SUPPLEMENTAL DATA

Durable Response After Tisagenlecleucel in Adults With Relapsed/Refractory Follicular Lymphoma: ELARA Trial Update

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METHODS

Biomarker analyses

Cytokine analysis: Approximately 5.0 mL of blood was collected at different time points for cytokine analysis. Serum was prepared within 4 hours of blood collection by centrifuging the samples at 860 RCF for 10 minutes and stored at –70°C. Tubes containing 500 µL frozen serum aliquots were shipped to BioAgilytix (Durham, NC) for analysis via pro-inflammatory multiplex panel according to manufacturer's instructions.

T-, B-, and natural killer cell counts: Approximately 5.0 mL of blood was collected at different time points in speckled Cyto-Chex® tubes and sent ambient to LabCorp for the measurement of T-, B-, and natural killer cell counts via flow cytometry.

Immunophenotyping: Approximately 5.0 mL of blood was collected for the immunophenotypic characterization by flow cytometry. Immunophenotyping was performed by Navigate BioPharma (Carlsbad, CA). Proportions of T-cell subsets, including naïve T cells, were identified by maturation markers (CCR7/CD45RA/CD45RO) and then correlated with clinical outcome. Naïve CD8+ T cells were separated into high vs low groups by using 3.5% as cutoff.

Fluorescent immunohistochemistry analysis: Formalin-fixed paraffin-embedded blocks and/or 4-µm-thick slides were sent to Navigate BioPharma (Carlsbad, CA) for the quantification of CD19 as well as T-cell markers (e.g., CD3, LAG3) expression on baseline tumor biopsies. Fluorescent images were acquired on the PhenoImager HT (Akoya Biosciences) at ×20 using various channels, including DAPI, Opal 520, Opal

570, and Opal 620 depending on the biomarker. Images were analyzed by proprietary analysis algorithms AQUA®.[1,2] LAG3 positive CD3 T cells were separated into high (highest quantile) vs low (three lower quantiles) groups by using 3% as cutoff.

Immunohistochemistry analysis: Quantification of CD19 baseline expression baseline tumor biopsies (formalin-fixed paraffin-embedded blocks and/or 4-µm-thick slides) was performed using immunohistochemistry method. Samples were sent to NeoGenomics Laboratories (Aliso Viejo, CA) and tested with CD19 antibody (Dako, M7296) on the Ventana Ultra platform according to vendor instructions.

Timing of archival patient tumor biopsies samples for LAG3+ progression-free survival/duration of response analysis

Of the 97 patients infused with tisagenlecleucel, 96 submitted central biopsies for analysis. One additional patient did not submit central sample; however, local assessment confirmed follicular lymphoma grade 3A and archival tumor biopsy from 2014 diagnosis was available at site. Thirty-five patients submitted newly obtained tumor biopsies (i.e., collected after patient informed consent was obtained) and 61 submitted archival tumor biopsies (i.e., collected prior to ELARA screening). For archival samples, the median time from biopsy to tisagenlecleucel infusion was 127 days (interquartile range [IQR], 98-210 days), with only 13 patients having a biopsy collected >1 year prior to tisagenlecleucel infusion. For all infused patients, the median time from biopsy to tisagenlecleucel infusion was 108 days (IQR, 81-166 days).

Statistical analyses

Efficacy outcomes were measured in all patients who received infusion of tisagenlecleucel and had measurable disease at baseline as per independent review committee. Safety outcomes were assessed in all patients who received tisagenlecleucel. The cellular kinetic analysis set included all patients who received tisagenlecleucel infusion and provided ≥1 cellular kinetic parameter.

RESULTS

Baseline characteristics

Baseline characteristics for all patients who received tisagenlecleucel infusion are shown in **Supplemental Table 1.**

Efficacy

Among all patients in the efficacy analysis set, the overall response rate was 90.4% (85/94; 95% CI, 82.6-95.5) according to local assessment, with a complete response rate of 73.4% (69/94; 95% CI, 63.3-82.0). The concordance rate between local and independent review committee assessments was high (86.2%).

