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Early Increase in Peripheral Lymphocyte Count Predicts Objective Response and Progression-Free Survival in Advanced Hepatocellular Carcinoma Treated with Durvalumab Plus Tremelimumab

M Rojas[1*], L Acosta[1], E Brito[1]

[1]Department of Medical Oncology, School of Medicine, University of Caracas, Caracas, Venezuela.

***E-mail** ✉ caracas.medonc.83@outlook.com

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ABSTRACT

The combination therapy of Durvalumab and Tremelimumab (Dur/Tre) has shown promise in treating advanced hepatocellular carcinoma (HCC). Nonetheless, reliable factors that predict therapeutic benefit and survival outcomes remain largely undefined. This study retrospectively assessed variables influencing clinical outcomes and treatment response in HCC patients receiving Dur/Tre. A total of 30 patients were retrospectively analyzed in this single-institution study to identify indicators of Dur/Tre effectiveness in advanced HCC. Clinical parameters associated with the objective response rate (ORR), progression-free survival (PFS), overall survival (OS), and immune-mediated adverse events (imAEs) were evaluated. Particular emphasis was placed on the two-week change in circulating lymphocyte count from baseline (Δlymphocyte). Seventeen participants (56.7%) presented with BCLC stage C disease. The median observation period was 11 months. The ORR reached 30.0%, and the disease control rate (DCR) was 53.3%. Median PFS was 3.7 months, while OS had not yet been determined. A higher Δlymphocyte was independently correlated with improved objective response (hazard ratio [HR] 1.004; p = 0.016). Patients with Δlymphocyte ≥ +245/μL exhibited significantly longer PFS (HR 0.308; 95% CI 0.095-0.998; p = 0.049), with median PFS not reached compared to 1.96 months in those below +245/μL (log-rank p = 0.003). An early rise in lymphocyte count following Dur/Tre administration appears to be a strong indicator of favorable therapeutic response and prognosis in advanced HCC.

Keywords: Durvalumab, Tremelimumab, Hepatocellular carcinoma, Lymphocyte dynamics, Prognostic biomarker, Immune checkpoint inhibitor

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Introduction

Hepatocellular carcinoma (HCC) is one of the most prevalent cancers and ranks sixth among the leading causes of cancer mortality globally [1]. For patients with unresectable or metastatic disease, systemic approaches such as molecular targeted agents (MTAs) and immune checkpoint inhibitors (ICIs) now represent standard management options [2]. The multikinase inhibitor sorafenib was the first MTA approved for advanced HCC, achieving modest objective responses but a relatively high disease control rate [3]. Regorafenib later became available as a second-line treatment, extending overall survival in cases progressing on sorafenib [4, 5]. Lenvatinib followed as a first-line alternative, offering similar OS but superior response and progression-free survival compared to sorafenib [6, 7]. Additional second-line options include ramucirumab [8] and cabozantinib [9].

Checkpoint inhibitors have further reshaped systemic therapy for HCC, offering durable and occasionally dramatic responses. The IMbrave150 trial demonstrated that combining the PD-L1 inhibitor atezolizumab with the VEGF inhibitor bevacizumab improved overall survival relative to sorafenib [10, 11]. Likewise, the HIMALAYA study showed that Durvalumab (anti-PD-L1) plus Tremelimumab (anti-CTLA-4) extended survival compared with sorafenib monotherapy [12, 13].

However, predictive biomarkers for ICI response in advanced HCC remain elusive. While PD-L1 expression serves as a response marker in some cancers [14], its prognostic relevance in HCC is inconsistent. Prior work has suggested that the pretreatment neutrophil-to-lymphocyte ratio (NLR) can forecast outcomes in patients treated with Atezolizumab plus Bevacizumab (Atez/Bev) [15-17]. Moreover, NLR changes early in therapy have been linked to treatment response and prognosis [18].

The STRIDE regimen (Single Tremelimumab Regular Interval Durvalumab) — which employs a one-time high priming dose of Tremelimumab — is a defining feature of Dur/Tre therapy. Evidence indicates that peripheral lymphocyte numbers, particularly proliferating CD8+ T cells (Ki67+), rise by day 15 post-treatment and correlate with response [19]. Based on this, we postulated that lymphocyte alterations during Dur/Tre therapy could reflect treatment efficacy more clearly than in Atez/Bev therapy.

Thus, this study analyzed early changes in peripheral lymphocyte counts—specifically at baseline and two weeks after starting Dur/Tre—and their association with therapeutic outcomes and prognosis in patients with advanced HCC.

