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Prognostic value of plasma thymus- and activation-regulated chemokine levels after the second cycle of chemotherapy in pediatric Hodgkin lymphoma

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Running head: Interim TARC predicts outcome in pediatric cHL

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Data sharing statement:

Summary data supporting the findings are provided in the supplementary material. Additional data are available from the corresponding author upon reasonable request.

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To the editor:

Thymus- and activation-regulated chemokine (TARC) is a well-established biomarker in classical Hodgkin lymphoma (cHL), secreted by Hodgkin-Reed-Sternberg cells, causing plasma elevations correlating with disease burden, stage, and treatment response[1,2]. Despite its established role in adult cHL, the prognostic value of early-treatment TARC dynamics in pediatric patients remains unclear. Early response assessment (ERA) in pediatric cHL largely relies on imaging, particularly on interim positron emission tomography (PET). However, PET-based stratification has limitations and complementary biomarkers capable of improving early risk-stratification are needed. Therefore, we aimed to determine whether interim TARC levels predict relapse risk in pediatric cHL and provide independent prognostic information beyond established clinical factors.

Inclusion criteria were age ≤ 25 years and treatment with the EuroNet-PHL-C2 protocol, adopted by the Associazione Italiana di Ematologia e Oncologia Pediatrica (AIEOP) for frontline treatment of pediatric cHL. The EuroNet-PHL-C2 trial aimed to reduce radiotherapy after two OEPA induction cycles, omitting radiotherapy in low-risk patients (TL1) with adequate ERA-PET response (AR), and by comparing intensified (DECOPDAC21) versus standard (COPDAC28) consolidation in intermediate- and advanced-stage patients (TL2+3). Exclusion criteria were HIV positivity and atopic dermatitis. Our cohort showed a balanced sex distribution (male = 82, female = 86) and median age at diagnosis of 15.3 years (range 5.4-23.2). Baseline clinical and treatment-related characteristics of the study cohort were comparable with those of the remaining patients in the Italian PHL-C2 cohort not included in the present analysis (Table S1). Plasma TARC levels were measured in all available samples, although not all time-points were available for all patients. Blood samples from 168 patients were collected at diagnosis (DIA; n = 168), after second cycle of chemotherapy (II CT; n = 153), at end of therapy (ST; n = 30), at relapse (REL; n = 7), and at end of therapy after relapse (ST REL; n = 2). A general overview of the TARC kinetics in the total cohort can be found in Figure S1A. However, due to sample availability and limited event numbers, all inferential analyses were restricted to DIA and II CT. The study was conducted in accordance with the Declaration of Helsinki and approved by AIFA (Agenzia Italiana del Farmaco) on March 9, 2018 (EudraCT 2012-004053-88, EM-04) and by the Regional Unique Ethics Committee of Friuli Venezia Giulia on May 17, 2018 (EudraCT 2012-004053-88, EM-04). Informed consent was obtained from all subjects, or their legal guardians involved in the study.

Peripheral blood samples were collected in sodium citrate, processed within 24 hours by sequential centrifugation (820 × *g* for 10 min and 2,500 × *g* for 10 min) and stored at -80 °C until analysis. Plasma TARC concentrations were measured using a commercial ELISA (Human CCL17/TARC; Invitrogen, EHCCL17), with absorbance read at 450 nm and concentrations calculated by four-parameter logistic model. Samples were analyzed according to the laboratory sample identification order, independent of clinical characteristics. Whenever possible, serial samples from the same patient were measured on the same ELISA plate to minimize inter-assay variability. Plasma samples were thawed only once before analysis.

Statistical analyses were performed using SAS (version 9.4) and figures created using GraphPad Prism (version 8.0.2). Patients were stratified by relapse status, and comparisons between groups were performed using the Mann-Whitney U test. ROC analysis was performed to evaluate discriminative ability, and the optimal cut-off was defined as the maximum Youden index. TARC levels and clinical variables included in the study were analyzed according to progression-free survival (PFS). PFS was defined as the time from diagnosis to the first event of relapse, progression or death. Patients without events were censored at the last follow-up. PFS was estimated using the Kaplan-Meier method and compared using the log-rank test. Variables significantly associated with PFS in univariable analyses were included in a multivariable Cox proportional hazards model. Among the 168 patients of the study cohort, 20 experienced relapse or progressive disease with no death events during the study period.

At DIA, TARC levels were markedly elevated, with a median of 18,935.7 pg/mL (n = 168). Higher median baseline TARC levels at diagnosis were observed in patients who subsequently relapsed compared with those who remained in remission, although this difference did not reach statistical significance (23,693.4 pg/mL vs. 18,250 pg/mL; n = 20 vs. n = 148; p = 0.1124).

