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Data will be made available upon reasonable request to the corresponding author

Running Title: CAR T-cells are not impacted by prior bispecifics

ABSTRACT

In this retrospective study, CAR T-cells remained effective in relapsed/refractory LBCL patients after prior exposure to bispecific antibodies (BsAbs) targeting different antigens. These results are relevant to clinical practice, particularly given the increasing use of BsAbs in earlier treatment lines.

LETTER TO BLOOD

The development of T-cell-engaging therapies, particularly chimeric antigen receptor (CAR) T-cells and bispecific antibodies (BsAbs), has revolutionized the treatment of patients with relapsed or refractory (R/R) large B cell lymphoma (LBCL). Recently, BsAbs have shown impressive efficacy results in heavily pretreated patients, leading to regulatory approval of glofitamab and epcoritamab as single agents for R/R LBCL exposed to at least 2 prior lines of systemic therapy [1-5]. Ongoing trials are exploring their use in combination with other agents in earlier treatment lines, increasing the number of patients exposed to BsAbs before CAR T-cells [6-8]. However, the relatively similar mechanism of action between both strategies has raised the concern of potential resistance to immune-killing after progressing to BsAbs, together with T-cell exhaustion which could affect subsequent CAR T-cell outcomes. This study evaluates efficacy and toxicity of anti-CD19 CAR T-cells in patients with R/R LBCL previously exposed to BsAbs, addressing a key clinical question and aiding treatment sequencing in this setting [9-11].

In the first part of the study, we conducted a retrospective analysis of 47 patients with R/R LBCL treated with CD19-targeted CAR T-cells after prior BsAb exposure at 11 French and 4 Spanish centers between 2018 and January 2023; patients exposed to CD19/CD3 BsAbs were excluded. All patients provided informed consent; the study was approved by DESCAR-T's ethics committee (French Data Protection Agency #2208143; HDH publication #20221220174727). Baseline characteristics of the study cohort are summarized in Table 1. The best overall (ORR) and complete response rate (CRR) achieved with prior BsAb treatment was 46% and 19%, respectively. The median PFS was 3.1 months (95% CI 2.7-4.4 months) and 6-month PFS was 21% (95% CI 11%-34%). Cytokine release syndrome (CRS) after BsAbs occurred in 27 (57%) patients, mostly grade 1-2 (only one grade 3 event) with no reported immune effector cell-associated neurotoxicity syndrome (ICANS). In 26 (55%) patients, BsAb therapy was the last regimen before CAR T-cells.

In terms of the subsequent CAR T-cell therapy, 22 (47%) patients received axicabtagene ciloleucel (axi-cel), 20 (42%) tisagenlecleucel (tisa-cel) and 5 (11%) lisocabtagene maraleucel (liso-cel). The best overall (complete) response rate to CAR T-cells was 85% (43%) (Table S1), without significant differences between patients who had previously responded [partial response or CR] or not (stable disease or progressive disease) to BsAb treatment [86% (41%) vs 84% (44%), p=1.0] (Table S2). At a median follow-up of 10.5 months, median PFS was 6.6 months (95% CI 2.6-not reached [NR]) and median OS was not reached (95% CI 9.0-NR). The estimated 1-year PFS and OS were 42% (95% CI 25.9-57.7) and 55% (95% CI 37.5-70.6), respectively (Figure 1).

The median time from the last dose of BsAb therapy to leukapheresis was 51 days (range 13-512), while the median time from leukapheresis to CAR-T infusion was 43 days. The ORR (CR) of patients previously exposed to BsAb within 50 days of leukapheresis was similar to patients who had a longer washout [82% (32%) vs 84% (52%) (p=0.36)]. The same comparable outcomes were observed for PFS and OS (Figure S1). Efficacy results did not differ significantly when the analysis was restricted to patients previously exposed to single-agent CD20/CD3 BsAbs and when it focused exclusively on axi-cel recipients (data not shown).

