Response Rates of Extra-Nodal Diffuse Large B Cell Lymphoma to anti CD19-CAR T Cells - a Real Word Retrospective Multi-Center Study

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Abstract

Chimeric antigen receptor T-cells (CAR-T) are widely used for the treatment of relapsed/refractory Diffuse large B cell lymphoma (DLBCL). The data for CAR-T cell therapy in patients with extra-nodal(EN) lymphoma is restricted. We included 126 consecutive patients with DLBCL treated with commercially available CAR-T cells (tisagenlecleucel, n=100, 79.4% and axicabtagene ciloleucel, n=26, 20.6%). At lymphodepletion , 72/126(57%) had EN disease, 42/126(33%) patients had nodal disease (ND)-only and 12/126(10%) showed no disease assessed by PET-CT. There were no significant differences in CAR-T related toxicities and in the median PFS between EN patients and ND [10.76(95% CI: 7.8-13.6) vs 14.1 (95% CI:10-18.1) months, p =0.126)]. Similarly, median OS was not significantly different [15.36 (95% CI 12.5-18.2) vs. 18.4 (95% CI 14.8-22.1) months , p =0.100]. Subgroup analysis according to the number of EN involved sites showed that median PFS and OS were significantly higher in patients with ≤ 2 EN sites [12.3 months (95% CI 9-15.5)] vs 4.28 months (95% CI 0.6-7.9), p=0.010] compared to patients with ≥3 EN sites, respectively[16.5 months (95% CI 13.4-19.6) vs 8.7 months (95% CI 4.6-12.8), p=0.05]. In multivariate cox regression analysis, increased number sites of EN disease and high LDH at lymphodepletion negatively impacted PFS (p=0.021 and <0.001, respectively), while sex, type of product administered, age and performance status did not predict PFS and OS. Of note, all the patients with involvement of gastrointestinal tract (n= 9), urinary tract (n= 9), or pharynx (n= 3) at lymphodepletion progressed or had an early relapse.

In conclusions, both short and long term outcomes were similar in patients with EN and patients with ND at lymphodepletion, however patients with specific sites of EN disease may demonstrate grim prognosis.

Introduction

Chimeric antigen receptor(CAR)-T cell therapy has created a revolution in the treatment of Diffuse large B cell lymphoma(DLBCL). Since commercially available from 2017, we are widening our knowledge and experience on patient and disease related factors that contribute to treatment response. Identifying patients that are less likely to respond to CAR-T cells is markedly important in the era of novel agents and taking into consideration the toxicities associated with this therapy.

DLBCL can present with nodal or extra-nodal involvement. Extra-nodal(EN) lymphoma is considered clinically, genetically and prognostically distinct from nodal lymphoma(ND)[1–4]. A retrospective study revealed that EN DLBCL was associated with older age and poorer performance status[3]. Biologically, using immunophenotype, a higher proportion of patients with non-germinal center B cell like(non-GCB) profile was associated with EN involvement[5]. Importantly, EN disease is associated with poor prognosis and lack of treatment response with specific involvement of two EN sites being associated with worse progression free survival(PFS) and overall survival(OS)[5]. The response of EN lymphoma to CAR-T cells is sparsely reported and defined.

A retrospective study in a cohort of 32 patients treated with anti-CD19 CAR-T cells showed that soft tissue infiltration of lymphoma(n=12) at lymphodepletion was the only factor correlated with adverse prognosis[6]. Furthermore, real life data analysis from 116 patients treated with axi-cel and tisa-cel show that involvement of two or more EN sites at lymphodepletion was associated with reduced PFS and OS[7]. Several cases of DLBCL treated with CAR-T cells have described penetration of CAR-T cells to pleural cavity and cerebrospinal fluid(CSF) suggesting that CAR-T may enhance EN lymphoma response[8,9].

In this multi-center study, we provide a comprehensive description of the response and associated toxicity of diverse EN organs to commercially available CAR-T cells and compare to patients with ND.