Materials and Methods

Patient selection

This retrospective investigation was conducted at a single institution and included individuals with advanced hepatocellular carcinoma (HCC) who underwent Durvalumab plus Tremelimumab (Dur/Tre) therapy between March 2023 and October 2024. The diagnosis of HCC was established through contrast-enhanced computed tomography (CT) or Gd-EOB-DTPA magnetic resonance imaging (MRI), and in some cases, confirmed by liver biopsy. Macrovascular invasion was defined as tumor thrombus within the portal vein, hepatic vein, or bile duct.

A total of 32 patients received Dur/Tre, including 12 who had previously been treated with Atezolizumab plus Bevacizumab (Atez/Bev). Two patients were excluded due to a follow-up duration shorter than 30 days or the absence of post-treatment imaging evaluation using CT or Gd-EOB-DTPA MRI. Thus, the final cohort comprised 30 patients with unresectable HCC. The start of Dur/Tre therapy was considered the beginning of follow-up, while the end was marked by either the last recorded visit (for surviving patients) or death during observation. Data collection was censored on 28 October 2024.

Treatment protocol and response evaluation

Treatment decisions regarding Dur/Tre initiation were made by the attending physician according to the Japan Society of Hepatology (JSH) treatment algorithm [20]. The standard Dur/Tre regimen included Durvalumab 1500 mg combined with Tremelimumab 300 mg on day 1, followed by Durvalumab 1500 mg every four weeks, in accordance with the manufacturer's protocol.

Initial response assessment was conducted approximately 4-8 weeks after treatment initiation using contrast-enhanced CT and/or Gd-EOB-DTPA MRI, taking into account tumor marker trends and patient condition. Tumor responses were categorized following the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 [21]:

Complete Response (CR): disappearance of all measurable lesions.

Partial Response (PR): ≥30% reduction in total target lesion diameters from baseline.

Progressive Disease (PD): ≥20% increase in total diameters relative to the smallest sum observed (including baseline if lowest), with an absolute increase of ≥5 mm, or appearance of new lesions.

Stable Disease (SD): changes insufficient for PR or PD classification.

The Objective Response Rate (ORR) represented the sum of CR and PR, while the Disease Control Rate (DCR) included CR, PR, and SD. Progression-Free Survival (PFS) was defined as the interval from Dur/Tre initiation to confirmed PD (per RECIST v1.1) or death from any cause. Cases without progression at last contact were censored at the latest follow-up. Overall Survival (OS) was measured from therapy initiation until death or the final follow-up date.

Laboratory assessments

Routine hematologic and biochemical tests were performed using standardized procedures. Ratios including the neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), and platelet-to-lymphocyte ratio (PLR) were calculated using peripheral blood cell counts. The albumin-bilirubin (ALBI) score was computed

based on serum albumin and total bilirubin levels according to the formula: ALBI score = ($\log_{10}$ bilirubin [μmol/L] × 0.66) + (albumin [g/L] × −0.085) as described previously [22].

Adverse event evaluation

Treatment-related adverse events were classified using the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 (https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm, accessed 31 October 2024). Immune-mediated adverse events (imAEs) were diagnosed at the discretion of the treating physician. Dur/Tre was discontinued if patients developed severe imAEs or showed radiologic or clinical disease progression. After cessation, the attending physician decided on subsequent therapeutic strategies.

Statistical analysis

Continuous variables were expressed as median with interquartile range (IQR). Chi-square or Fisher's exact tests were used for categorical comparisons of ORR and DCR. Spearman's rank correlation was applied to assess relationships among variables. PFS and OS were analyzed via the Kaplan-Meier method, with comparisons performed using the log-rank test. Predictive factors for ORR were examined by logistic regression, while Cox proportional hazards models were used for PFS and OS. Variables with $p < 0.05$ in univariate analysis were subsequently tested in multivariate analysis.

The prognostic parameters included age, sex, treatment line (first or later), presence of imAEs, liver disease etiology (viral/non-viral), AFP level (≥400 vs. <400 ng/mL), white blood cell count, lymphocyte, monocyte, and neutrophil counts, as well as NLR, LMR, and PLR at baseline. The same indices were also evaluated two weeks after Dur/Tre administration (NLR2w, LMR2w, PLR2w), along with the absolute changes in neutrophil (Δneutrophil), monocyte (Δmonocyte), and lymphocyte (Δlymphocyte) counts from baseline to two weeks. Receiver Operating Characteristic (ROC) analysis determined cutoff values for predicting objective response in the logistic model. A p-value < 0.05 was considered significant. Statistical processing was carried out using SPSS version 25.0 (IBM Corp., Tokyo, Japan).