Incidence of any grade CRS and ICANS after CAR T-cells was 79% (grade ≥3 in 6%) and 23% (grade ≥3 in 2%), respectively. There were no differences in the rate of CRS after CAR T-cells according to previous CRS occurrence with BsAbs (78% vs 79%, p=1.0) (Table S3). Within the first month post-CART, 66% of patients experienced grade ≥3 neutropenia and 45% grade ≥3 thrombocytopenia. During follow-up, 18 (38%) patients died due to disease progression (n=12), infections (n=5; 2 septic shock, 1 pneumonia, 1 COVID-19 infection, 1 multiple organ failure after CMV infection) or unknown cause (n=1).

In the second part of the study, we generated a BsAb-naïve control group with 42 patients treated with axi-cel or tisa-cel among 980 patients from the DESCAR-T registry, via a 1:1 propensity score matching (PSM) analysis including 13 baseline covariates of clinical and prognostic relevance, to compare their outcomes with those of the BsAb-exposed group [12].

The 5 patients who received liso-cel were not included in this analysis due to the lack of an available control partner. Key characteristics of the PSM cohorts are shown in Table S4; after matching, SMD remained >0.1 for 6 covariates. The BsAb-exposed group achieved a higher ORR compared with the control group (86% vs 55%, p=0.02) but CRR, 1-year PFS and 1-year OS were not statistically different between BsAb-exposed and naïve patients (43% vs 38% [p=0.5], 43% vs 29% [p=0.1] and 55% vs 37% [p=0.08]) (Figure S2). Concerning the safety profile, there was a comparable rate of CRS and ICANS (any grade and grade ≥2) in both groups (Table S5).

In this retrospective, multicenter study evaluating efficacy and toxicity of CAR T-cell therapy in patients exposed to BsAbs before leukapheresis, key efficacy outcomes including response rate and survival were consistent with those of a matched control group, the pivotal trials and real-world data studies [13-19]. We found no evidence of intrinsic cross-resistance between CAR T-cells and BsAbs when both T-cell-engaging approaches did not target the same antigen. Our results strongly suggest (i) a lack of correlation between primary resistance to BsAbs and resistance to CAR T-cells in a given patient, in view of the similar ORR and CRR to CAR-T irrespective of prior response to BsAbs, and (ii) a similar efficacy of CAR T-cells in patients previously exposed to BsAb compared to a population of BsAb-naïve patients. Also, the interval between the last dose of BsAbs and leukapheresis did not seem to influence CAR T-cell efficacy. Taking into account that the half-life of BsAbs is relatively short (10-20 days) [20, 21], the washout period in our study seemed to provide sufficient BsAb clearance to allow T-cell fitness recovery before leukapheresis. However, further studies with larger cohorts including shorter washout periods are warranted to confirm these findings.

Preliminary data on the use of CD20/CD3 BsAbs in patients relapsing after CAR T-cells appear promising, with CR rates around 30% in R/R LBCL, suggesting that BsAbs are a potential salvage option after CAR-T failure [1-5, 22]. In this study, CAR T-cells also appear to be effective in patients who progress after exposure to BsAbs, highlighting that both

sequencing modalities seem effective. Importantly, no new safety signals or increased CRS/ICANS incidence were observed with CAR-T therapy in BsAb-exposed patients.

Beyond its retrospective nature, our study has some limitations. The small number of patients and the heterogeneity of treatments require larger prospective studies to confirm these results, but the overall findings appear to be consistent in the different subgroup analysis. In addition, despite the inclusion of numerous covariates with prognostic relevance in our PSM analysis, unidentified confounding factors could potentially persist.

In conclusion, our data suggest that CAR T-cell therapy remains effective in R/R LBCL patients after prior exposure to BsAbs when the target antigen is different. Also, lack of response to previous BsAbs does not predict for lower response rates after CAR T-cells. These results are relevant to clinical practice to inform on treatment sequencing, and reassuring in view of the increasing use of BsAbs in earlier treatment lines.

This study was approved by DESCAR-T ethics committee (French Data Protection Agency #2208143; HDH publication #20221220174727).

