression (n=1) 7.8 months after treatment with zamto-cel and cardiac failure (n=1) 14.6 months after treatment with zamto-cel. The second patient had initially achieved a PR but subsequently relapsed at month 6 after zamto-cel treatment. Hereafter, the patient was treated with Pola-BR and showed a CR for 3 months after succumbing to the cardiac failure.

Cytokine Analysis

Given the high frequency of CRS, serum cytokine levels of IFN-gamma were assessed, IL-6, IL-10, MCP-1, IL-2 and TNF alpha at baseline, day 0, days 1-6, day 9, day 14, and week 3. In patients with CRS, levels of IL-2 (t_{max} = d1), IL-6 (t_{max} = d9) and TNF alpha (t_{max} = d2) were elevated compared to patients without CRS (t_{max} not detectable). The other cytokines revealed inconsistent levels over time. While IL-2 and TNF alpha levels returned to baseline by day 14, IL-6 levels were still elevated in patients with CRS when compared to patients without CRS at week 3 (**Figure 1b**).

Efficacy

The efficacy analysis included all 12 infused patients. At DL1, the BOR was 50% with 3 patients achieving a CR. The remaining 3 patients at DL1 had stable disease (SD) as best response and progressed subsequently. Among the 6 patients at DL2 the BOR was 100% including 2 patients with CR and 4 patients with PR. BOR for the entire study cohort was 75% (5 CR, 4 PR). The median time to first objective response was 42 days (range, 36 – 97) while median time to CR was 105 days (range 97 – 188). Based on investigator assessment extended definition, the median PFS time was not evaluable at DL1 and 6.3 months (95% CI: 3.2-NE) at DL2 respectively. The PFS rate at month 36 was 66.7% (95% CI: 19.5% - 90.4%) at DL1 and 33.3% (95% CI: 4.6% - 67.6%) at DL2 (**Figure 2a,** PFS shown for the overall population). With a median follow up time of 24.6 months, the OS rate at month 60 was 75.0% (95% CI: 12.8% - 96.1%) at DL1 and 66.7% (95% CI: 5.4% - 94.5%) at DL2 (**Figure 2a,** OS shown for the overall population). Among the patients with CR as best response per investigator assessment, 4/5 patients had PET-negative CR at month 3, while 1 patient converted from PR to CR at month 6 (**Figure 2b**). Of the 5 patients in CR, all completed a 2-year FU visit without clinical evidence of relapse. During the FU period until year 5 three patients were lost to follow-up and 2 patients completed the 5-year follow-up visit without any clinical evidence of relapse. All patients with PR or SD as best response (n=7) progressed within the first 180 days after infusion (**Figure 2c**). Out of the 7 patients with documented progression, 4 received a biopsy as part of the clinical routine. These available biopsies showed persistent expression of both CD19 and CD20. The median duration of response at

DL2 was 4.8 months and was not reached at DL1 (**Figure 2d** shows DOR for the overall population).

In vivo expansion of zamto-cel

Since clinical response after zamto-cel was not associated with the total number of CAR^+ T cells being infused, we hypothesized that in vivo expansion of zamto-cel will show better correlation with clinical response[3]. To this end, we analyzed zamto-cel expansion in peripheral blood from all 12 patients (**Supplementary Figure 5a**). All patients with CR had a C_{max} ≥450 cells/µL with a mean C_{max} of 1,092.5 cells/µL (460.1-3,147.0), while patients with no CR had significantly lower values with a mean C_{max} of 111. 3 cells/µL (3.9-458.8) (**Figure 2e+f**). The robust segregation of response by C_{max} was consistent across $CD4^+$ and $CD8^+$ zamto-cel populations (**Supplementary Figure 5b**). Patients without CR had a mean AUC_{D0-28} of 944.1 d×cells/µl (28.0 - 3,504.7), whereas the mean AUC_{D0-28} in CR was 8,245.5 d×cells/µl (95% CI: 2,396.0 - 21,295.6) (**Supplementary Figure 5c**). Two of five CR patients still showed measurable zamto-cel concentrations above the detection limit in peripheral blood two years after administration of zamto-cel. In the non-CR patients, no CAR T cell concentrations above the detection limit could be determined on average after 25.4 days (95% CI: 13.0-30.0).