At II CT, TARC levels declined substantially in both groups, indicating response to treatment. However, patients who subsequently relapsed compared with those who remained in remission maintained significantly higher median TARC concentrations (777.6 pg/mL vs. 384.7 pg/mL; n = 17 vs. n = 136; p = 0.0033) (Figure 1A). Importantly, the relative reduction from baseline was comparable between groups (-96.7% vs. -97.9%), indicating that relative decline alone does not capture relapse risk (Figure S1B).

An exploratory ROC analysis at II CT identified a cut-off ≥ 757.4 pg/mL (AUROC = 0.7154; p = 0.0038) for relapse prediction (Figure 1B). Patients with TARC ≥ 757.4 pg/mL had a markedly inferior PFS compared with those below this threshold (59% vs. 94%; p < 0.0001) (Figure 1C). Median follow-up time was 4.15 years.

In univariable analyses, elevated TARC levels at II CT (≥ 757.4 pg/mL), bulky disease (tumor volume ≥ 200 mL), B-symptoms, inadequate ERA-PET response (IR) and higher treatment level were associated with inferior PFS. In a multivariable Cox proportional hazards model including established clinical risk factors, TARC ≥ 757.4 pg/mL at II CT remained an independent prognostic factor (hazard ratio (HR) = 6.1; 95% CI: 2.2-16.5; p = 0.0004), while bulky disease also retained independent significance (HR = 4.1; 95% CI: 1.3-12.8; p = 0.015) (Table 1). Other variables, including B-symptoms, treatment level and ERA-PET lost statistical significance in multivariable analysis. Survival curves for these markers can be found in Figure S1C-E.

Erythrocyte sedimentation rate (≥ 30 mm/h), histological subtype and extranodal involvement were not significant in univariable and multivariable analyses. Late-response-assessment-PET (LRA-PET) was performed only in patients with IR on ERA-PET. Therefore, the number of evaluable LRA-PET cases is limited by design and was not included in the analysis.

Patients with bulky disease had a lower 5-year PFS compared with those without (75% vs. 93%; p = 0.0006) (Figure 1D). Combining TARC levels at II CT and bulky disease improved risk stratification compared with either marker alone. Patients with both TARC ≥ 757.4 pg/mL and bulky disease had a markedly lower 5-year PFS (41%), whereas patients with only one risk factor showed intermediate risk (87%). In contrast, patients without either risk factor had an excellent 5-year PFS (97%; p < 0.0001), defining a clear three-tier risk model (Figure 1E).

Our findings identify plasma TARC levels at II CT as a strong independent prognostic biomarker in pediatric cHL providing information beyond conventional clinical risk factors. The combination of circulating TARC levels with bulky disease further improves risk stratification and may support early response-adapted treatment strategies. Complementary aspects of disease biology may be reflected by tumor volume (bulky disease) and biological tumor activity (circulating TARC levels)[3]. Despite marked reductions at II CT, absolute TARC levels remained higher in patients who subsequently relapsed, suggesting persistent disease activity not fully captured by relative decline.

Our findings are consistent with previous studies, linking elevated inflammatory and immune-related markers such as miR-122-5p, IL-6, and sCD30 to adverse disease features in cHL[4,5]. Integrating such liquid biopsy markers across treatment time-points may enable development of composite risk scores for response monitoring.

Given the exploratory nature of the ROC analysis, small cohort size, and absence of an independent validation cohort, the identified cut-off should be considered exploratory and requires validation in larger multicenter cohorts to confirm robustness and generalizability.

In conclusion, we identify a prognostic threshold based on TARC levels after II CT which may reflect disease activity independent of subsequent treatment stratification according to risk level and PET response assessment. Early TARC monitoring may refine response-adapted risk stratification and support timely treatment intensification.

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Table 1: Univariable log-rank (Mantel-Cox) and multivariable Cox proportional hazards analyses of clinical parameters and TARC plasma levels after two cycles of chemotherapy (II CT) for progression-free survival (PFS) defined as time from diagnosis to relapse, progression or death. Patients without events were censored at the last follow-up. AR, adequate response; CI, confidence interval; ERA-PET, early-response assessment by positron emission tomography; HR, hazard ratio; II CT, after the second cycle of chemotherapy; IR, inadequate response; ns, not significant; PFS, progression-free survival; SE, standard error; TARC, thymus- and activation-regulated chemokine.