Contributions

Conception and design: G.C., G.I., P.B., F.M. and R.H. Provision of study materials or patients: G.C., G.I., A.C., E.B., T.G., G.C., M.K., R.G., N.M-C., C.C-Ll., P.A., M.G., C.S., V.C., S.G., A.C., E.D., K.B., P.B., F.M. and R.H. Collection and assembly of data: G.C., G.I., J.I-T., J.A-C., P.B., F.M. and R.H. Data analysis and interpretation: G.C., G.I., T.F., B.L., F.B., P.B., F.M. and R.H. Manuscript writing: All authors Final approval of manuscript: All authors Accountable for all aspects of the work: All authors

Conflicts of Interest

G.C.: Honorary/Travel Grant: Gilead, Roche, Sobi and Abbvie G.I. Consultancy and Honoraria: Novartis, Roche, Kite/Gilead, Bristol-Myers Squibb, Abbvie, Janssen, Sandoz, Miltenyi, AstraZeneca E.B.: Amgen, BMS (Research Funding); Kite, Gilead, Novartis, Roche,

Incyte, Miltenyi Biotech, Takeda, Sanofi (Honoraria); Roche, Gilead, ADC Therapeutics, Takeda, Novartis, Incyte (Membership on an entity's Board of Directors or advisory committees). T.G.: Gilead/Kite, Takeda (Honoraria, Membership on an entity's Board of Directors or advisory committees and Other: Support for attending meetings/travel, participation in a data safety monitoring board or advisory board). G.C.: MabQi, Ownards Therapeutics, Abbvie, Roche, BMS (Membership on an entity's Board of Directors or advisory committees); Gilead, Novartis, Miltenyi, Sanofi, Abbvie, Takeda, Roche, Janssen, Celgene, Novartis, BMS (Honoraria). R.G.: GILEAD, SANDOZ, SANOFI (Other: Congress); SANOFI (Other: Teaching) C.C-Ll.: IXAKA LIMITED (Consultancy); GILEAD KITE (Honoraria). P.A. Consulting/advisory: Roche, Genmab, Janssen, BMS, AbbVie, AstraZeneca, BeiGene; Honoraria: Roche, Genmab, Janssen, BMS, AbbVie, AstraZeneca, Gilead, Incyte. V.C.: Honorarium: Kite, a Gilead Company, Bristol Myers Squibb, Novartis, Incyte, Kyowa Kirin, Abbvie, Ideogen, Takeda. Travel fees: Pfizer, Kite, a Gilead Company, Bristol Myers Squibb, Novartis; Research funding paid to institution : Astra Zeneca, Bristol Myers Squibb, Novartis, Ideogen S.G.: Gilead Honoraria E.D.: Stemline Therapeutics (Consultancy and Honoraria); ImmunoGen (Consultancy and Honoraria); Chugai (Research Funding). K.B.: Kite/Gilead (Consultancy and Honoraria); Takeda (Consultancy and Honoraria); Roche (Consultancy and Honoraria). P.B.: Allogene: Honoraria; Amgen: Honoraria; BMS: Honoraria; Kite/Gilead: Honoraria; Janssen: Honoraria; Jazz Pharmaceuticals: Honoraria; Miltenyi: Honoraria; Novartis: Honoraria; Nektar: Honoraria. F.M.: Roche (Consultancy and Membership on an entity's Board of Directors or advisory committees); Gilead (Consultancy and Membership on an entity's Board of Directors or advisory committees); Abbvie (Consultancy and Membership on an entity's Board of Directors or advisory committees); Novartis (Membership on an entity's Board of Directors or advisory committees); BMS (Membership on an entity's Board of Directors or advisory committees); Genmab (Membership on an entity's Board of Directors or advisory committees); AstraZeneca (Membership on an entity's Board of Directors or advisory committees); Janssen (Membership on an entity's Board of Directors or advisory