Safety

Similar to previous reports, of the 97 patients evaluated for safety, 99% (96/97) of patients experienced any-grade adverse events (AEs) at any time post infusion and 81.4% (79/97) patients had grade ≥3 AEs, most commonly neutropenia (43.3%). The incidence of any-grade cytokine release syndrome (CRS) was 48.5% (47/97) and no

CRS grade ≥3 any time post infusion was suspected to be tisagenlecleucel related. One patient died (Day 375) after developing hemophagocytic lymphohistiocytosis (HLH) >1 year post tisagenlecleucel infusion. However, the patient did not have CRS during or immediately preceding HLH.

Treatment-related grade ≥3 AEs occurred in 48.5% (47/97) patients. Serious AEs were experienced by 46.4% (45/97) of patients and serious AEs suspected to be related to study drug were reported in 28.9% (28/97) of patients. Any-grade and grade ≥3 AEs of special interest were experienced by 95.9% (93/97) and 79.4% (77/97) patients, respectively, regardless of tisagenlecleucel relationship any time post infusion. All-grade and grade ≥3 AEs of special interest suspected to be related to tisagenlecleucel any time post infusion were reported in 75.3% (73/97) and 46.4% (45/97) patients, respectively.

Neurological Events: Any-grade serious neurological events any time post infusion, regardless of tisagenlecleucel relationship, included encephalopathy (3.1%), tremor (3.1%), dyskinesia (1%), and muscular weakness (1%). Any-grade serious neurological adverse reactions any time post infusion, regardless of tisagenlecleucel relationship, occurred in 12 (12.4%) patients; 3 (3.1%) experienced grade ≥3 (**Supplemental Table 3**). Median onset from infusion to first neurological event was 8.5 days (range, 4-345) post infusion. Immune effector cell-associated neurotoxicity syndrome of any grade occurred in 4/97 (4.1%) patients, while 1 patient had grade ≥3 immune effector cell-associated neurotoxicity syndrome (**Supplemental Table 3**).

Deaths: A total of 13 (13.4%) deaths that occurred >30 days after tisagenlecleucel infusion have been reported (study indication, n=7; other causes, n=6 [HLH on Day 375, euthanasia due to worsening neurological symptoms in a patient with possible progressive multifocal leukoencephalopathy on Day 302, post-allogenic stem cell transplant complications on Day 1049, urothelial bladder cell carcinoma (G3) on Day 784, metastatic squamous cell carcinoma on Day 897; and pneumonia on Day 721). No deaths occurred within 30 days after tisagenlecleucel infusion.

REFERENCES

1. Dolled-Filhart M, Gustavson M, Camp RL, et al: Automated analysis of tissue microarrays. *Methods Mol Biol*. 2010;664:151-162.
2. Johnson DB, Bordeaux J, Kim JY, et al: Quantitative spatial profiling of PD-1/PD-L1 interaction and HLA-DR/IDO-1 predicts improved outcomes of anti–PD-1 therapies in metastatic melanoma. *Clin Cancer Res*. 2018;24(21):5250-5260.

SUPPLEMENTAL TABLES

Supplemental Table 1. Baseline Patient Demographics, Disease History, and Clinical Characteristics

Parameter	Infused Patients (N=97)
Median age (range), years	57 (29-73)
≥65 years, n (%)	24 (24.7)
Male, n (%)	64 (66.0)
ECOG PS ≥1 prior to infusion, n (%)	42 (43.3)
Stage at study entry III-IV, n (%)	83 (85.6)
Bone marrow involvement, n (%)	37 (38.1)
Bulky disease,[a] n (%)	63 (64.9)
FLIPI high at study entry (≥3), n (%)	58 (59.8)
Median no. of prior therapies (range)	4 (2-13)
≥3 lines	73 (75.2)
≥5 lines	27 (27.8)
POD24 from first anti-CD20 mAb-containing therapy,[b] n (%)	61 (62.9)
Refractory disease[c] to last line of therapy, n (%)	76 (78.3)
Best response SD/PD	
Refractory to ≥2 regimens, n (%)	69 (71.1)
Double refractory[d]: Anti-CD20 mAb + alkylating agent	66 (68.0)