Immunophenotypic analysis of exhaustion, activation and proliferative analysis

Enhanced persistence of zamto-cel in some patients with CR encouraged us to next characterize DP features of zamto-cel in patients with CR vs. non-CR. Thus, we profiled LP starting material and final zamto-cel DPs from patients with CR vs. non-CR via flow cytometry. Immunophenotype of the DP in comparison with LP showed low expression of exhaustion markers Lag-3 and PD-1 in $CD4^+$ and $CD8^+$ CAR^+ T cells. We observed increased CD25 expression in DPs vs. LP, however, without differences between CR vs. non-CR patients. In contrast, the inducible activation marker 4-1BB was not increased on $CD4^+$ or $CD8^+CAR^+$ cells in the DP (**Supplementary Figure 6a+b**). Interestingly, the proliferative ability of zamto-cel as assessed by CD27 and CD127/IL7R expression showed a trend for increased expression in CR patients ($CAR^+CD4^+CD27^+$ in CR [mean=86.2, 95% CI: 73.57 - 91.59] vs non-CR [mean=60.98, 95% CI: 8.93 - 92.14; p=0.14], $CAR^+CD4^+CD127/IL7R^+$ in CR [mean=82.09, 95% CI: 76.97 - 88.93] vs non-CR [59.34, 95% CI: 29.87 - 82.48; p=0.035], $CAR^+CD8^+CD27^+$ in CR [mean=88.85, 95% CI: 82.09 - 94.46] vs. non-CR [mean=66.33, 95% CI: 16.55 - 94.01; p=0.05] and $CAR^+CD8^+CD127/IL7R^+$ in CR [mean=26.72, 95% CI: 15.52 - 43.95] vs. non-CR [12.36, 95% CI: 1.93 - 49.96; p=0.1]) (**Figure 3a+b, Supplementary Figure 6c**).

High-dimensional profiling via scRNA-seq in zamto-cel products

Given the higher levels of both CD27 and CD127 in zamto-cel DP from patients with CR, we performed scRNA-seq of zamto-cel DP to obtain a fine-grained profile of $CD27^+$ and $CD127^+$ CAR^+ cells. First, we validated our in silico approach to call CAR^+ cells by correlating their fraction with CAR^+ cells identified via flow cytometry. We

found a strong correlation between in silico and in vitro identified CAR^+ cells in zamto-cel DP (r=1, p=0.00021) (**Supplementary Figure 7a**). Next, we identified $CAR^+CD27^+CD127^+$ cells in zamto-cel products (**Supplementary Figure 7b**), which we further investigated via differential gene expression analysis. We observed genes related to protein biosynthesis (*RPS28, RPS27, RPS24, EEF2, EEF1A1, RPL19, RPL30, RPS27A, RPS14, RPL10, RPL18*), and regulation of migration in naïve T cells (*KLF2*)[33] to be highly expressed. In contrast, genes associated with cell division (*KIFC1, PCLAF, TYMS, CDK1, TOP2A, CENPF, MKI67* and others) showed low expression in $CAR^+CD27^+CD127^+$ cells from zamto-cel DP (**Figure 3c**).

Memory subsets in Drug Products and during expansion phase

We hypothesized that the elevated expression of CD27 and CD127 yields higher levels of T_{cm} cells[22,23]. Therefore, we analyzed memory subsets in DPs from patients with CR and non-CR. In patients with CR, we observed higher levels of CAR^+CD8^+ T_{cm} (91.3% ± 4.7% in CR vs. 79.8% ± 16.3% in non-CR patients) (**Figure 3d**) and CAR^+CD4^+ T_{cm} (89.8% ± 7.8% in CR vs. 75.2% ± 25.3% in non-CR patients) in DPs, while the cellular composition for other memory subsets was comparable in patients with CR vs. non-CR (**Supplementary Figure 8a+b**). To understand whether the higher number of T_{cm} cells translates into enhanced persistence, we profiled memory subsets from patients with CR vs. non-CR in vivo over time. While persistence of all memory subsets in both $CD4^+$ and $CD8^+$ CAR T cells was improved in patients with CR (**Supplementary Figure 8c+d)**, T_{cm} cells showed remarkably high levels post treatment (CR=502.7 cells/µLvs. non-CR=27 cells/µL average C_{max}) (**Figure 3e**).

Discussion

The development of zamto-cel was driven by the attempt to reduce relapse in B-NHL through dual targeting of CD20 and CD19[16]. Preclinical data suggests, that CD19 is heterogeneously expressed in CAR T cell naïve B-NHL when compared to healthy B cells and thereby determines CAR activity[11]. In turn, loss of the CD19 antigen post axi-cel has been observed in 60% of patients with r/r B-NHL[14].Genomic alterations (deletion, frameshift and missense mutations) as well as alternative splicing of CD19 have been described in B-ALL after treatment with CD19 directed CAR T cells[10]. In contrast, in r/r DLBCL after treatment with rituximab-based chemoimmunotherapy only 7% of cases show mutations in MS4A1 (CD20)[35]. Thus, the development of tandem CARs against CD19 and CD20, which signal in an “OR” gated manner resulting in sufficient CAR activation by only one antigen[34] provides an exciting avenue for the treatment of r/r B-NHL.