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REFERENCES

1. Dickinson, M.J., et al., *Glofitamab for Relapsed or Refractory Diffuse Large B-Cell Lymphoma.* N Engl J Med, 2022. **387**(24): p. 2220-2231.
2. Thieblemont, C., et al., *Epcoritamab, a Novel, Subcutaneous CD3xCD20 Bispecific T-Cell-Engaging Antibody, in Relapsed or Refractory Large B-Cell Lymphoma: Dose Expansion in a Phase I/II Trial.* J Clin Oncol, 2022: p. JCO2201725.
3. Bannerji, R., et al., *Odronextamab, a human CD20xCD3 bispecific antibody in patients with CD20-positive B-cell malignancies (ELM-1): results from the relapsed or refractory non-Hodgkin lymphoma cohort in a single-arm, multicentre, phase 1 trial.* Lancet Haematol, 2022. **9**(5): p. e327-e339.
4. Budde, L.E., et al., *Single-Agent Mosunetuzumab Shows Durable Complete Responses in Patients With Relapsed or Refractory B-Cell Lymphomas: Phase I Dose-Escalation Study.* J Clin Oncol, 2022. **40**(5): p. 481-491.
5. Patel, K., et al., *A Phase 1 Study of Plamotamab, an Anti-CD20 x Anti-CD3 Bispecific Antibody, in Patients with Relapsed/Refractory Non-Hodgkin's Lymphoma: Recommended Dose Safety/Efficacy Update and Escalation Exposure-Response Analysis.* Blood, 2022. **140**(Supplement 1): p. 9470-9472.

6. Falchi, L., S.A. Vardhana, and G.A. Salles, *Bispecific antibodies for the treatment of B-cell lymphoma: promises, unknowns, and opportunities.* Blood, 2023. **141**(5): p. 467-480.
7. Topp, M.S., et al., *Glofitamab Plus R-CHOP Induces High Response Rates and a Favorable Safety Profile in Patients with Previously Untreated Diffuse Large B-Cell Lymphoma (DLBCL): Results from a Phase Ib Study.* Blood, 2022. **140**(Supplement 1): p. 1775-1777.
8. Falchi, L., et al., *First-line treatment (Tx) with subcutaneous (SC) epcoritamab (epco) + R-CHOP in patients (pts) with high-risk diffuse large B-cell lymphoma (DLBCL): Phase 1/2 data update.* Journal of Clinical Oncology, 2022. **40**(16_suppl): p. 7523-7523.
9. Lemoine, J., M. Ruella, and R. Houot, *Born to survive: how cancer cells resist CAR T cell therapy.* J Hematol Oncol, 2021. **14**(1): p. 199.
10. Philipp, N., et al., *T-cell exhaustion induced by continuous bispecific molecule exposure is ameliorated by treatment-free intervals.* Blood, 2022. **140**(10): p. 1104-1118.
11. Piccione, E.C., et al., *Immune correlates of response to Glofitamab: biomarker findings from a pivotal phase II expansion study in patients with relapsed or refractory (R/R) Diffuse Large B-cell Lymphoma (DLBCL).* HemaSphere, 2022. **6**: p. 1096-1097.
12. Bachy, E., et al., *A real-world comparison of tisagenlecleucel and axicabtagene ciloleucel CAR T cells in relapsed or refractory diffuse large B cell lymphoma.* Nat Med, 2022. **28**(10): p. 2145-2154.
13. Abramson, J.S., et al., *Lisocabtagene maraleucel for patients with relapsed or refractory large B-cell lymphomas (TRANSCEND NHL 001): a multicentre seamless design study.* Lancet, 2020. **396**(10254): p. 839-852.
14. Neelapu, S.S., et al., *5-Year Follow-Up Supports Curative Potential of Axicabtagene Ciloleucel in Refractory Large B-Cell Lymphoma (ZUMA-1).* Blood, 2023.