Parameter	Infused Patients (N=97)
Refractory to PI3K inhibitors	14 (14.4)
Prior autologous HSCT, n (%)	35 (36.1)
Relapsed ≤12 months after autologous HSCT	15 (15.5)
Median DOR from last line of therapy, months (n=35)	7.5
Received bridging chemotherapy	44 (45.4)

[a]Bulky disease defined per IRC as imaging showing any nodal or extranodal tumor mass that is >7 cm in diameter or involvement of at least 3 nodal sites, each with a diameter >3 cm. [b]POD24: Patients primary refractory or experiencing progression of disease within 24 months from initiation of a first-line anti-CD20 mAb-containing treatment. [c]Refractory is defined as failure to respond to previous treatment (SD/PD as best response) or PD within 6 months of prior therapy completion. [d]Double refractory is defined as no response or progressed <6 months after treatment with monoclonal anti-CD20 antibodies and alkylating agents.

CD, cluster of differentiation; DOR, duration of response; ECOG PS, Eastern Cooperative Oncology Group performance status; FLIPI, Follicular Lymphoma International Prognostic Index; HSCT, hematopoietic stem cell transplant; IRC, independent review committee; mAb, monoclonal antibody; PD, progressive disease; PI3K, phosphatidylinositol 3-kinase; POD24, progression of disease within 24 months from first immunochemotherapy; SD, stable disease.

Supplemental Table 2. Best Overall Response

	IRC Assessment
Best Overall Response, n (%)	
Overall response rate (ORR: CR+PR)	81 (86.2) 95% CI, 77.5-92.4
CR	64[a] (68.1) 95% CI, 57.72-77.3
PR	17 (18.1)
SD	3 (3.2)
PD	9 (9.6)
UNK[b]	1 (1.1)

[a]One patient in CR downgraded to PR due to a determination that their confirmatory bone marrow test was performed outside of the strict 14-day testing window, per protocol. [b]This patient received a lower dose than the assigned range of CAR-positive viable T cells. The investigator started a new anticancer treatment before Month 3.

CAR, chimeric antigen receptor; CR, complete response; IRC, independent review committee; ORR, overall response rate; PD, progressive disease; PR, partial response; SD, stable disease; UNK, unknown.

Supplemental Table 3. COVID-19 AEs, Anytime Post Infusion

	All Patients,[a] N=97	
	All Grades, n (%)	Grade ≥3, n (%)
COVID-19	8 (8.2)	3 (3.1)
COVID-19 pneumonia	1 (1.0)	1 (1.0)
Post-acute COVID-19 syndrome	1 (1.0)	0

[a]All patients infused with tisagenlecleucel.

AE, adverse event.

Supplemental Table 4. Selected AESI, Anytime Post Infusion Suspected to Be Related to Tisagenlecleucel

	All Patients,[a] N=97	
	All Grades, n (%)	Grade ≥3, n (%)
No. of patients with at least 1 AE	73 (75.3)	45 (46.4)
CRS[b,c]	47 (48.5)	0
Hematological disorders including cytopenias	45 (46.4)	43 (44.3)
Neutropenia	23 (23.7)	23 (23.7)
Anemia	13 (13.4)	7 (7.2)
Thrombocytopenia	6 (6.2)	5 (5.2)
Infections	16 (16.5)	9 (9.3)
Hypogammaglobulinemia	11 (11.3)	1 (1)
Serious neurological adverse events	8 (8.2)	2 (2.1)
ICANS	4 (4.1)	1 (1)
Encephalopathy	3 (3.1)	1 (1)
Dyskinesia	1 (1)	0
Muscular weakness	1 (1)	0
Tremor	1 (1)	0

[a]All patients infused with tisagenlecleucel. [b]CRS was graded using Lee scale 2014. [c]Refers to first CRS episode only.