Zamto-cel was manufactured fully automated on the Miltenyi CliniMACS Prodigy closed-production system to transduce activated T cells with pLTG1497. Final products were harvested after a 12-day process. Importantly, all 12 patients who underwent leukapheresis were infused without cryopreservation. In contrast, previously tested tandem CAR T cell products targeting CD20 and CD19 in r/r B-NHL have been manufactured either within an open process, required prolonged culture and / or cryopreservation before infusion[15,17,18]. A shortened ex vivo culture has been

proven to result in more naïve and memory-enriched T cell phenotypes[36] which positively impact tumor cell killing[24]. Cryopreservation results in impaired quality of final products, which negatively affects outcome[15]. A prolonged ex vivo expansion as well as cryopreservation can be avoided by local manufacturing. In an academic context, on-site manufacturing is facilitated by a closed-system platform to generate GMP grade cell therapeutic products[37].

The major adverse events of CAR T cell treatment comprise acute toxicities such as CRS and ICANS[38], but also non-immediate hematological impairments, e.g. prolonged neutropenia and B cell aplasia leading to increased rates of infection and non-relapse mortality[39,40]. Treatment with zamto-cel resulted in no DLTs thus, defining $2.5x10^6$ CAR^+ T cells/kg BW as recommended dose for further clinical evaluation. No incidences of CRS and ICANS ≥ grade 3 occurred. A comparably low rate of relevant CRS and ICANS has only been reported for one other tandem CAR product targeting CD20 and CD19 in r/r B-NHL[18]. Only two patients (16.7%) showed an ongoing neutropenia ≥ grade 3, which was persistent beyond day 28 (both considered not related to zamto-cel per investigator assessment), while thrombocytopenia or anemia were not reported beyond day 28. Even though definitions of prolonged cytopenia are not uniform across studies, one third (30-38%) of r/r B-NHL patients treated with commercial anti-CD19 CAR T cells present with persistent grade ≥ 3 neutropenia and 21-29% with thrombocytopenia after day 21[39,41] resulting in significant morbidity and utilization of health care resources.
We observed responses in 9 patients, of whom 5 achieved a CR (42%). None of the CR patients progressed over the follow up period. While other CAR products targeting CD20 and CD19 in r/r B-NHL with comparable long-term follow-up data have achieved slightly higher ORRs of around 80 %[15,17], durable CR was only reported in 58%[42] and 70%[43], respectively. Interestingly, relapse biopsies after CD20/CD19 directed CAR T-cell therapy indicated no loss of CD20 and CD19 antigens[15]. CR with zamto-cel did not associate with dose level but in vivo expansion, which is in line with previous findings for axi-cel in r/r B-NHL[3]. These two findings emphasize the importance of identifying product metrics to predict successful engraftment, expansion and persistence of tandem CD20 and CD19 directed CAR T cells in B-NHL.

Profiling final products from treated patients, we identified CD27 and CD127/IL7R overexpression in zamto-cel to associate with CR. CD27 overexpression in $CD8^+$ anti-CD19 CAR T cell products has been highlighted as prognostically relevant in r/r B-NHL[22,23]. When incorporated into second generation CARs as a costimulatory domain, CD27 enhances persistence and improves resistance against apoptosis via Bcl-X_L expression[44]. Naïve and central memory T cells express CD127/IL7R, and stromal cells provide IL-7 to both populations for homeostatic proliferation thereby enabling T cell maintenance and growth despite lack of antigen- and CD28-mediated (co-)stimulation[45]. Hence, overexpression of CD127/IL7R in CAR T-cell products has been hypothesized to be advantageous and to boost engraftment and expansion after lymphodepletion[46]**.**Our high-dimensional profiling indicates that the combined

expression of CD27 and CD127/IL7R in zamto-cel is linked to a naïve state with maintained expression of ribosomes, which can immediately be engaged to facilitate the T cell activation program[47]. In patients with CR, our longitudinal analysis indicated not only enhanced expansion but also persistence especially of central memory CAR^+ T cells and thus provides orthogonal evidence that high levels of endogenous CD127/IL7R results in superior clinical activity of CAR T cells. Our work expands the significance of sustained memory and persistence via CD27 and CD127/IL7R to a tandem CAR targeting r/r B-NHL and provides a direction for future development of CAR T cell products with improved persistence in r/r B-NHL.

Our work provides evidence that zamto-cel is safe with early signs of long-term efficacy. Given the broader activity of zamto-cel at DL2 as compared to DL1 (ORR=100% vs. 50%) and no additional toxicity at higher dose, two pivotal phase-II trials exploring zamto-cel efficacy at DL2 are currently being conducted. DALY 2-US studies zamto-cel as third-line therapy in r/r DLBCL, and DALY 2-EU investigates zamto-cel as second-line therapy in adult and elderly patients with r/r DLBCL ineligible for HDC and ASCT.