Results and Discussion

Patient characteristics

Details of patient demographics and clinical findings at the initiation of durvalumab plus Tremelimumab (Dur/Tre) therapy and two weeks afterward are presented in **Table 1**. The median age of participants was 75 years, with 24 individuals (80.0%) being male. Dur/Tre was used as the initial systemic treatment in 15 patients (50.0%), while the remaining 15 (50.0%) received it as a second or later-line therapy.

The underlying causes of hepatocellular carcinoma (HCC) included viral infection in 14 cases (46.7%)—specifically hepatitis B in 8 and hepatitis C in 6—and non-viral etiologies in 16 patients (53.3%), comprising alcohol-related liver injury (ALD) in 9 and metabolic dysfunction-associated steatotic liver disease (MASLD) in 7. Cirrhosis was documented in 19 patients (63.3%), attributed to hepatitis B in 6 (31.6%), hepatitis C in 3 (15.8%), ALD in 6 (31.6%), and MASLD in 4 (21.0%).

Most patients (27, 90.0%) were classified as Child-Pugh A, while 6 (20.0%) were modified ALBI grade 1. Seventeen participants (56.7%) had Barcelona Clinic Liver Cancer (BCLC) stage C disease. The median largest tumor diameter measured 34 mm, and all tumors were beyond the up-to-seven criteria, defined as the sum of the largest tumor diameter (cm) plus the number of liver tumors [23].

Macrovascular involvement occurred in 8 patients (26.7%), and extrahepatic metastases were identified in 17 (56.7%). Laboratory medians were as follows: WBC 5750/μL, lymphocytes 1287/μL, monocytes 351/μL, and neutrophils 3763/μL. The corresponding ratios were NLR2w = 3.05 (IQR 2.06-4.13), LMR2w = 3.16 (IQR 2.13-4.03), and PLR2w = 119.9 (IQR 72.4-163.8).

Changes from baseline to week 2 yielded median Δneutrophil = +286.9/μL (IQR −419.1-1225.2), Δmonocyte = +57.3/μL (IQR −10.3-153.1), and Δlymphocyte = +35.3/μL (IQR −132.3-369.8). The median follow-up period for the cohort was 11.07 months (IQR 4.85-15.75).

Table 1. Baseline and two-week characteristics of patients receiving durvalumab plus Tremelimumab.