AE, adverse event; AESI, AE of special interest; CRS, cytokine release syndrome; ICANS, immune effector cell-associated neurotoxicity syndrome.

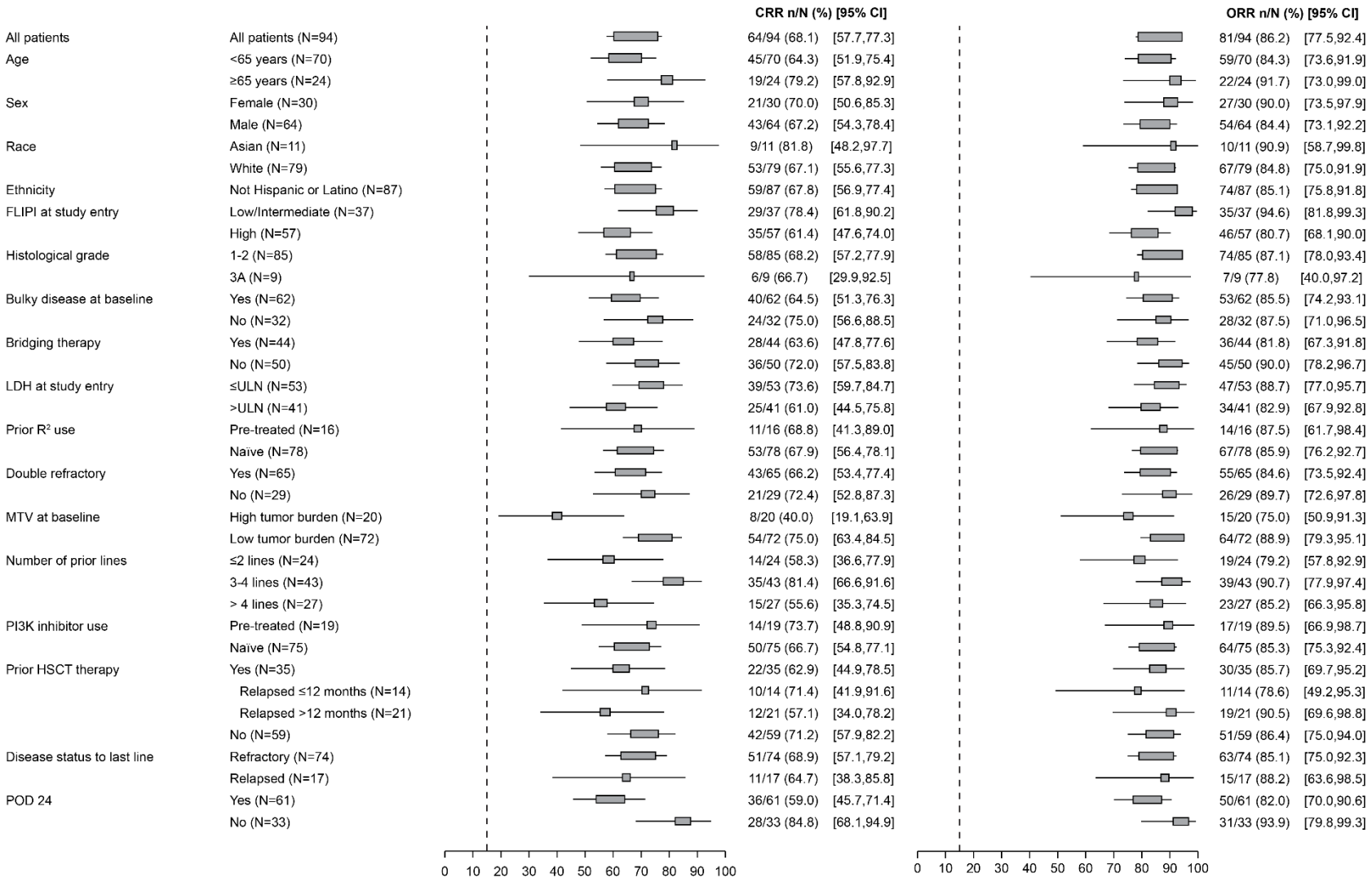
Supplemental Table 5. Summary of Cellular Kinetic Parameters by Response at Month 3