15. Neelapu, S.S., et al., *Axicabtagene Ciloleucel CAR T-Cell Therapy in Refractory Large B-Cell Lymphoma.* N Engl J Med, 2017. **377**(26): p. 2531-2544.
16. Schuster, S.J., et al., *Tisagenlecleucel in Adult Relapsed or Refractory Diffuse Large B-Cell Lymphoma.* N Engl J Med, 2019. **380**(1): p. 45-56.
17. Bethge, W.A., et al., *GLA/DRST real-world outcome analysis of CAR T-cell therapies for large B-cell lymphoma in Germany.* Blood, 2022. **140**(4): p. 349-358.
18. Jacobson, C.A., et al., *Real-World Evidence of Axicabtagene Ciloleucel for the Treatment of Large B Cell Lymphoma in the United States.* Transplant Cell Ther, 2022. **28**(9): p. 581 e1-581 e8.
19. Van Le, H., et al., *Use of a real-world synthetic control arm for direct comparison of lisocabtagene maraleucel and conventional therapy in relapsed/refractory large B-cell lymphoma.* Leuk Lymphoma, 2023. **64**(3): p. 573-585.
20. Hutchings, M., et al., *Glofitamab, a Novel, Bivalent CD20-Targeting T-Cell-Engaging Bispecific Antibody, Induces Durable Complete Remissions in Relapsed or Refractory B-Cell Lymphoma: A Phase I Trial.* J Clin Oncol, 2021. **39**(18): p. 1959-1970.
21. Hutchings, M., et al., *Dose escalation of subcutaneous epcoritamab in patients with relapsed or refractory B-cell non-Hodgkin lymphoma: an open-label, phase 1/2 study.* Lancet, 2021. **398**(10306): p. 1157-1169.
22. Iacoboni, G., et al., *Salvage Treatment with Novel Agents Is Preferable to Standard Chemotherapy in Patients with Large B-Cell Lymphoma Progressing after Chimeric Antigen Receptor T-Cell Therapy.* Blood, 2022. **140**(Supplement 1): p. 378-380.

Table 1.- Demographic and clinical characteristics of patients at time of lymphodepleting chemotherapy.

Characteristics	Patients n=47
Age, median years (range)	65 (31-82)
Male gender - no (%)	31 (66)
ECOG performance status >1 - no (%)	4 (9)
Disease stage III or IV — no. (%)	42 (89)
Histology- no (%)	
- DLBCL NOS	38 (81)
- tFL	5 (11)
- THRLBCL	2 (4)
- PMBL	1 (2)
- HGBL	1 (2)
Number of prior therapies, median (range)[a]	3 (2-9)
Previous ASCT- no (%)	9 (19)
Bulky disease (>5cm)- no (%)	17 (36)
CRP ≥ 30 mg/L – no (%)[b]	18 (39)
LDH ≥ 2xULN – no (%)	27 (57)
CAR T-cell therapy— no. (%)	
- Axi-cel	22 (47)
- Tisa-cel	20 (42)
- Liso-cel	5 (11)
Days from the last BsAb dose to leukapheresis - median (range)	51 (13-512)
Days from the last BsAb dose to CAR T-cell	97 (47-572)

infusion – median (range)	
Days from leukapheresis to CAR T-cell infusion - median (range)	43 (34-103)
Bridging therapy - no (%)	42 (89)
- Immunochemotherapy	29 (62)
- Targeted therapy	9 (19)
- Radiotherapy	3 (6)
- Not specified	1 (2)
Response to bridging - no (%)[c]	
- Responder (CR/PR)	11 (28)
- Non responder (SD/PD)	28 (72)
Prior BsAb exposure	
Type of BsAb – no (%)[d]	
- CD20/CD3	43 (91)
- CD22/CD3	4 (9)
Combination with BsAb - no (%)	5 (11)
- Lenalidomide	2 (4)
- Chemotherapy	2 (4)
- Polatuzumab vedotin	1 (2)
- Missing	1 (2)

Table 1_Abbreviations: IQR interquartile range, DLBCL diffuse large B-cell lymphoma, HGBL high grade B-cell lymphoma, PMBL primary mediastinal B-cell lymphoma, THRLBCL T cell histiocyte rich large B cell lymphoma, tFL transformed follicular lymphoma, ASCT autologous stem cell transplant, CRP C-reactive protein, ULN Upper Limit of Normal, LDH lactate dehydrogenase, ECOG Eastern Cooperative Oncology Group, CAR chimeric antigen

receptor, CR complete response, PR partial response, SD stable disease, PD progressive disease, BsAb Bispecific antibody

[a] The median number of prior lines of therapy before BsAbs was 2 (range 1-6)

[b] Missing data for 1 patient

[c] Evaluable response in 39 patients (3 patients with missing data)

[d] The CD20/CD3 bispecifics (n=43/47) included glofitamab (n=23), epcoritamab (n=3), mosunetuzumab (n=8), odronextamab (n=6), plamotamab (n=3).