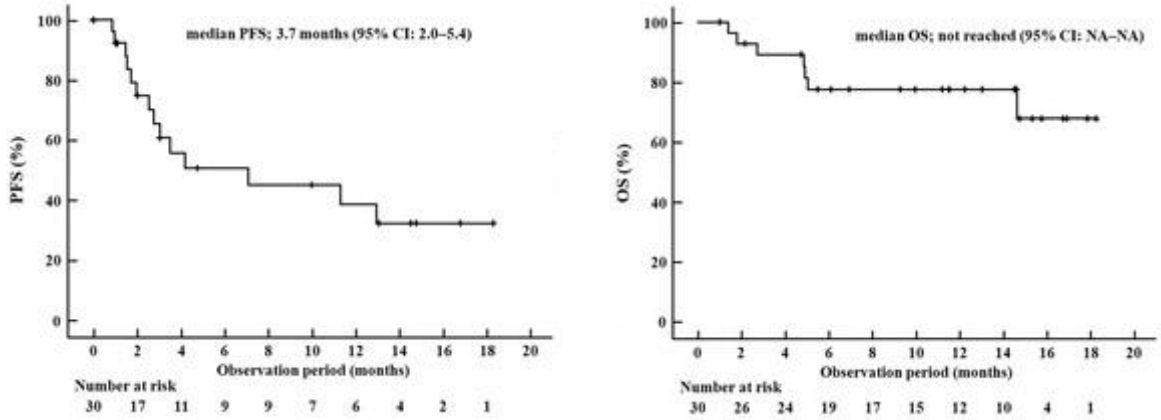
Characteristic	Value (n = 30)
Age, years	75 (69, 77)
Sex, male/female, n	24/6
ECOG performance status, 0/1, n	26/4
Dur/Tre treatment line, 1st/later, n	15/15
Etiology, hepatitis B/hepatitis C/non-viral, n	8/6/16
Liver cirrhosis, present/absent, n	19/11
Child-Pugh class, A/B, n	27/3
Modified ALBI grade, 1/2a/2b/3, n	6/8/14/2
Maximum tumor diameter, mm	34 (22, 72)
Macrovascular invasion, present/absent, n	8/22
Extrahepatic metastasis, present/absent, n	13/17
BCLC stage, A/B/C, n	1/12/17
Prior Atez/Bev exposure, yes/no, n	12/18
Laboratory parameters at Dur/Tre initiation	
Albumin, g/dL	3.5 (3.2-3.8)
Total bilirubin, mg/dL	0.9 (0.6-1.2)
AFP, ng/mL	16.7 (5.2-7929.4)
DCP, mAU/mL	636.0 (55.5-3864.7)
Platelet count, ×10⁴/μL	15.7 (11.3-19.2)
WBC count, /μL	5750 (4275-7050)
Neutrophil count, /μL	3763 (2502-5131)
Monocyte count, /μL	351 (260-469)
Lymphocyte count, /μL	1287 (1052-1582)
NLR	3.27 (1.96-4.47)
LMR	3.57 (2.80-4.79)
PLR	122.9 (91.3-167.6)
Laboratory parameters at 2 weeks post-Dur/Tre initiation	
Platelet count, ×10⁴/μL	14.5 (10.8-21.0)
WBC count, /μL	6350 (5000-8925)
Neutrophil count, /μL	4187 (2648-5560)
Monocyte count, /μL	468 (297-646)
Lymphocyte count, /μL	1360 (1052-1786)
NLR	3.05 (2.06-4.13)
LMR	3.16 (2.13-4.03)
PLR	119.9 (72.4-163.8)
Follow-up duration, months	11.07 (4.85-15.75)

Values are expressed as median (interquartile range). Abbreviations: Dur/Tre, durvalumab + Tremelimumab; ECOG PS, Eastern Cooperative Oncology Group performance status; ALBI, albumin-bilirubin; BCLC, Barcelona Clinic Liver Cancer; Atez/Bev, atezolizumab + bevacizumab; AFP, alpha-fetoprotein; DCP, des-γ-carboxy prothrombin; WBC, white blood cell; NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; PLR, platelet-to-lymphocyte ratio.

Therapeutic efficacy

At the time of analysis, six patients remained on Dur/Tre. Among those who discontinued, one did so following complete response (CR) and conversion surgery, one due to liver failure linked to tumor progression, thirteen because of progressive disease (PD), and nine owing to treatment-related adverse events (AEs).

The median progression-free survival (PFS) for all participants was 3.7 months (95% CI 2.0-5.4) **(Figure 1a)**. The median overall survival (OS) had not yet been reached, so a 95% CI could not be determined **(Figure 1b)**.



a) b)

Figure 1. Kaplan-Meier plots showing (a) progression-free and (b) overall survival in the full cohort.

Abbreviations: PFS, progression-free survival; OS, overall survival; CI, confidence interval; NA, not applicable.
The best radiological outcomes were CR 6.7%, PR 23.3%, SD 23.3%, and PD 46.7%. One individual with multinodular HCC achieved a radiologic CR on Dur/Tre, underwent curative resection, and was found to have no viable carcinoma histologically, constituting a pathological CR achieved solely by Dur/Tre. No other participants underwent conversion surgery, locoregional intervention, or additional systemic therapy before disease progression.
The objective response rate (ORR) and disease control rate (DCR) were 30.0% and 53.3%, respectively. No statistically significant variation in ORR or DCR was noted between first-line and later-line Dur/Tre recipients.

Factors associated with objective response
To identify determinants of objective response, univariate and multivariate logistic regression analyses were carried out **(Table 2)**. In univariate testing, PLR2w (HR = 0.973; 95% CI 0.951-0.995; p = 0.017) and Δlymphocyte (HR = 1.004; 95% CI 1.001-1.006; p = 0.016) showed significant correlations with treatment response. However, neither retained significance after multivariate adjustment.
A negative correlation was also identified between PLR2w and Δlymphocyte (r = −0.538, p = 0.002).

Table 2. Logistic regression analysis identifying variables associated with objective response.