Parameter	Statistics	CR/PR (N=78)	SD/PD/Unknown (N=13)	All Patients (N=91)
$AUC_{0\text{-}28d}$, (copies/µg × days)	n	67	9	76
	Geo-mean	51,600	20,100	46,200
	Geo-CV%	308	7220	454
	Fold difference (responders over nonresponders)		2.6	
$AUC_{0\text{-}84d}$, (copies/µg × days)	n	66	8	74
	Geo-mean	80,500	67,100	78,900
	Geo-CV%	273	555	289
	Fold difference (responders over nonresponders)		1.2	
$AUC_{0\text{-}180d}$, (copies/µg × days)	n	64	6	70
	Geo-mean	105,000	89,200	104,000
	Geo-CV%	250	496	260
	Fold difference (responders over nonresponders)		1.2	
C_{max}, copies/µg	n	71	11	82
	Geo-mean	4950	2100	4410
	Geo-CV%	472	1610	565
	Fold difference (responders over nonresponders)		2.4	
T_{max}, days	n	71	11	82
	Median	10.0	13.1	10.7
	Min, max	1.91, 562	7.73, 54.8	1.91, 562
T_{last}, days	n	75	12	87
	Median	336	107	210
	Min, max	13.0, 925	18.7, 918	13.0, 925

n, number of patients with nonmissing values. Geo-CV% = sqrt (exp (variance for log transformed data)-1) × 100.