Methods

Patients

Four medical centers were involved in this retrospective study. Every center independently searched the database for adult, DLBCL patients treated with commercially available CAR-T cells from June 2019 up to October 2021.All patients signed informed consent for the reporting of outcomes and the study was performed in accordance with the declaration of Helsinki and was approved by institutional review board. The medical records of all consecutive patients were analyzed for general characteristics of the patients and the disease. We excluded patients with no positron emission tomography - computed tomography (PET-CT) prior to lymphodepletion and patients with primary mediastinum B cell lymphoma. Cell of origin was extracted from the medical files and based on Hans algorithm[10]. Baseline PET-CT prior to lymphodepletion and CAR-T cell administration was used in order to assess both the disease burden using the Lugano response criteria[11] and, specifically, the allocation whether patient had no EN involvement, 1-2 EN sites, or >2 EN sites. Based on these criteria we designed an organ based response criteria to ensure consistency between centers(Sup Table 1)[11]. The EN lymphoma cohort included patients with EN involvement only or combined nodal and EN organs. The description of the EN organs involved in PET-CT at lymphodepletion was independently extracted by two reviewers. The toxicity associated with CAR-T cell administration was recorded using the ASTCT consensus guidelines[12].

Preparative regimen and supportive care

Once product was available for infusion, patients were admitted to the Bone Marrow Transplantation ward in designated rooms with high efficiency particulate air (HEPA) filters. Prior to infusion of CAR-T, patients were given cyclophosphamide (300-500 mg/m2) and Fludarabine (30 mg/m2) for 3 days (days -5 to -3). Cells were thawed and infused according to manufacturer recommendations.

Definitions

EN organs were defined as any organ other than lymph node, Waldeyer’s ring, tonsils or spleen. Patients were classified as nodal disease(ND) if no EN organs were involved by lymphoma. Sup table 1 describes the criteria for EN response.

Statistical analysis

Continuous variables are reported using median and range. Categorical data is presented as percentage. Response rates, PFS and OS of patients with EN disease at lymphodepletion were compared to patients presenting with a ND only. PFS and OS were derived using the Kaplan-Meier estimator. Multivariant logistic regression model was used to identify predictors of survival. Statistical significance was defined as 2-sided $p<0.05$.

Results

Baseline characteristics:

Between June 2019 and October 2021, 126 consecutive patients with DLBCL were treated with commercially available CAR-T cells in four different hospitals. Mean age at time of lymphodepletion for all patients was 65.8 (range; 63.4-68) years. The infused product was tisagenlecleucel in 100(79.4%) patients and axicabtagene ciloleucel in 26(20.6%) patients. At lymphodepletion therapy prior to CAR-T cells, 72/126(57%) had EN disease, 42/126(33%) had nodal disease(ND) and 12/126(10%) showed no disease assessed by PET-CT. 12 patients (9.5%) had more than 2 EN sites, 60 (47.5%) had 1-2 EN sites. More patients with EN involvement had higher eastern cooperative oncology group performance status(ECOG-PS) compared to those with ND (61% vs.29% , p= 0.008). All other baseline characteristics of disease and patients were similar between the two groups, Table 1.

Outcomes of patients treated with CAR-T cells with extra-nodal disease compared to nodal disease:

Response rates:

For all patients included, overall response rate(ORR) and complete response(CR) at 1 months after CAR-T infusion were 63% and 44% respectively.

There was no difference in both the rate of overall response and in the rate of CR at 1 month after CAR-T infusion between EN group and ND (58.1% vs. 64.3%, p=.51 , and 39.2% vs. 43%, p=.81, respectively)

Survival:

For all patients included, at a median follow-up of 7.5 months (range 0.2-27.3), 78 patients(62%) are alive and 53 patients(42%) are in CR. The median PFS and OS for all patients was 12.5 months(95% CI: 10.2-14.8) and 17 months (95% CI: 14.8-19.2) respectively. There was no significant difference in median PFS between the EN patients and ND [10.76(95% CI: 7.8-13.6) vs 14.1 (95% CI:10-18.1) months, p =0.126)] (Figure 1A). Similarly, median OS was not significantly different [15.36 (95% CI 12.5-18.2) vs. 18.4 (95% CI 14.8-22.1) months, p =0.100](Figure 1B).