Variable	Univariate Analysis			Multivariate Analysis		
	HR	(95% CI)	p-value	HR	(95% CI)	p-value
Age, years	1.002	(0.928-1.083)	0.953			
Sex, male vs. female	0.333	(0.053-2.115)	0.244			
Etiology, viral vs. non-viral	0.880	(0.183-4.226)	0.873			
Dur/Tre line, 1st vs. later	1.375	(0.286-6.603)	0.691			
At Dur/Tre initiation						
WBC count, /μL	1.000	(1.000-1.000)	0.619			
Neutrophil count, /μL	1.000	(0.999-1.000)	0.498			
Monocyte count, /μL	1.000	(0.996-1.004)	0.947			
Lymphocyte count, /μL	1.000	(0.999-1.002)	0.916			
NLR	0.782	(0.470-1.299)	0.342			
LMR	1.043	(0.684-1.589)	0.846			
PLR	0.995	(0.982-1.008)	0.421			
At 2 weeks post-Dur/Tre						
WBC count, /μL	1.000	(1.000-1.000)	0.640			
Neutrophil count, /μL	1.000	(1.000-1.000)	0.409			
Monocyte count, /μL	0.999	(0.995-1.002)	0.451			

Lymphocyte count, /μL	1.001	(1.000-1.003)	0.068			
NLR	0.472	(0.203-1.096)	0.081			
LMR	1.653	(0.965-2.831)	0.067			
PLR	0.973	(0.951-0.995)	0.017	0.981	(0.957-1.004)	0.108
ΔNeutrophil, /μL	1.000	(0.999-1.000)	0.593			
ΔMonocyte, /μL	0.995	(0.988-1.003)	0.203			
ΔLymphocyte, /μL	1.004	(1.001-1.006)	0.016	1.002	(0.999-1.005)	0.130

Abbreviations: HR, hazard ratio; CI, confidence interval; Dur/Tre, durvalumab plus Tremelimumab; WBC, white blood cell; NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; PLR, platelet-to-lymphocyte ratio; Δneutrophil, Δmonocyte, and Δlymphocyte represent the cell-count differences between baseline and two weeks post-Dur/Tre initiation.

Receiver operating characteristic (ROC) analyses were carried out to determine suitable thresholds for predicting objective response using PLR2w and Δlymphocyte. Both variables were significantly correlated with treatment response: PLR2w demonstrated an AUC of 0.831 (95% CI: 0.627-1.000; p = 0.005), and Δlymphocyte showed an AUC of 0.761 (95% CI: 0.571-0.951; p = 0.031). From these curves, the most accurate cut-off levels were 98.6 for PLR2w and +244.5/μL for Δlymphocyte. Accordingly, participants were classified into two subgroups based on these values—PLR2w < 98.6 vs. ≥ 98.6 and Δlymphocyte < +245/μL vs. ≥ +245/μL.

The objective response rate (ORR) was markedly higher among those with lower PLR2w values (below 98.6; n = 10) than in the elevated PLR2w group (≥ 98.6; n = 20), at 80.0% versus 5.0% (p < 0.001). Similarly, patients with an increase in Δlymphocyte exceeding +245/μL (n = 10) achieved an ORR of 70.0%, compared with 10.0% in the lower group (n = 20; p = 0.002). Disease control rate (DCR) followed a similar pattern—90.0% versus 35.0% for low versus high PLR2w (p = 0.006), and 90.0% versus 35.0% for high versus low Δlymphocyte (p = 0.006) **(Table 3)**.

Table 3. Radiological best response (per RECIST v1.1) to durvalumab plus Tremelimumab therapy stratified by platelet-to-lymphocyte ratio at 2 weeks (PLR2w) and the change in lymphocyte count (Δlymphocyte).

	Whole Cohort	**High PLR2w**	**Low PLR2w**	***p* Value**	**High Δlymphocyte**	**Low Δlymphocyte**	***p* Value**
n	30	20	10		10	20	
CR	2 (6.7)	0 (0.0)	2 (20.0)		2 (20.0)	0 (0.0)	
PR	7 (23.3)	1 (5.0)	6 (60.0)		5 (50.0)	2 (10.0)	
SD	7 (23.3)	6 (30.0)	1 (10.0)		2 (20.0)	5 (25.0)	
PD	14 (6.7)	13 (65.0)	1 (10.0)		1 (10.0)	13 (65.0)	
ORR	26.7%	5.0%	80.0%	<0.001	70.0%	10.0%	0.002
DCR	53.3%	35.0%	90.0%	0.006	90.0%	35.0%	0.006

Data are shown as n (%). Abbreviations: CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ORR, objective response rate; DCR, disease control rate; PLR, platelet-to-lymphocyte ratio; PLR2w, platelet-to-lymphocyte ratio two weeks after treatment initiation; Δlymphocyte, change in lymphocyte count from baseline to week 2.