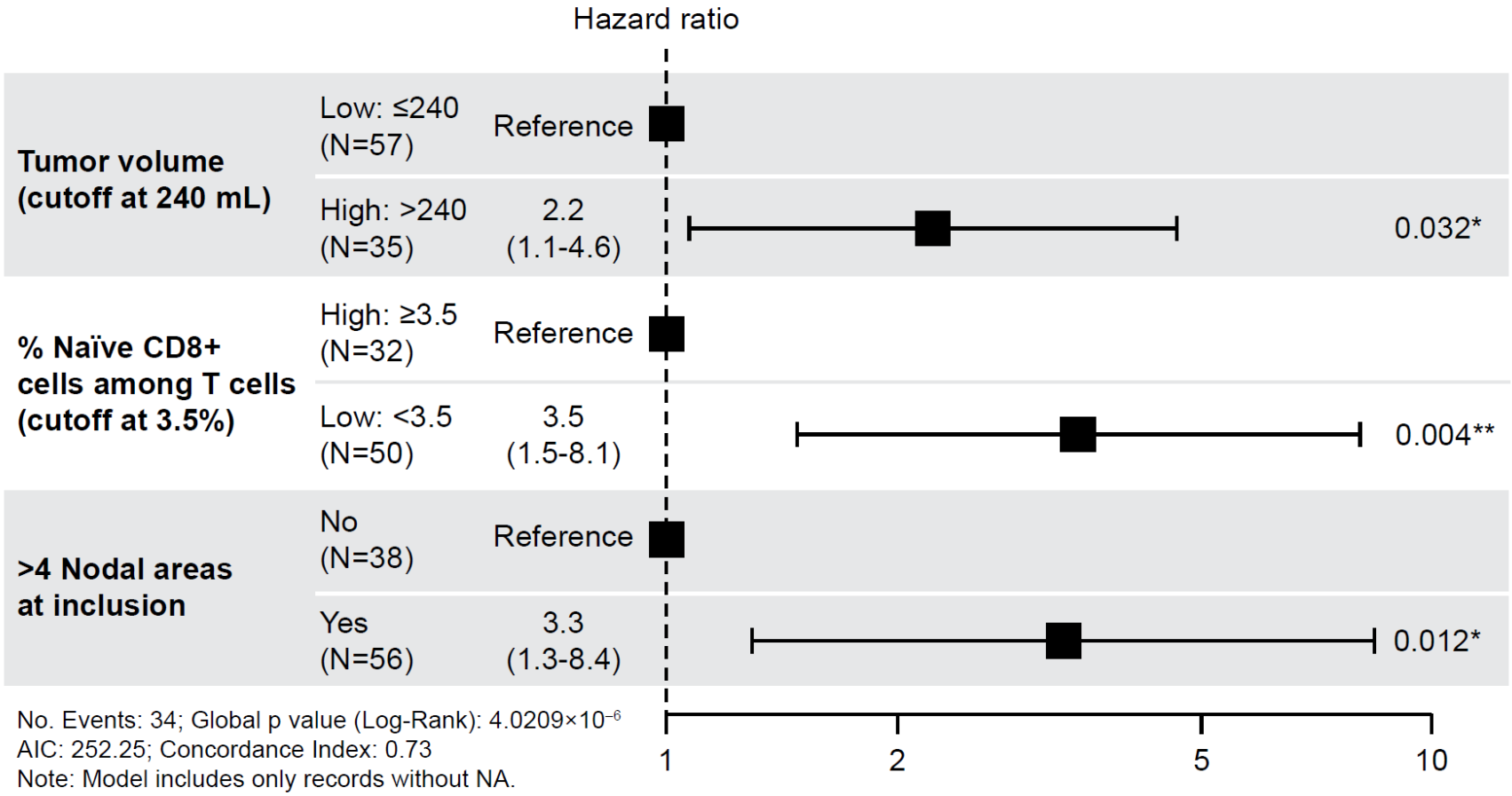
AUC, area under the curve; C_{max}, maximum transgene level; CR, complete response; CV, coefficient of variation; exp, exponent; Geo, geometric; max, maximum; min, minimum; PD, progressive disease; PR, partial response; SD, stable disease; T_{last}, time to last quantifiable concentration following dosing; T_{max}, time to maximum transgene level.