Subgroup analysis according to the number of EN involved sites showed that median PFS was significantly higher in patients with no EN disease [14.6 months (95% CI 10.9-18.3)] or ≤ 2 EN sites [12.3

months (95% CI 9-15.5)] compared to patients with ≥3 EN sites[4.28 months (95% CI 0.6-7.9), p=0.01] (Figure 2A). Similarly, OS was prolonged in patients with ≤ 2 EN sites compared to ≥3 EN sites[16.5 months (95% CI 13.4-19.6) vs [8.7 months (95% CI 4.6-12.8), p=0.05] (Figure 2B).

In multivariate cox regression analysis, the number of sites of EN disease and high LDH at lymphodepletion were predictive for inferior PFS (p=0.021 and <0.001, respectively), while sex, type of product administered, age, and performance status did not predict PFS(Table 3). Similarly, both higher LDH and ≥3 EN disease were associated with inferior OS(p<.004 and p<.001, respectively), while all other factors were not.

Characteristics of CAR-T cells related response in extra-nodal lymphoma:

Among EN patients(n=72), 69% had combined nodal and EN lymphoma involvement and 31% had isolated EN lymphoma at the time of lymphodepletion. Overall , 127 EN organs were involved in 72 patients at the time of lymphodepletion. The most common EN organ was bone/bone marrow(BM) involvement in 27 patients(21% of all organs)(Figure 3). Lung involvement was the second most common organ identified in 13% among EN organs and liver, skin and muscular organs were described in 9-10%. Ocular, parotid, prostate and pharynx were rarely recognized in our cohort (Figure 3). EN organs that were more likely to respond to CAR-T cells were liver(n=13) and breast(n=4). conversely, all the patients with involvement of gastrointestinal tract (n= 9),urinary tract (n= 9) or pharynx (n= 3) at lymphodepletion progressed or relapsed less than 3 months from CAR-T administration(Figure 4).

Eleven patients had CNS involvement(15% among EN patients) at lymphodepletion. Of these patients, 7/11 showed relapse or progression following CAR-T cells (64%) with 5/11 (45%) demonstrating early relapse less than 3 months from CAR-T infusion.

Characteristics of CAR-T cells related toxicity in extra-nodal lymphoma:

Incidence of overall cytokine release syndrome(CRS) was higher in patients with EN lymphoma compared to ND, however this did not reach statistically significance (76% vs.57% , p= 0.06)(Table 2). Severe CRS was comparable (18% vs. 14%, respectively). Among the 13 patients with severe CRS and EN disease, the most commonly involved organs were BM/bone involvement(n=5) and liver involvement(n=5).

Incidence of overall immune effector-cell associated neurotoxicity syndrome (ICANS) was similar in the ND and the EN group. Among the 4 patients with severe ICANS in the EN group, 2 had CNS involvement and 2 had liver involvement. Of note, among patients with CNS involvement, high grade ICANS was noted in 2/11(18%).

Discussion

The aim of this study was to consistently explore the characteristics and response rates of DLBCL involving EN sites at lymphodepletion therapy prior to CAR-T cells. In this multi-center study, we showed

that both short and long-term outcomes were similar in patients with EN lymphoma and patients with ND with no enhanced toxicity.

The most frequent involved site in primary EN lymphoma is variable between different studies and most commonly reported organs are GI, bone or BM[1–4]. In our cohort we assessed the frequency of EN sites at lymphodepletion and show that the most common organ was bone and BM followed by lung involvement. Analyzing treatment response segregated by organs involved we did not identify any organ that was significantly associated with treatment response. However, all patients with involvement of GI/urinary/kidney or pharynx at lymphodepletion experienced relapse or progression less than 3 months from CAR-T infusion. To our knowledge there is only one study(n=32 patients) assessing EN specific involvement prior to CAR-T and identified that most patients presented with soft tissue(37.5%) and BM(25%) lymphoma involvement[6]. In multivariate analysis, soft tissue involvement by lymphoma was the only factor associated with PFS and OS but not other EN organs. This trial is not comparable to our trial due to major distinctions. Most notably, our trial assessed commercially available CAR-T cells and analyzed treatment response based on EN site at lymphodepletion and not at diagnosis.

Secondary CNS lymphoma involvement is an area of unknown ground in the CAR-T field with specific concerns from neurologic toxicity. There are now several small series retrospectively evaluating real life data on CAR-T cells for secondary CNS lymphoma. Response appears encouraging with toxicity rate equivalent to non-CNS patients[8,13–17]. Our cohort included 11 patients with secondary CNS lymphoma further supporting the data from other centers on the feasibility and efficiency of CAR-T cells in CNS lymphoma.