				Univariable			Multivariable	
Characteristics		**Patients**	**PFS events**	**5-year PFS (%)**	**SE (%)**	**p-value**	**p-value**	**HR (95% CI)**
TARC at II CT (*15)	< 757.4 pg/mL	125	7	94	2	<0.0001	0.0004	6.1 (2.2-16.5)
	≥ 757.4 pg/mL	28	10	59	10			
Bulky disease (≥ 200 mL)	No	110	6	93	3	0.0006	0.015	4.1 (1.3-12.8)
	Yes	58	14	75	6			
Symptoms	A	101	6	93	3	0.0024	ns	
	B	67	14	77	5			
Treatment level	1+2	101 (26+75)	8	91	3	0.0366	ns	
	3	67	12	81	5			
ERA-PET (*4)	AR	118	10	90	3	0.0146	ns	
	IR	46	10	78	6			

*Missing values.

Figure 1: PFS analysis based on II CT TARC levels and bulky disease in pediatric cHL. (A) At II CT, TARC levels were significantly higher in patients who subsequently relapsed compared with those in CR ($p = 0.0033$). **(B)** ROC analysis demonstrated moderate discriminative performance of TARC for relapse prediction (AUROC = 0.7154, $p = 0.0038$). **(C)** 5-year PFS stratified by TARC level at II CT using a cut-off of 757.4 pg/mL, distinguishing patients with high (≥ 757.4 pg/mL; 59%) and low (< 757.4 pg/mL; 94%; $p < 0.0001$) TARC levels. **(D)** 5-year PFS stratified by bulky disease (≥ 200 mL), separating patients with bulky disease (75% PFS) from those without (93% PFS; $p = 0.0006$). **(E)** Combined risk stratification using II CT TARC and bulky disease identifies three risk groups: high risk (both risk factors; 41% PFS), intermediate risk (one risk factor; 87% PFS), and low risk (no risk factors; 97% PFS; $p < 0.0001$). PFS was defined as time from diagnosis to the first event of relapse, progression or death. Patients without an event were censored at last follow-up. AUROC, area under the receiver operating characteristic curve; cHL, classical Hodgkin lymphoma; CR, complete remission; II CT, after the second cycle of chemotherapy; PFS, progression-free survival; REL, relapse; ROC, receiver operating characteristic; TARC, thymus- and activation-regulated chemokine.