When Δlymphocyte data were instead categorized simply as “plus” (increase) or “minus” (decrease), response trends remained consistent. The ORR in patients showing a positive Δlymphocyte (n = 17) was 47.0%, compared with only 7.6% in the negative group (n = 13; p < 0.001).

Prognostic factors for progression-free survival (PFS)

Univariate and multivariate Cox regression analyses were applied to identify predictors of PFS. Initial blood indices—including total white cell count, lymphocyte, monocyte, and neutrophil counts, as well as baseline NLR, LMR, and PLR—showed no significant relationship with PFS. In contrast, univariate analyses indicated several meaningful associations: the occurrence of immune-mediated adverse events (imAEs) (HR 0.239; 95% CI: 0.086-0.667; p = 0.006), NLR2w (HR 1.129; 95% CI: 1.003-1.271; p = 0.044), PLR2w (HR 1.008; 95% CI: 1.001-1.016; p = 0.023), and a Δlymphocyte ≥ +245/μL (HR 0.215; 95% CI: 0.072-0.647; p = 0.006). Multivariate

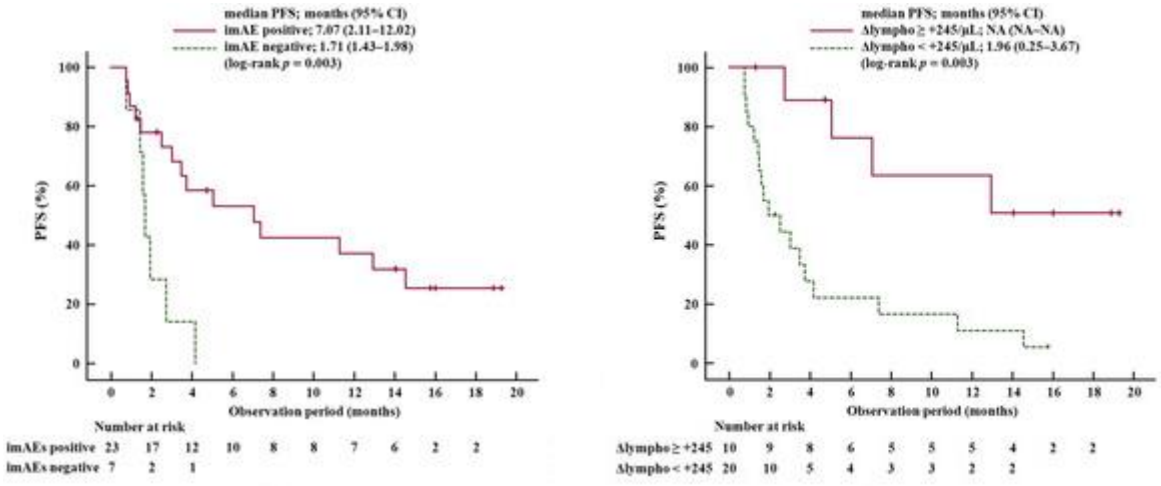
modeling confirmed that imAE presence (HR 0.321; 95% CI: 0.112-0.923; p = 0.035) and high Δlymphocyte (HR 0.308; 95% CI: 0.095-0.998; p = 0.049) independently predicted improved PFS **(Table 4)**.

Table 4. Cox regression for predictors of progression-free survival.