Our finding that several EN sites may be more resistant to CAR-T trafficking may be of both biologically and clinical importance. CAR-T cells are suggested to migrate and penetrate into EN organs but the data are still limited. Recently, applying single cell analysis by cytof technology revealed a spatiotemporal plasticity of CAR-T cells[18]. Upregulation of trafficking and activation molecules such as CD4 and CD8 integrin-β7, CD4 granzyme B, and CD11a were demonstrated in the CAR-T cell product. Tumor associated fibroblasts and macrophages, may also play a role in the penetration of CAR-T to the EN sites. CNS and BM penetration of CAR-T cells has been described in several clinical cases[19–21]. Furthermore, pleural fluid penetration of CAR-T cells has also been reported[9]. Nevertheless, the true trafficking and migration of CAR-T cells into different organs is unidentified and should be further explored. The fact that certain sites may be more CAR-T-sensitive, while other may be more resistant, should be further explored in term of cytokines and adhesion molecules expression, as well as tumor microenvironment diversions.

From the clinical outcome perspective, there were no significant differences in PFS and OS between patients with EN and ND-only lymphoma in our trial. However, multivariate cox regression analysis identified that the number of EN organs involvement and high LDH at lymphodepletion were predictive of inferior PFS and OS. This is in accordance with the French group showing that two or more EN sites at lymphodepletion were associated with poor survival[7]. Thus, in patients with multiple EN sites at lymphodepletion especially involving GI, urinary or pharynx, post CAR-T immunotherapy might be

required in order to deepen response and enhance survival. Prospective studies are needed to identify a genetic aberration that might suggest an optimal add on therapy in these poor prognosis patients.

This study has several limitations. First the retrospective nature and biases associated with multi-center analysis. Nevertheless, all 4 centers follow the same guidelines for staging and clinical surveillance monitoring. Second, results applying for specific sites are subjected to bias because of the small number of cases of each organ involved by lymphoma. Third, the definition of EN lymphoma and response in some cases was challenging and arguable. To overcome this, we have defined specific characteristics of treatment response based on the different organs to increase consistency between the centers.

In conclusion, survival of patients with EN involvement appears similar to patients with ND at lymphodepletion prior to CAR-T cells. Three or more EN sites and high LDH were associated with reduced survival. Patients with involvement of GI/urinary/ pharynx at lymphodepletion demonstrate grim prognosis. Larger studies should further validate these findings. If confirmed, patients with specific sites of EN disease may be candidate for future trials with post-CAR-T consolidation with immunotherapy strategies.

Declarations

The authors declare no conflict of interest.

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Tables

Table 1

Datum		Nodal (n=42)	Extra-nodal (n=72)	P value
Age in years (mean ± S.D.)		65 (± 15)	67 (± 11)	0.4
Sex – Female n(%)		21 (50)	42 (58)	0.48
Lymphoma cell of origin – non-GCB n(%) *		21(54)	37 (53)	0.92
LDH U/L (mean ± S.D.)		521(± 778)	532(± 562)	0.93
Transformed Lymphoma - n(%)		13 (31)	21(29)	0.77
Previous treatment >2 lines – n(%)		16 (38)	29 (40)	0.15
Performance status – ECOG PS>1 – n(%)		**12(29)**	**44(61)**	**0.008**
Product Tisagenlecleucel - n(%)		33(79)	61(85)	0.61
PET-CT at lymphodepletion – n(%)	CR	0 (0)	0(0)	0.16
	PR	15 (36)	20 (27)	
	SD	9 (21)	8 (11)	
	PD	18 (43)	44(61)	

Table 2

Datum	Nodal (n=42)	Extra-nodal (n=72)	P value
Any grade CRS- n(%)	**24 (57)**	**55 (76)**	**0.06**
High grade CRS(3-4) - n(%)	6(14)	13(18)	0.64
Any grade ICANS -n(%)	10(24)	12(17)	0.50
High grade ICANS(3-4) -n(%)	3 (7)	4 (5)	0.71