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Conflict of interest

H.B.-W.: Research support: Wilson and Wolf (G-Rex Grant Program); Honoraria: Miltenyi Biotec.

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P.G: Travel grants: Johnson & Johnson;

Author contributions

H.B.-W., P.G., P.B., N.K., C. Schmid, C. Scheid, F.A., U.H., T.H, C.W., S.B., I.B. G.Z. and R.O. contributed to data acquisition and/or analysis. P.B., B.F., P.v.H., S.H., . and C.B. contributed to study conception and/or design and data analysis. H.B.-W., P.G., S.H., C.B., P.v.H., S.M., M.H. and T.O. contributed to data interpretation. All authors contributed to writing the manuscript

References

1. Westin JR, Oluwole OO, Kersten MJ, et al. Survival with Axicabtagene Ciloleucel in Large B-Cell Lymphoma. N Engl J Med. 2023;389(2):148-157.
2. Abramson JS, Solomon SR, Arnason J, et al. Lisocabtagene maraleucel as second-line therapy for large B-cell lymphoma: primary analysis of the phase 3 TRANSFORM study. Blood. 2023;141(14):1675-1684.
3. Neelapu SS, Jacobson CA, Ghobadi A. Five-year follow-up of ZUMA-1 supports the curative potential of axicabtagene ciloleucel in refractory large B-cell lymphoma. Blood. 2023;141(19):2307-2315.
4. Weber EW, Parker KR. Transient rest restores functionality in exhausted CAR-T cells through epigenetic remodeling. Science. 2021;372(6537):eaba1786.
5. Labanieh L, Mackall CL. CAR immune cells: design principles, resistance and the next generation. Nature. 2023;614(7949):635-648.
6. Jain MD, Zhao H, Wang X. Tumor interferon signaling and suppressive myeloid cells are associated with CAR T-cell failure in large B-cell lymphoma. Blood. 2021;137(19):2621-2633.
7. Scholler N, Perbost R, Locke FL. Tumor immune contexture is a determinant of anti-CD19 CAR T cell efficacy in large B cell lymphoma. Nat Med. 2022;28(9):1872-1882.
8. Locke FL, Filosto S, Chou J, et al. Impact of tumor microenvironment on efficacy of anti-CD19 CAR T cell therapy or chemotherapy and transplant in large B cell lymphoma. Nat Med. 2024;30(2):507-518.
9. Stahl D, Gödel P, Balke-Want H, et al. CSF1R+ myeloid-monocytic cells drive CAR-T cell resistance in aggressive B cell lymphoma. Cancer Cell. 2025.
10. Sotillo E, Barrett DM, Black KL, et al. Convergence of Acquired Mutations and Alternative Splicing of CD19 Enables Resistance to CART-19 Immunotherapy. Cancer Discov. 2015;5(12):1282-1295.
11. Majzner RG, Rietberg SP, Sotillo E, Dong R. Tuning the Antigen Density Requirement for CAR T-cell Activity. Cancer Discov. 2020;10(5):702-723.
12. Majzner RG, Mackall CL. Tumor Antigen Escape from CAR T-cell Therapy. Cancer Discov. 2018;8(10):1219-1226.
13. Cordoba S, Onuoha S, Thomas S, Pignataro DS, Hough R, Ghorashian S. CAR T cells with dual targeting of CD19 and CD22 in pediatric and young adult patients with relapsed or refractory B cell acute lymphoblastic leukemia: a phase 1 trial. Nat Med. 2021;27(10):1797-1805.
14. Spiegel JY, Patel S. CAR T cells with dual targeting of CD19 and CD22 in adult patients with recurrent or refractory B cell malignancies: a phase 1 trial. Nat Med. 2021;27(8):1419-1431.
15. Shah NN, Johnson BD, Schneider D, Zhu F, Szabo A. Bispecific anti-CD20, anti-CD19 CAR T cells for relapsed B cell malignancies: a phase 1 dose escalation and expansion trial. Nat Med. 2020;26(10):1569-1575.
16. Schneider D, Xiong Y, Wu D, et al. A tandem CD19/CD20 CAR lentiviral vector drives on-target and off-target antigen modulation in leukemia cell lines. Journal for ImmunoTherapy of Cancer. 2017;5(1):42.