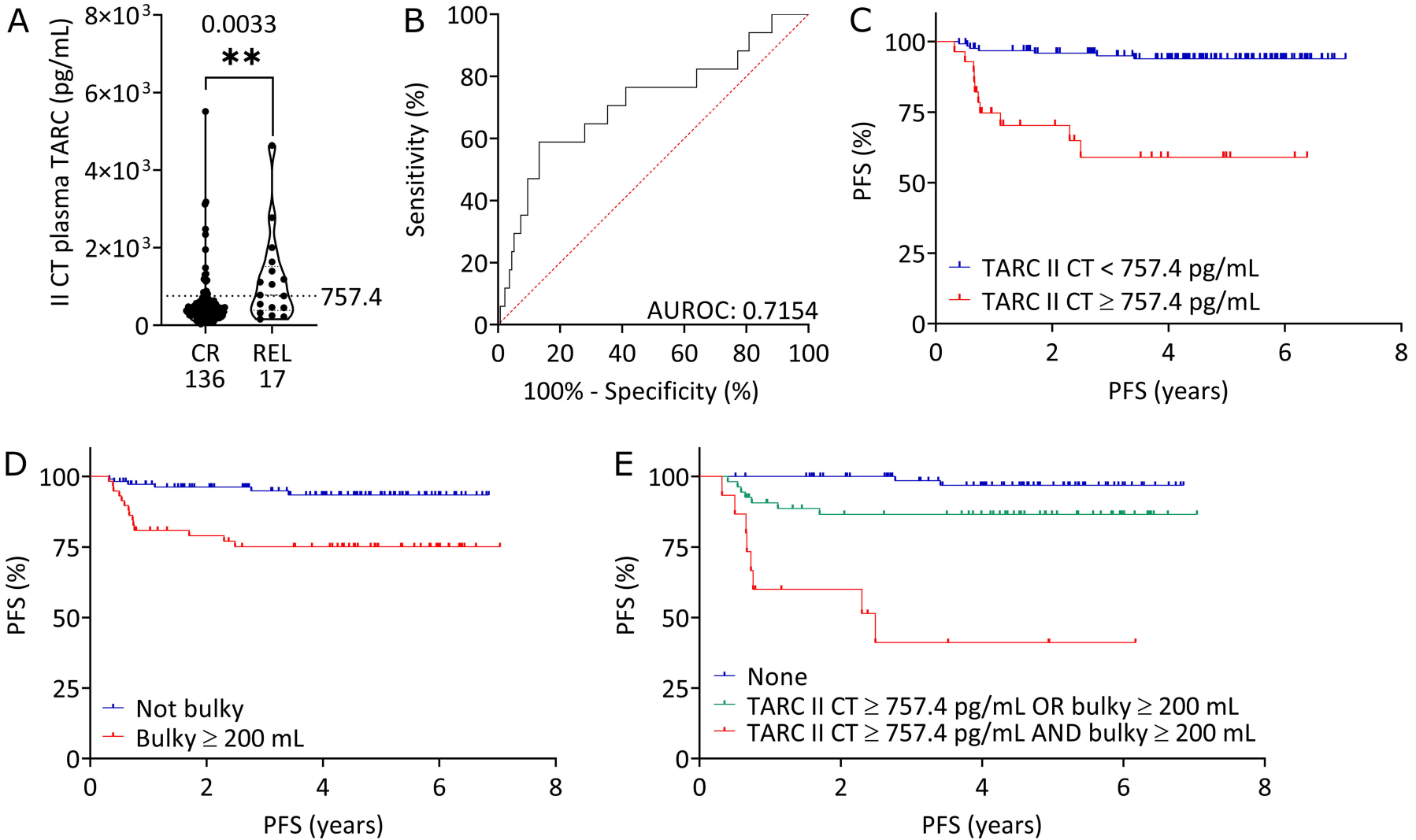
A
8×10³
6×10³
4×10³
2×10³
0
II CT plasma TARC (pg/mL)
0.0033
**
757.4
CR
136
REL
17
B
Sensitivity (%)
100
80
60
40
20
0
AUROC: 0.7154
0
20
40
60
80
100
100% - Specificity (%)
C
PFS (%)
100
75
50
25
0
TARC II CT < 757.4 pg/mL
TARC II CT ≥ 757.4 pg/mL
0
2
4
6
8
PFS (years)
D
PFS (%)
100
75
50
25
0
Not bulky
Bulky ≥ 200 mL
0
2
4
6
8
PFS (years)
E
PFS (%)
100
75
50
25
0
None
TARC II CT ≥ 757.4 pg/mL OR bulky ≥ 200 mL
TARC II CT ≥ 757.4 pg/mL AND bulky ≥ 200 mL
0
2
4
6
8
PFS (years)

Table S1: Baseline characteristics in the study cohort compared with the Italian PHL-C2 patients not included in the study. The study cohort (n = 168) is a subset of the entire Italian PHL-C2 cohort (n = 554). All p-values therefore compare the study cohort with the 386 patients not included in the study, which are mutually exclusive groups. The entire Italian PHL-C2 cohort columns (554 = 168 + 386) are reported for descriptive purposes only and were not used for hypothesis testing. All comparisons were done with the two-sided Fisher's exact test. AR, adequate response; ERA-PET, early-response assessment by positron emission tomography; IR, inadequate response.

		Italian EuroNet-PHL-C2 cohort (n = 554)	Study cohort (n = 168)	Italian EuroNet-PHL-C2 - study cohort (n = 386)	
Characteristic		Patients n (%)			p-value
Bulky disease (≥ 200 mL)	No	328 (59.5)	110 (65.5)	218 (56.9)	0.073
	Yes	223 (40.5)	58 (34.5)	165 (43.1)	
Symptoms	A	333 (60.1)	101 (60.1)	232 (60.1)	1.000
	B	221 (39.9)	67 (39.9)	154 (39.9)	
Treatment level	1+2	337 (60.8)	101 (60.1)	236 (61.1)	0.850
	3	217 (39.2)	67 (39.9)	150 (38.9)	
ERA-PET	AR	376 (68.6)	118 (72.0)	258 (67.2)	0.315
	IR	172 (31.4)	46 (28.0)	126 (32.8)	