Table 3

Factor		Hazard ratio	95% CI	p-value
Progression-free survival				
Sex		0.657	0.362-1.193	0.167
Product type		0.923	0.426-2	0.839
Age at lymphodepletion		0.990	0.965-1.016	0.453
LDH at lymphodepletion		1.001	1.000-1.001	0.001
ECOG PS		1.321	0.694-2.515	0.39
EN disease at lymphodepletion				
	END≤2	1.675	0.842-3.330	0.141
	END≥3	2.908	1.172-7.216	0.021
Overall survival				
Sex		0.757	0.464-1.236	0.266
Product type		1.172	0.638-2.150	0.609
Age at lymphodepletion		0.996	0.975-1.018	0.712
LDH at lymphodepletion		1.001	1.000-1.001	0.001
ECOG PS		0.880	0.519-1.492	0.634
EN disease at lymphodepletion				
	END≤2	1.369	0.792-2.367	0.26
	END≥3	3.064	1.434-6.541	0.004

Figures

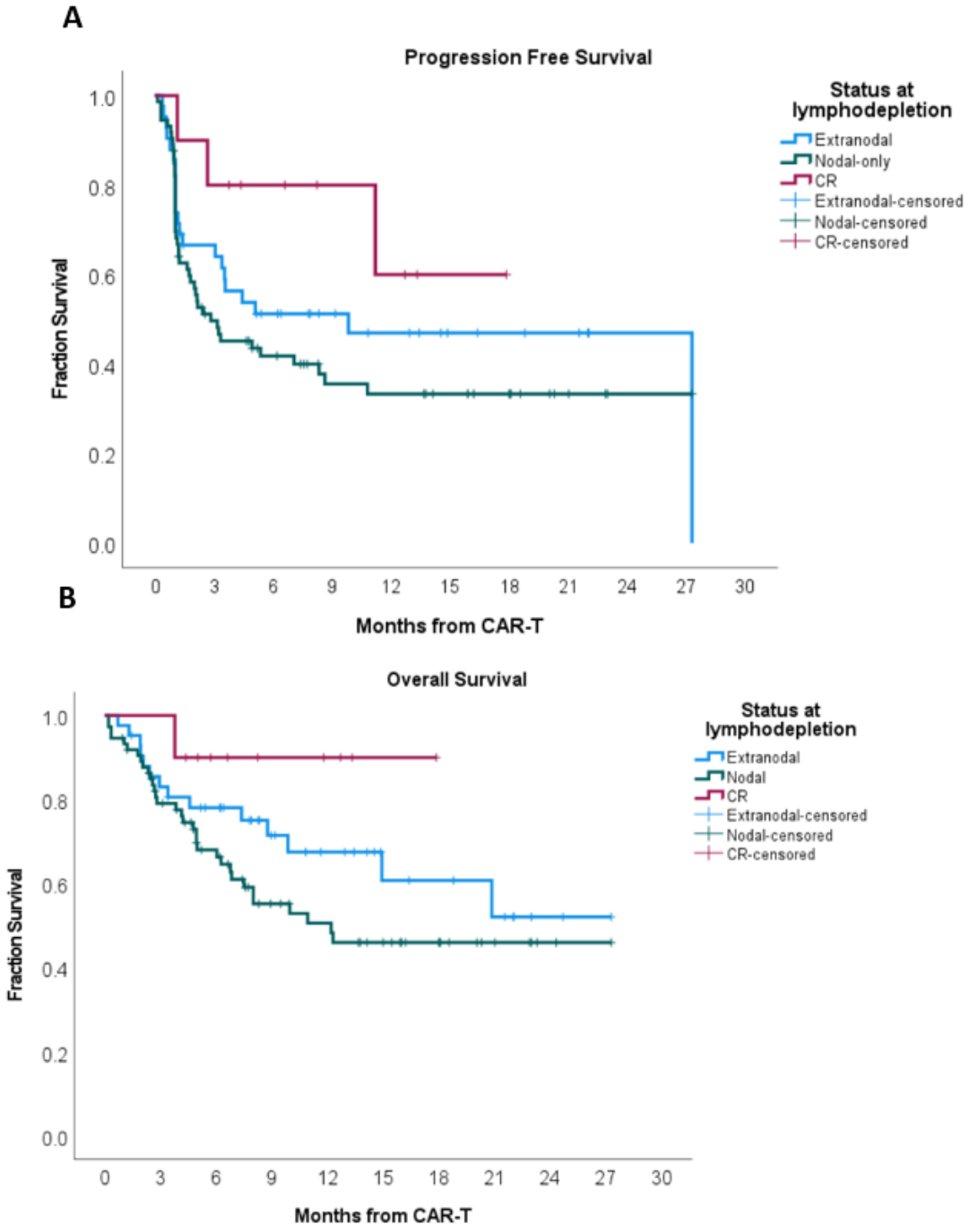

Figure 1

Progression free survival and overall survival -extra-nodal and nodal lymphoma

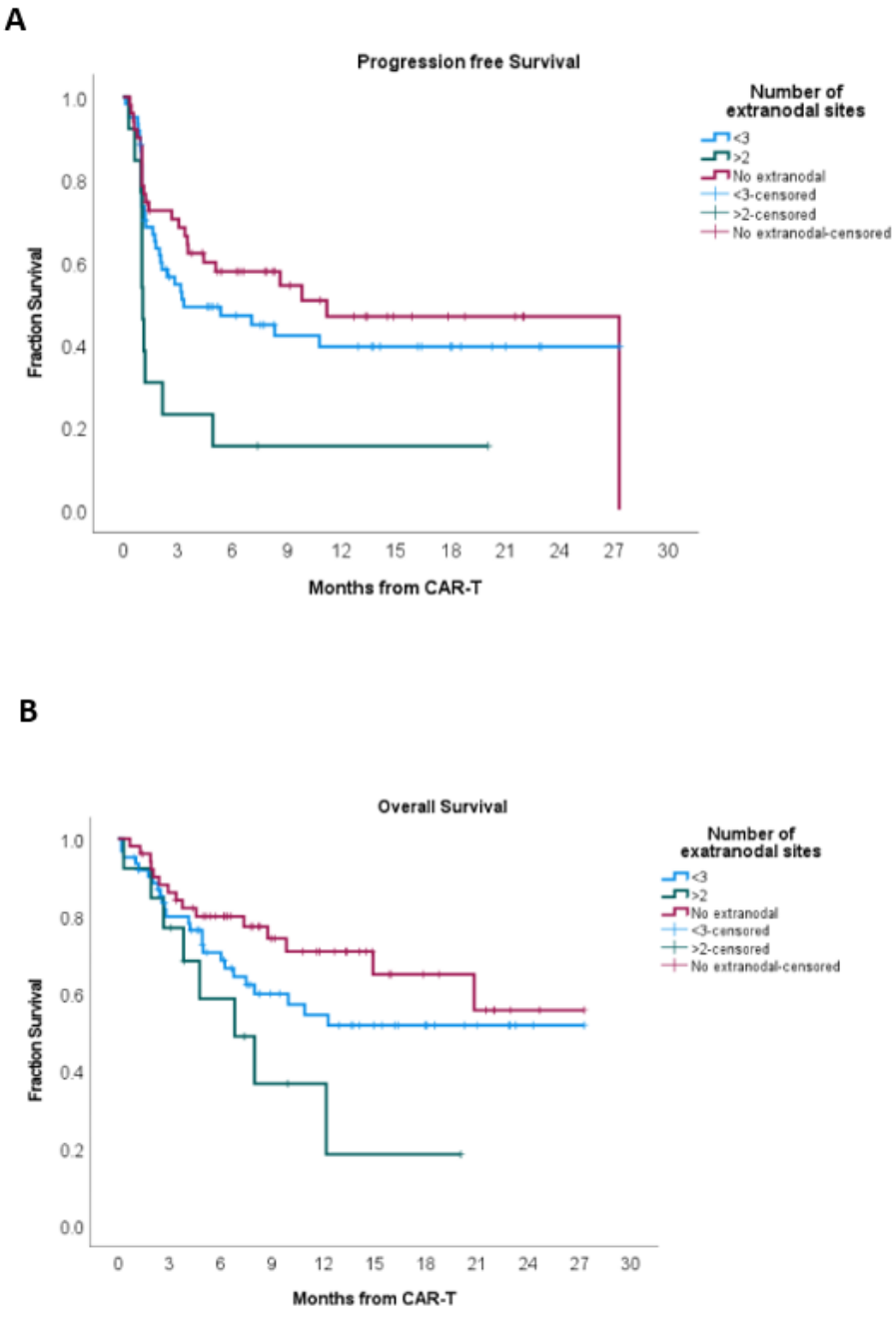

Figure 2

Progression free survival and overall survival -multiple extra-nodal and nodal lymphoma