17. Tong C, Zhang Y, Liu Y, et al. Optimized tandem CD19/CD20 CAR-engineered T cells in refractory/relapsed B-cell lymphoma. Blood. 2020;136(14):1632-1644.
18. Larson SM, Walthers CM, Ji B, et al. CD19/CD20 Bispecific Chimeric Antigen Receptor (CAR) in Naive/Memory T Cells for the Treatment of Relapsed or Refractory Non-Hodgkin Lymphoma. Cancer Discov. 2023;13(3):580-597.
19. Wudhikarn K, Palomba ML. Infection during the first year in patients treated with CD19 CAR T cells for diffuse large B cell lymphoma. Blood Cancer J. 2020;10(8):79.
20. Hinrichs CS, Borman ZA, Gattinoni L, et al. Human effector CD8+ T cells derived from naive rather than memory subsets possess superior traits for adoptive immunotherapy. Blood. 2011;117(3):808-814.
21. Graef P, Buchholz Veit R, Stemberger C, et al. Serial Transfer of Single-Cell-Derived Immunocompetence Reveals Stemness of CD8+ Central Memory T Cells. Immunity. 2014;41(1):116-126.
22. Fraietta JA, Lacey SF, Orlando EJ, et al. Determinants of response and resistance to CD19 chimeric antigen receptor (CAR) T cell therapy of chronic lymphocytic leukemia. Nat Med. 2018;24(5):563-571.
23. Deng Q, Han G, Puebla-Osorio N, et al. Characteristics of anti-CD19 CAR T cell infusion products associated with efficacy and toxicity in patients with large B cell lymphomas. Nat Med. 2020;26(12):1878-1887.
24. Dickinson MJ, Barba P, Jäger U, et al. A Novel Autologous CAR-T Therapy, YTB323, with Preserved T-cell Stemness Shows Enhanced CAR T-cell Efficacy in Preclinical and Early Clinical Development. Cancer Discov. 2023;13(9):1982-1997.
25. Hendriks J, Gravestein LA, Tesselaar K, van Lier RAW, Schumacher TNM, Borst J. CD27 is required for generation and long-term maintenance of T cell immunity. Nat Immunol. 2000;1(5):433-440.
26. Jaeger-Ruckstuhl CA, Lo Y, Fulton E, et al. Signaling via a CD27-TRAF2-SHP-1 axis during naive T cell activation promotes memory-associated gene regulatory networks. Immunity. 2024;57(2):287-302.e212.
27. Melchionda F, Fry TJ, Milliron MJ, McKirdy MA, Tagaya Y, Mackall CL. Adjuvant IL-7 or IL-15 overcomes immunodominance and improves survival of the CD8+ memory cell pool. The Journal of Clinical Investigation. 2005;115(5):1177-1187.
28. Markley JC, Sadelain M. IL-7 and IL-21 are superior to IL-2 and IL-15 in promoting human T cell–mediated rejection of systemic lymphoma in immunodeficient mice. Blood. 2010;115(17):3508-3519.
29. Shum T, Omer B, Tashiro H, et al. Constitutive Signaling from an Engineered IL7 Receptor Promotes Durable Tumor Elimination by Tumor-Redirected T Cells. Cancer Discov. 2017;7(11):1238-1247.
30. Cheson BD, Fisher RI, Barrington SF, et al. Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. J Clin Oncol. 2014;32(27):3059-3068.
31. Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0, Published: November 27, 2017. US Department of Health and Human Services, National Institutes of Health, National Cancer Institute.
32. Lee DW, Santomasso BD, Locke FL, et al. ASTCT Consensus Grading for Cytokine Release Syndrome and Neurologic Toxicity Associated with Immune Effector Cells. Biol Blood Marrow Transplant. 2019;25(4):625-638.
33. Sebzda E, Zou Z, Lee JS, Wang T, Kahn ML. Transcription factor KLF2 regulates the migration of naive T cells by restricting chemokine receptor expression patterns. Nat Immunol. 2008;9(3):292-300.
34. Abbott RC, Hughes-Parry HE, Jenkins MR. To go or not to go? Biological logic gating engineered T cells. Journal for ImmunoTherapy of Cancer. 2022;10(4):e004185.