Variable	Univariate Analysis			Multivariate Analysis		
	HR	(95% CI)	p-value	HR	(95% CI)	p-value
Age, years	0.993	(0.949-1.039)	0.758			
Sex, male vs. female	1.212	(0.407-3.612)	0.730			
Etiology, viral vs. non-viral	0.987	(0.421-2.317)	0.977			
Dur/Tre line, 1st vs. later	0.832	(0.359-1.926)	0.667			
imAEs, present vs. absent	0.239	(0.086-0.667)	0.006	0.321	(0.112-0.923)	0.035
At Dur/Tre initiation						
AFP, ≥400 vs. <400 ng/mL	1.072	(0.436-2.638)	0.880			
WBC count, /µL	1.000	(1.000-1.000)	0.496			
Neutrophil count, /µL	1.000	(0.999-1.000)	0.399			
Monocyte count, /µL	1.000	(0.998-1.002)	0.933			
Lymphocyte count, /µL	1.000	(0.999-1.001)	0.963			
NLR	1.116	(0.882-1.413)	0.361			
LMR	1.005	(0.783-1.290)	0.969			
PLR	1.002	(0.996-1.007)	0.515			
At 2 weeks post-Dur/Tre						
WBC count, /µL	1.000	(1.000-1.000)	0.380			
Neutrophil count, /µL	1.000	(1.000-1.000)	0.182			
Monocyte count, /µL	1.000	(0.999-1.001)	0.629			
Lymphocyte count, /µL	0.999	(0.999-1.000)	0.113			
NLR	1.129	(1.003-1.271)	0.044	1.001	(0.854-1.172)	0.994
LMR	0.800	(0.606-1.057)	0.117			
PLR	1.008	(1.001-1.016)	0.023	1.005	(0.995-1.016)	0.317
ΔNeutrophil, /µL	1.000	(1.000-1.000)	0.222			
ΔMonocyte, /µL	1.001	(0.999-1.003)	0.281			
ΔLymphocyte, ≥+245 vs. <+245 /µL	0.215	(0.072-0.647)	0.006	0.308	(0.095-0.998)	0.049

Abbreviations: HR, hazard ratio; CI, confidence interval; Dur/Tre, durvalumab plus Tremelimumab; AFP, alpha-fetoprotein; imAEs, immune-mediated adverse events; WBC, white blood cell; NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; PLR, platelet-to-lymphocyte ratio; ΔNeutrophil, ΔMonocyte, ΔLymphocyte = change in cell counts from baseline to week 2.

Median PFS was 7.07 months (95% CI: 2.11-12.02) among those who developed imAEs, compared to 1.71 months (95% CI: 1.43-1.98) in those without (p = 0.003) **(Figure 2a)**. Stratification by Δlymphocyte showed that PFS was not reached in the high Δlymphocyte group, while it was 1.96 months (95% CI: 0.25-3.67) in the low group (p = 0.003) **(Figure 2b)**.



a) b)

Figure 2. Kaplan-Meier curves of PFS: (a) patients with imAEs (solid line) versus without (dotted line); (b) PFS categorized by Δlymphocyte cut-off (+245/μL). Abbreviations: PFS, progression-free survival; imAEs, immune-mediated adverse events; Δlymphocyte, change in lymphocyte count; CI, confidence interval; NA, not applicable.

The plus Δlymphocyte subgroup had a median PFS of 7.07 months (95% CI: 0.00-18.20), compared with 1.96 months (95% CI: 0.94-2.98) in the minus group (p = 0.032). After excluding one patient who underwent conversion surgery, 29 individuals remained for analysis; their median PFS values were 12.92 months (95% CI: 0.00-27.72) for high Δlymphocyte and 1.96 months (95% CI: 0.25-3.67) for low Δlymphocyte (p = 0.008).

Prognostic factors for overall survival (OS)

In univariate analyses, both imAE occurrence (HR 0.129; 95% CI: 0.028-0.600; p = 0.009) and AFP ≥ 400 ng/mL (HR 4.829; 95% CI: 1.143-20.409; p = 0.032) were significantly linked with OS. Multivariate results indicated that imAE presence (HR 0.111; 95% CI: 0.019-0.662; p = 0.016) and elevated AFP (HR 10.848; 95% CI: 1.804-65.229; p = 0.009) remained independent prognostic factors.

The median OS was not reached in patients who experienced imAEs, whereas it was 4.89 months (95% CI: 2.27-7.50) among those without (p = 0.002). Patients with AFP ≥ 400 ng/mL had a median OS of 12.32 months (95% CI: 0.00-26.84), while survival was not reached in those with AFP < 400 ng/mL (p = 0.018).

Predictive indicators for immune-mediated adverse events (imAEs)

The most frequent grade ≥3 toxicities were colitis/diarrhea (n = 5), hypopituitarism (n = 3), interstitial pneumonia (n = 2), and hepatitis (n = 2). According to univariate logistic regression, baseline WBC, neutrophil, monocyte counts, and NLR significantly correlated with imAE incidence. However, these associations were not maintained in multivariate analysis. No significant predictors were observed for grade ≥3 events.