SUPPLEMENTAL FIGURES



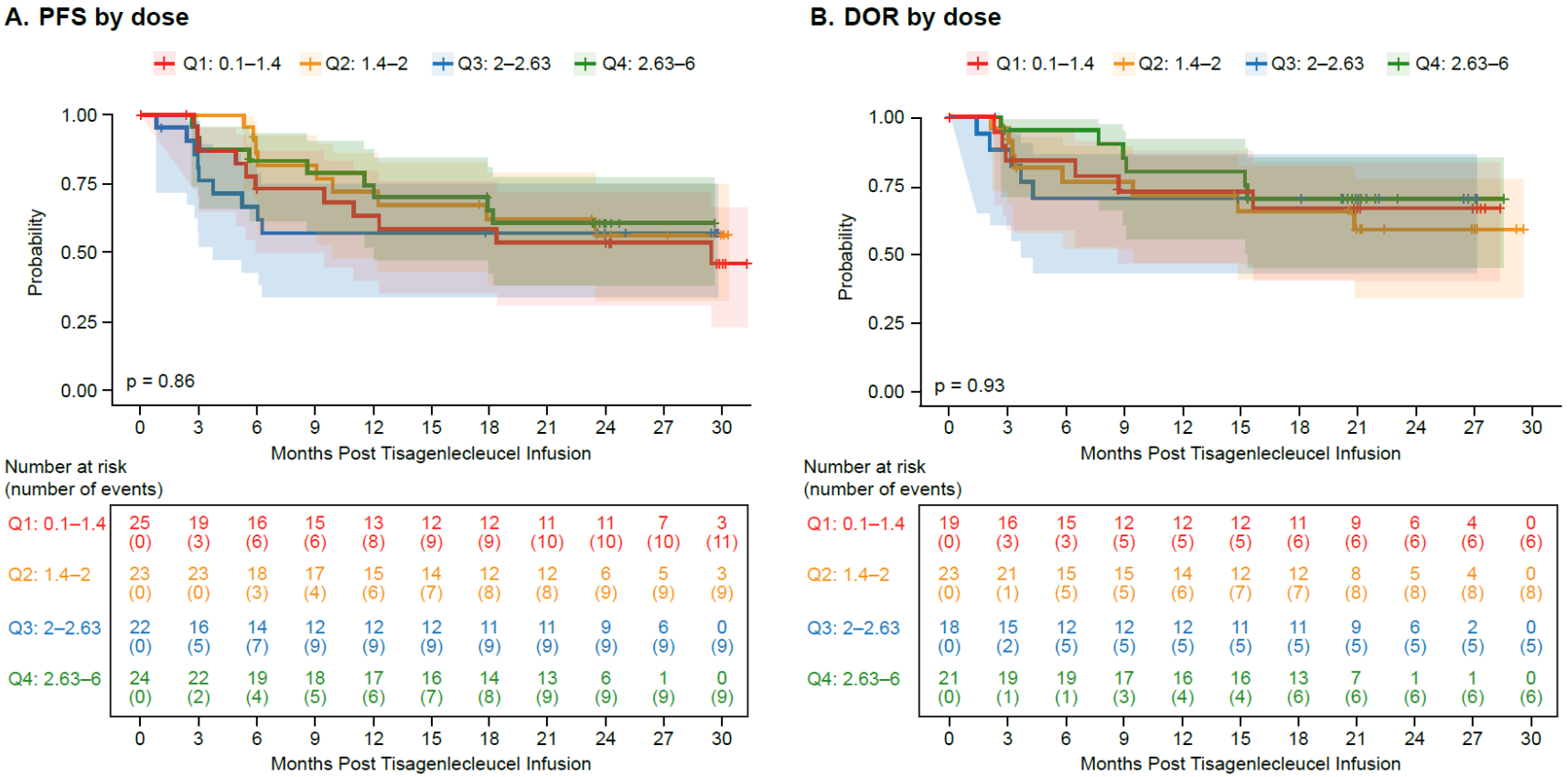
Supplemental Figure 1. Best response of CRR and ORR according to subgroup (EAS population).

Forest plot showing the effect of tisagenlecleucel treatment across major demographic and prognostic subgroups. Red boxes indicate key findings in high-risk prognostic subgroups in relation to CRR and ORR. [a]Patients primarily refractory or experiencing progression of disease within 24 months from initiation of a first-line anti-CD20 mAb-containing treatment. CD, cluster of differentiation; CRR, complete response rate; EAS, efficacy analysis set; FLIPI, Follicular Lymphoma International Prognostic Index; HSCT, hematopoietic stem cell transplant; LDH, lactate dehydrogenase; mAb, monoclonal antibody; MTV, metabolic tumor volume; ORR, overall response rate; PI3K, phosphatidylinositol 3-kinase; POD24, progression of disease within 24 months from first immunochemotherapy; R^2, lenalidomide + rituximab; ULN, upper limit of normal; US, United States.