35. Rushton CK, Arthur SE, Alcaide M, et al. Genetic and evolutionary patterns of treatment resistance in relapsed B-cell lymphoma. Blood Adv. 2020;4(13):2886-2898.
36. Ghassemi S, Durgin JS, Nunez-Cruz S, et al. Rapid manufacturing of non-activated potent CAR T cells. Nat Biomed Eng. 2022;6(2):118-128.
37. Dias J, Garcia J, Agliardi G, Roddie C. CAR-T cell manufacturing landscape—Lessons from the past decade and considerations for early clinical development. Mol Ther Methods Clin Dev. 2024; 32(2):101250.
38. Morris EC, Neelapu SS, Giavridis T, Sadelain M. Cytokine release syndrome and associated neurotoxicity in cancer immunotherapy. Nat Rev Immunol. 2022;22(2):85-96.
39. Rejeski K, Perez A, Sesques P, et al. CAR-HEMATOTOX: a model for CAR T-cell–related hematologic toxicity in relapsed/refractory large B-cell lymphoma. Blood. 2021;138(24):2499-2513.
40. Cordas dos Santos DM, Tix T, Shouval R, et al. A systematic review and meta-analysis of nonrelapse mortality after CAR T cell therapy. Nat Med. 2024;30(9):2667-2678.
41. Logue JM, Zucchetti E, Bachmeier CA, et al. Immune reconstitution and associated infections following axicabtagene ciloleucel in relapsed or refractory large B-cell lymphoma. Haematologica. 2021;106(4):978-986.
42. Zurko JC, Fenske TS, Johnson BD, et al. Long-term outcomes and predictors of early response, late relapse, and survival for patients treated with bispecific LV20.19 CAR T-cells. Am J Hematol. 2022;97(12):1580-1588.
43. Zhang Y, Wang Y, Liu Y, et al. Long-term activity of tandem CD19/CD20 CAR therapy in refractory/relapsed B-cell lymphoma: a single-arm, phase 1–2 trial. Leukemia. 2022;36(1):189-196.
44. Song DG, Ye Q, Poussin M, Harms GM, Figini M, Powell DJ, Jr. CD27 costimulation augments the survival and antitumor activity of redirected human T cells in vivo. Blood. 2012;119(3):696-706.
45. Mackall CL, Fry TJ, Gress RE. Harnessing the biology of IL-7 for therapeutic application. Nat Rev Immunol. 2011;11(5):330-342.
46. Lin FY, Stuckert A, Tat C, et al. Phase I Trial of GD2.CART Cells Augmented With Constitutive Interleukin-7 Receptor for Treatment of High-Grade Pediatric CNS Tumors. J Clin Oncol. 2024;42(23):2769-2779.
47. Wolf T, Jin W, Zoppi G, Vogel IA, Akhmedov M, Bleck CKE. Dynamics in protein translation sustaining T cell preparedness. Nat Immunol. 2020;21(8):927-937.

Main Table Legends

Table 1: Baseline characteristics of patients treated with zamto-cel.

All characteristics refer to the apheresis timepoint if not otherwise specified. BCL2: B cell lymphoma 2; DLBCL: diffuse large B cell lymphoma; FL: follicular lymphoma; IPI: international prognostic index. LDH: lactate dehydrogenase; MZL: marginal zone lymphoma; PMBCL: primary mediastinal B cell lymphoma. ULN: upper limit of norm.

Characteristic	Patients who received zamtocabtagene autoleucel (n=12)
Age	
Median – yr (range)	72 (20, 78)
<65 - n (%)	2 (16.7)
≥65 - n (%)	10 (83.3)
>70 - n (%)	8 (66.7)
Sex - n (%)	
Male	7 (58.3)
Female	5 (41.7)
ECOG performance score - n (%)	
0	10 (83.3)
1	2 (16.7)
Diagnosis of Primary Disease at screening - n (%)	
DLBCL, NOS	8 (66.7)
PMBCL	1 (8.3)
Transformed FL	2 (25.0)
MCL	1 (8.3)
IPI Score at screening - n (%)	
0	1 (8.3)
1	2 (16.7)
2	2 (16.7)
3	5 (41.7)
4	2 (16.7)
CD20 + at screening - n (%)	12 (100.0)
CD19 + at screening - n (%)	10 (83.3)
Missing	2 (16.7)
Bone marrow involvement at study entry - n (%)	
Negative	10 (83.3)
Indeterminate	1 (8.3)
Missing	1 (8.3)
LDH>ULN at screening - n (%)	3 (25.0)
Modified Ann arbour stage at screening - n (%)	
II	3 (25.0)
III	5 (41.7)
IV	4 (33.3)
Best Overall Response to previous lines of therapy - n (%)	

PD	4 (33.3)
SD	1 (8.3)
PR	3 (25)
CR	4 (33.3)
No of previous therapy lines - n (%)	
1	7 (58.3)
2	2 (16.7)
3	2 (16.7)
4	0 (0.0)
5	1 (8.3)

Main Figure Legends

Figure 1: Treatment Emergent Adverse Events (TEAEs) related to zamto-cel and levels of cytokines after zamto-cel

a, Frequency of TEAEs related to Investigational Medicinal Product (IMP). b, Serum cytokine levels in patients with CRS and without CRS.