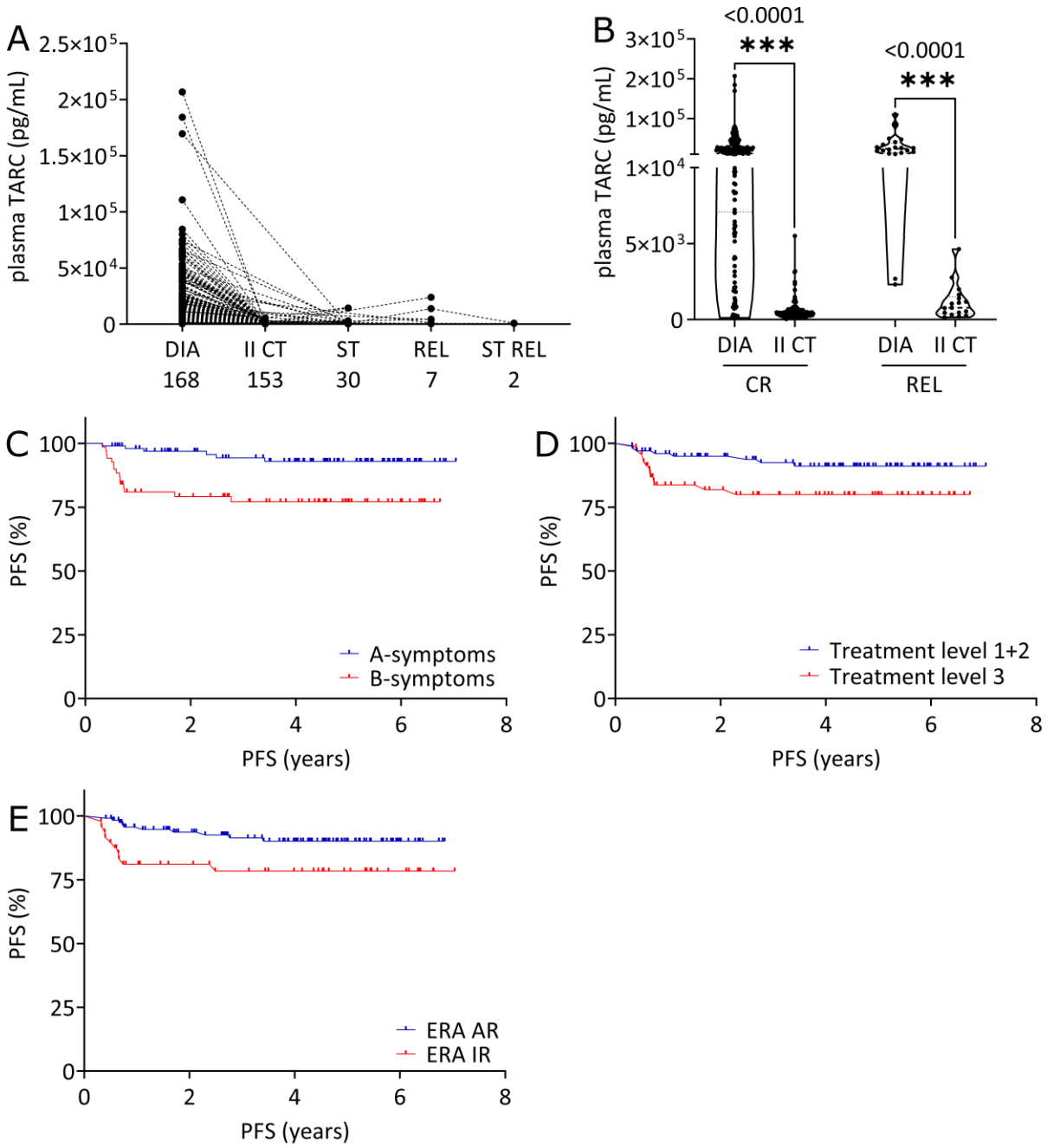

Figure S1: (A) TARC dynamics in the overall pediatric cohort at DIA, after II CT, at ST, at relapse (REL), and at end of therapy after relapse (ST REL). (B) TARC levels at DIA and II CT in patients who remained in continuous remission compared with those who relapsed (DIA: n = 148 vs. n = 20; II CT: n = 136 vs. n = 17). (C-E) 5-year PFS according to clinical risk factors that were significant in univariable log-rank (Mantel-Cox) analyses but not in multivariable Cox proportional hazards models: A vs. B symptoms (93% vs. 77%; p = 0.0024), treatment level 1+2 vs. 3 (91% vs. 81%; p = 0.0366) and ERA-PET response (90% vs. 78%; p = 0.0146; 4 missing values). AR, adequate response; CR, complete remission; DIA, day of diagnosis; ERA-PET, early-response assessment by positron emission tomography; II CT, after the second cycle of chemotherapy; IR, inadequate response; PFS, progression-free survival; REL, relapse; ST, stop therapy; ST REL, stop therapy after relapse; TARC, thymus- and activation-regulated chemokine.