Figure legends

Figure 1.- Outcomes after CAR T-cell therapy in patients previously exposed to bispecific antibody treatment. 1A) Overall Survival (OS) after CAR T-cell therapy (n=47); 1B) Progression-Free Survival (PFS) after CAR T-cell therapy (n=47); 1C) Duration of Response (DoR) after CAR T-cell therapy (n=38).

Figure 1

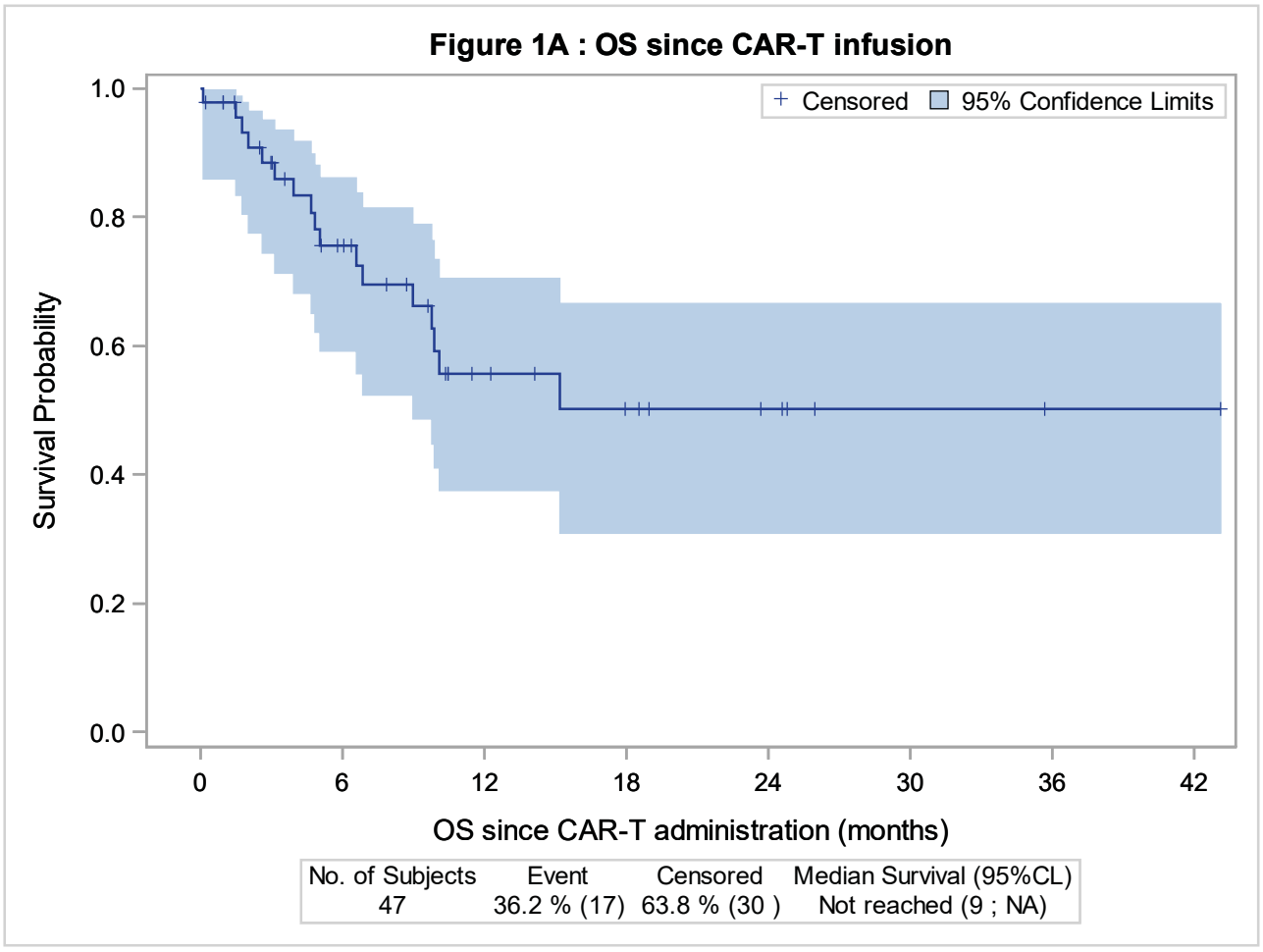

Figure 1

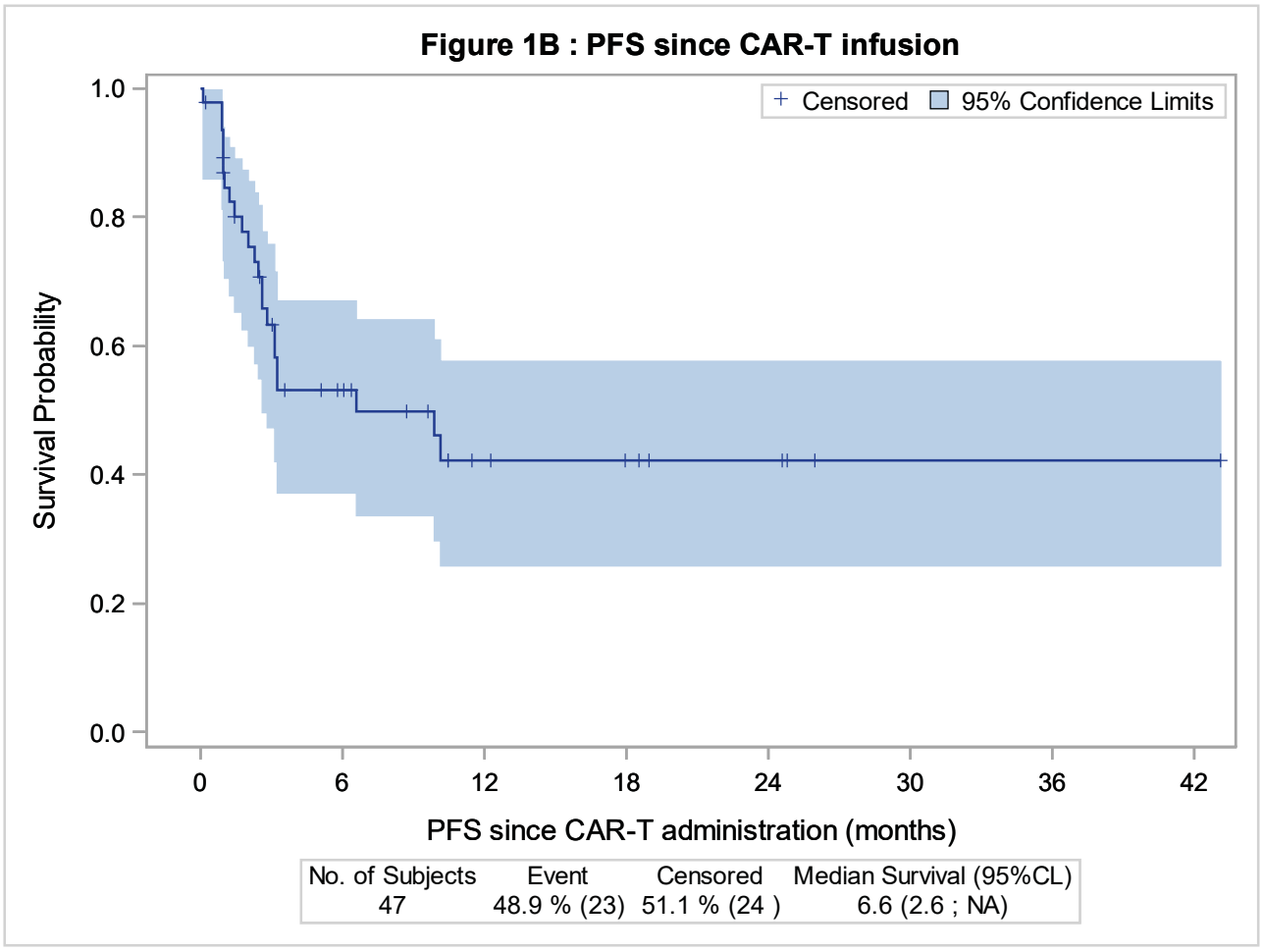

Figure 1