Figure 2: Efficacy of zamto-cel

a, PFS and OS among patients treated in cohort 1 and 2 (n=12), investigator assessment extended definition. b, Pre- and post-treatment PET-CT scans from patient 04_DL1 with CR. c, Swimmer‘s plot depicting response to treatment and follow up for each patient on study. Please note, that the three patients 01_DL1, 02_DL1 and 05_DL1 had SD as BOR. d, DOR among patients who showed CR or PR (n=9), investigator assessment. e, zamto-cel expansion in peripheral blood from treated individuals over time. f, C_{max} comparison of zamto-cel based on response. Statistical significance was assessed using Wilcoxon two-sample test with normal approximation (** $p<0.01$). C_{max}: maximum observed concentration of zamto-cel.

Figure 3: High-dimensional product characterization of zamto-cel

a+b, Expression of CD27 (a) and CD127 (b) in final drug product (DP) with representative quadrant plots from individuals with CR and non-CR respectively (left) and summarized data across all treated individuals (right). Gated on CAR+CD8+ cells. c, High-dimensional profiling of zamto-cell products (n=5) via scRNA-seq. Heatmap of top candidates of differentially expressed genes in CAR^+ T cells with high levels of CD27 and CD127 versus CAR^+ T cells with low and absent expression of CD27 and CD127 respectively. d, Memory phenotypes of DP in representative individuals (left) and summarized levels of Tcm cells (CD62L+/CD45RO+) in DP of treated individuals with CR and non-CR. e, Persistence of zamto-cel Tcm cells over time in peripheral blood from individuals with non-CR and CR. The mean is shown in violet (CR) and orange (non-CR) respectively. Statistical significance was assessed using Wilcoxon two-sample test with normal approximation.