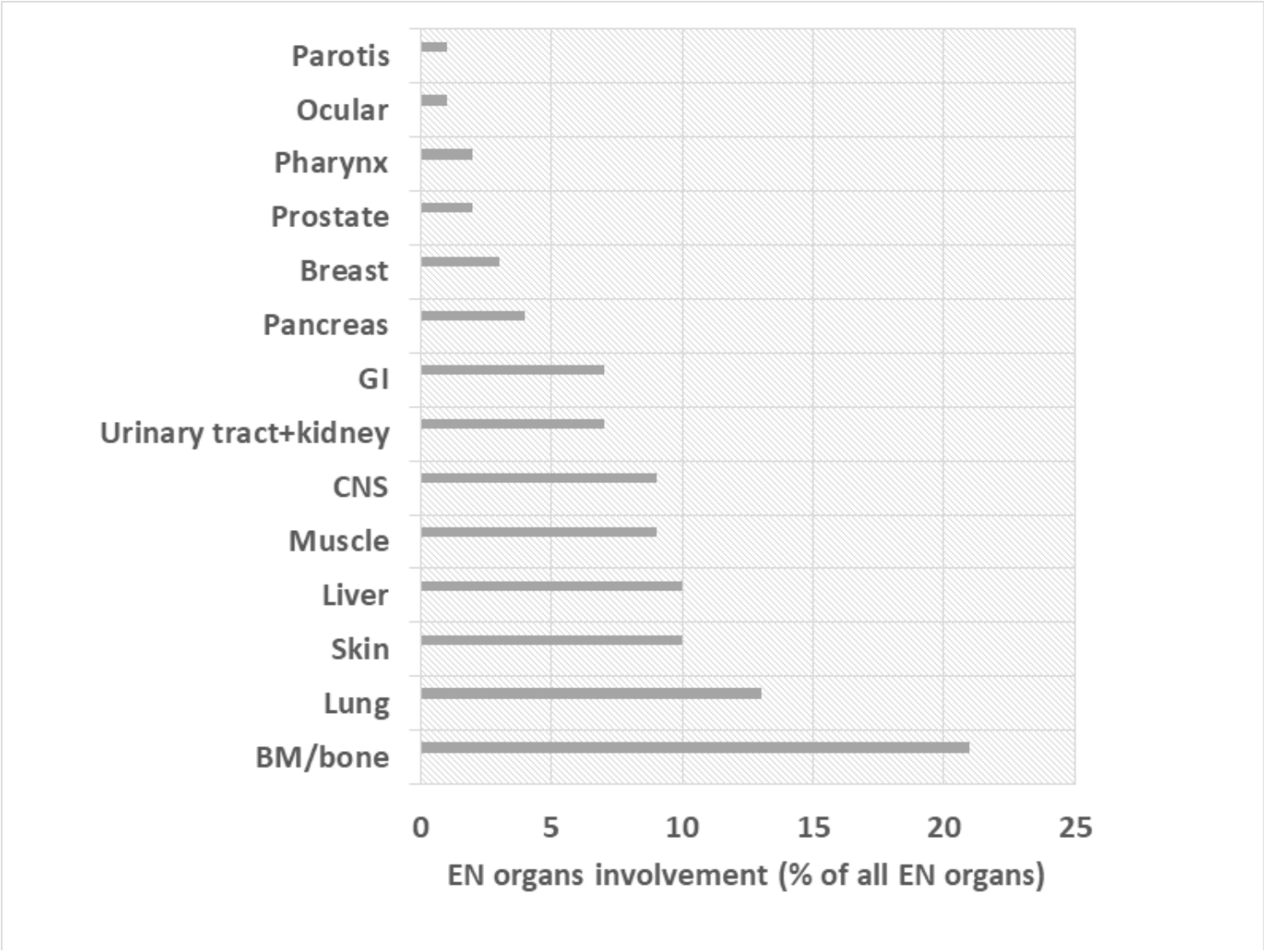

Figure 3

Multivariate analysis of survival

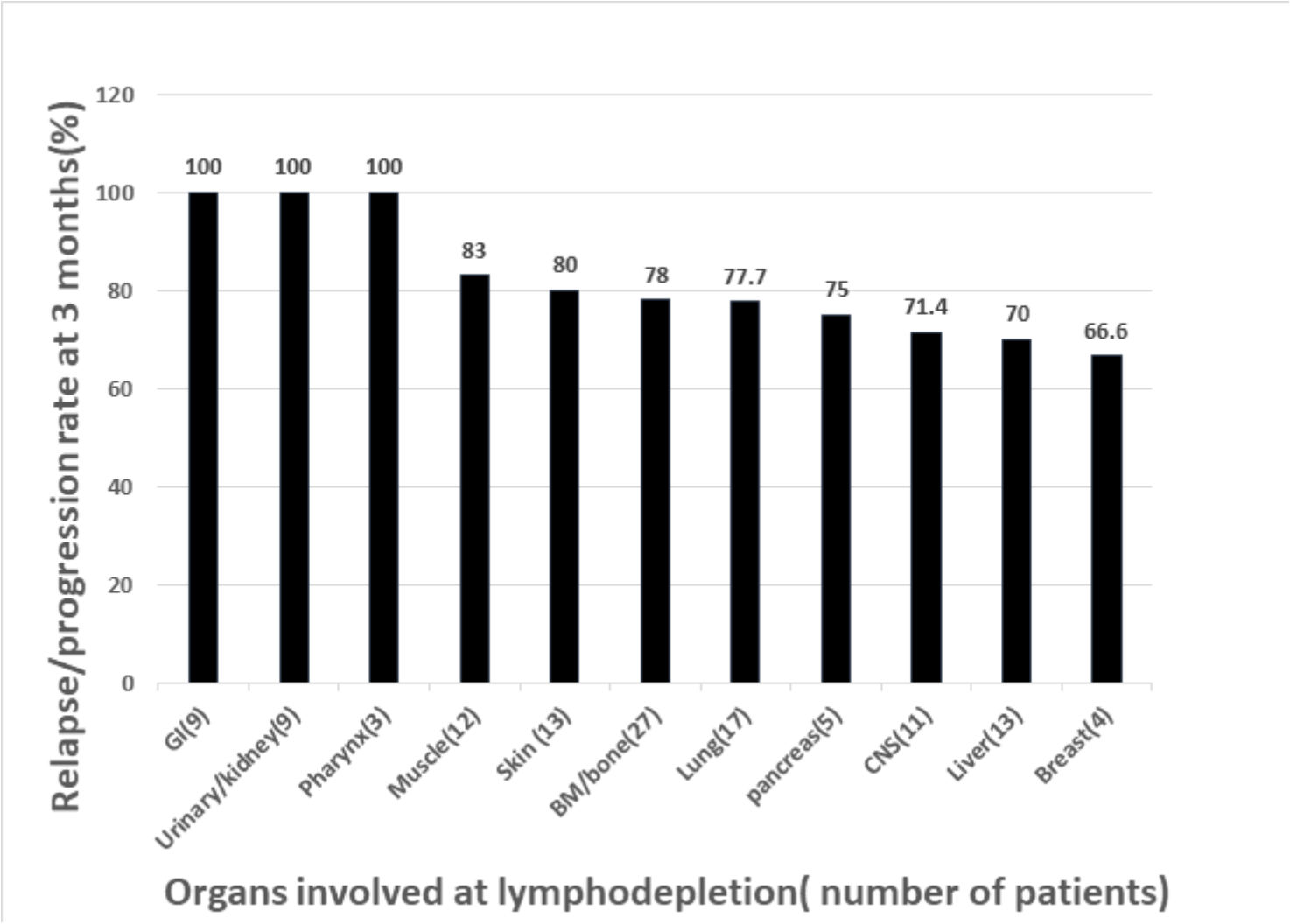

Figure 4

Response rates based on extra-nodal organ involved

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