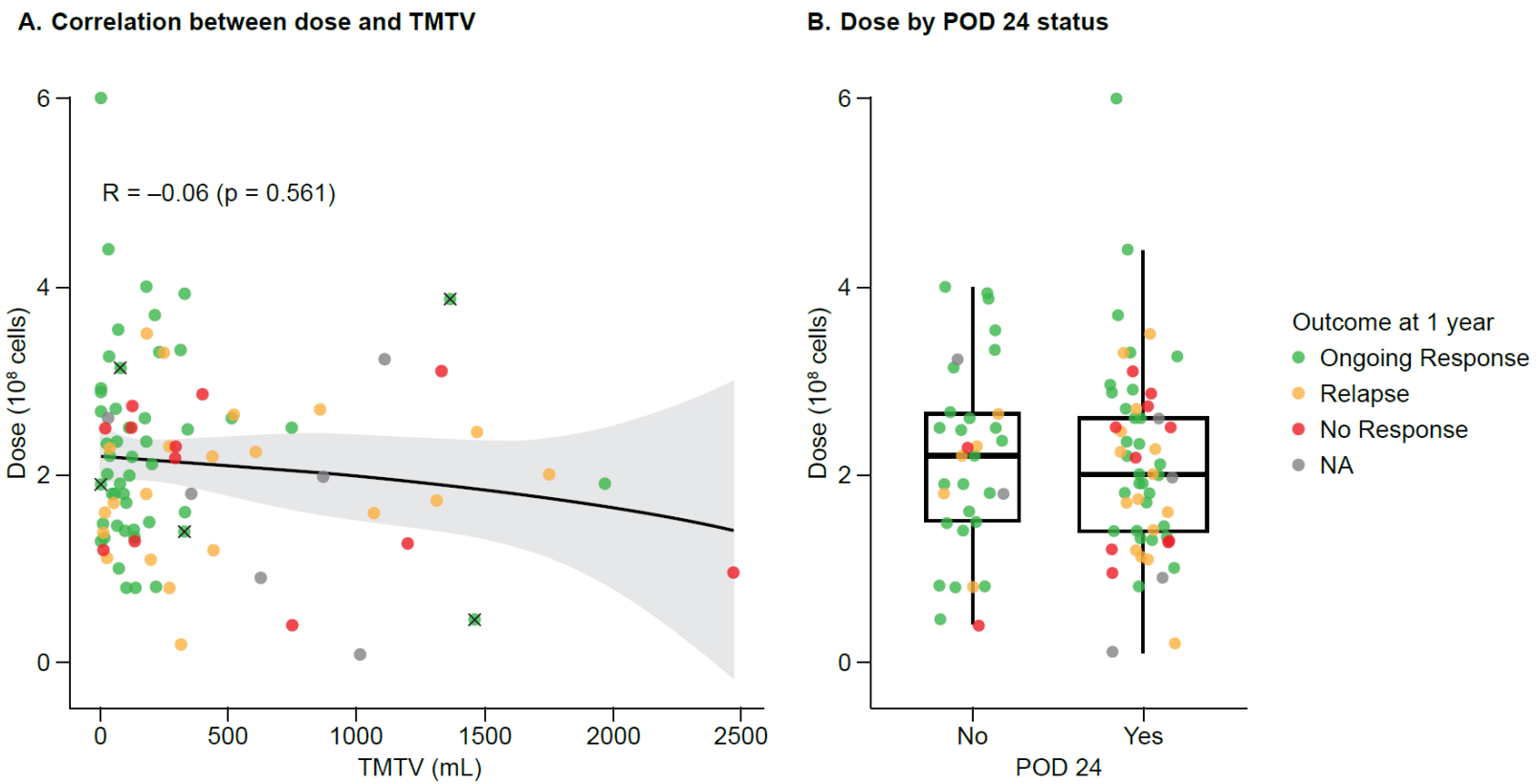
Supplemental Figure 2. Multivariate analysis of clinical factors significantly associated with PFS.

AIC, Akaike information criteria; CD, cluster of differentiation; NA, not available; PFS, progression-free survival.



Supplemental Figure 3. Dose was not significantly associated with clinical outcomes.

(**A**) PFS by dose. (**B**) DOR by dose. DOR, duration of response; PFS, progression-free survival; Q, quarter.



Supplemental Figure 4. Dose did not show strong correlations with baseline variables.

(A) Correlation between dose and TMTV. (B) Dose by POD 24 status. NA, not available; POD 24, progression of disease within 24 months from first immunochemotherapy; TMTV, total metabolic tumor volume.