Figure 1

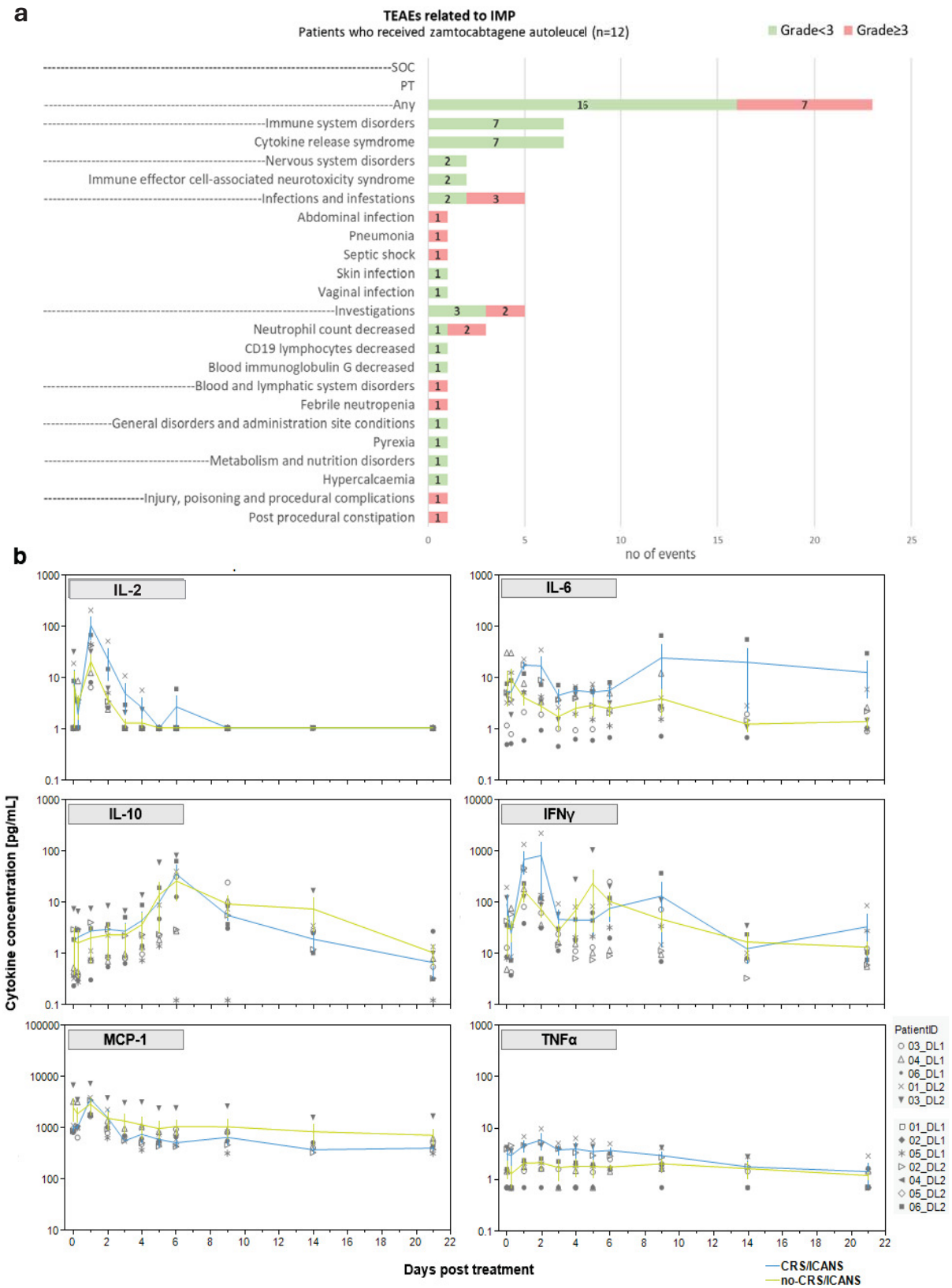

Figure 2

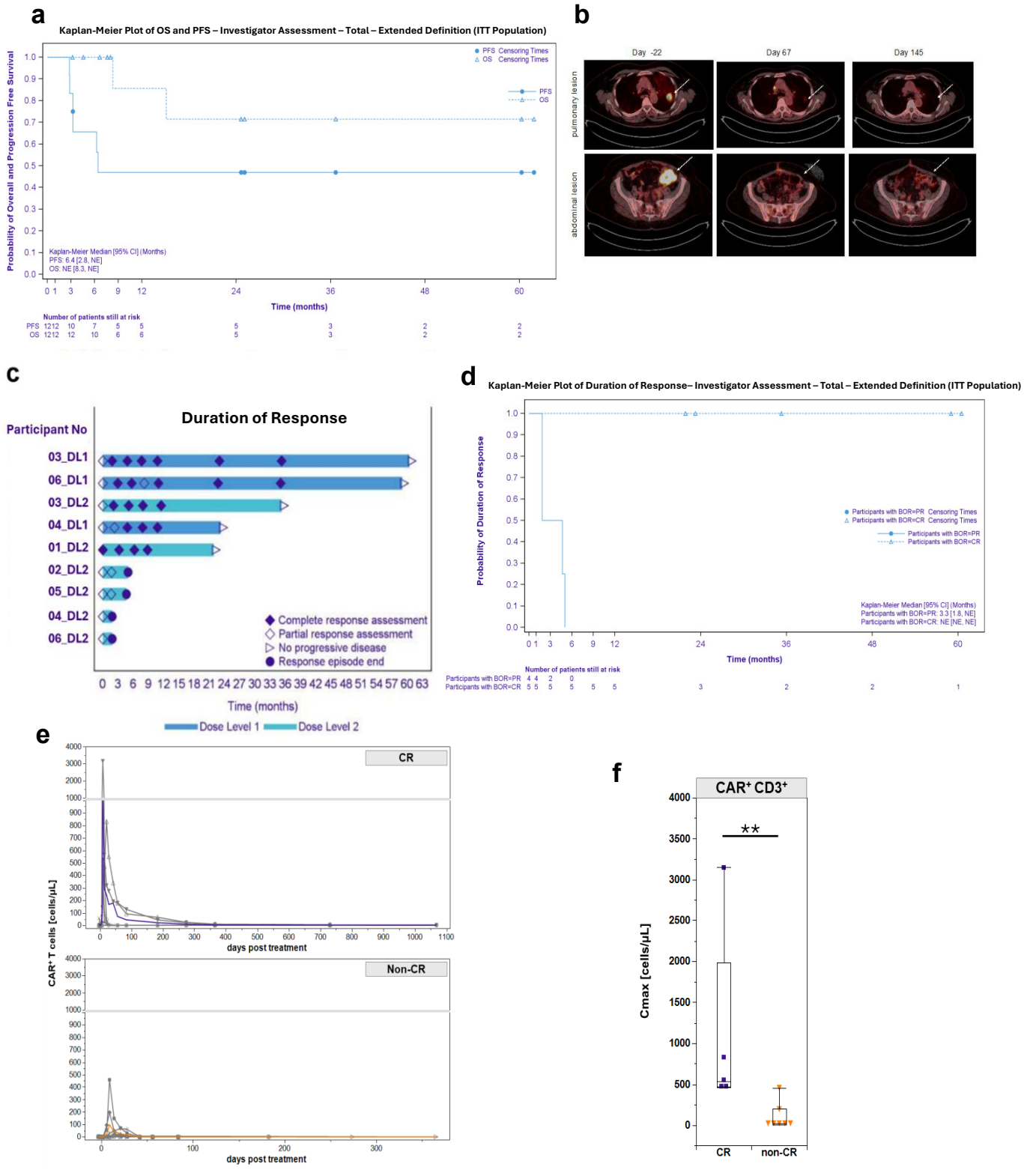

Figure 3