To date, several systemic therapeutic options have been introduced for the management of advanced hepatocellular carcinoma (HCC). Within the HIMALAYA trial, the STRIDE regimen demonstrated an objective response rate (ORR) of 20.1%, with a median time to response of 2.17 months [12]. This treatment protocol, incorporating an anti-CTLA-4 antibody, produced a distinct tail plateau on Kaplan-Meier survival analysis. More than half of the long-term survivors who received the STRIDE regimen achieved an objective response as defined by RECIST v1.1 [13]. The median progression-free survival (PFS) reported in HIMALAYA was 3.78 months [12], and real-world evidence from large cohort studies has indicated similar results, with median PFS ranging from 3.0 to 3.9 months [24, 25]. Consequently, the PFS outcomes in the current cohort appear consistent with prior research. As extending PFS often translates to improved overall survival (OS) in patients undergoing immune checkpoint inhibitor (ICI) therapy for HCC [26], achieving an early objective response following Durvalumab plus Tremelimumab (Dur/Tre) initiation may play a crucial role in enhancing OS. Additionally, early fluctuations in tumor markers such as alpha-fetoprotein (AFP) and des-gamma-carboxy prothrombin (DCP) after

Dur/Tre therapy have been proposed as potential biomarkers for forecasting treatment response and survival outcomes [27, 28].

Understanding anti-tumor immune responses requires considering both the local tumor microenvironment and the systemic immune landscape [29]. In this study, we focused on a straightforward peripheral biomarker — the lymphocyte count — as an accessible reflection of systemic immunity. Peripheral lymphocytes offer the advantages of noninvasive and dynamic assessment, yet their association with treatment outcomes in HCC remains complex and incompletely characterized. Therefore, the clinical significance of total lymphocytes and their subpopulations as indicators of ICI efficacy warrants continued evaluation in advanced HCC.

Among peripheral inflammatory indices, the neutrophil-to-lymphocyte ratio (NLR) is widely recognized as a marker of systemic inflammation and has been linked to ICI outcomes and prognosis in HCC [30, 31]. Elevated NLR is generally indicative of an unfavorable prognosis in advanced HCC. Neutrophils represent a key source of circulating vascular endothelial growth factor (VEGF); consequently, neutrophilia may facilitate angiogenesis and metastasis by promoting a VEGF-enriched microenvironment [32]. VEGF also exerts immunosuppressive activity by fostering an immune-evasive tumor milieu [33]. Conversely, the activation and proliferation of lymphocytes can promote tumor inhibition and prolong survival [34].

Previous findings indicated that patients with low baseline NLR (≤ 3) at the onset of Dur/Tre therapy exhibited significantly prolonged PFS [28]. However, in our analysis, baseline values of NLR, absolute lymphocyte and neutrophil counts, and the lymphocyte-to-monocyte ratio (LMR) and platelet-to-lymphocyte ratio (PLR) were not associated with either objective response or PFS. Based on prior evidence [19], we hypothesized that NLR, PLR, and lymphocyte levels measured two weeks after treatment initiation may more accurately represent the patient's immune status. Our findings supported this notion: both PLR at two weeks (PLR2w) and the change in lymphocyte count (Δlymphocyte) were significantly related to objective response, whereas NLR2w and Δneutrophil were not predictive.

Age-related decline in immune responsiveness has been noted in ICI therapy [35], and in our cohort, most patients (86.7%) were aged 65 years or older. Nonetheless, Δlymphocyte showed no significant association with age.

Patients were further classified by the ROC-derived cut-off values of PLR2w (98.6) and Δlymphocyte (+244.5/μL). Notably, the subgroup with low PLR2w and the group with elevated Δlymphocyte demonstrated significantly higher ORR and disease control rate (DCR). Moreover, Δlymphocyte ≥ +245/μL emerged as an independent predictor of prolonged PFS ($p = 0.049$). Despite the relatively short observation duration, PFS differed distinctly between patients with high and low Δlymphocyte ($p = 0.003$).

Univariate Cox regression identified NLR2w and PLR2w as significant correlates of PFS; however, their predictive value was lost in multivariate analysis. Given that PLR2w and Δlymphocyte were inversely correlated, the lower PLR2w observed likely reflected an increase in lymphocyte counts at two weeks post-treatment. Consequently, we considered Δlymphocyte ≥ +245/μL to represent the most reliable indicator of favorable PFS in patients receiving Dur/Tre.

Baseline AFP levels exceeding 400 ng/mL were identified as an independent determinant of unfavorable overall survival (OS). Prior studies have noted that the emergence of immune-mediated adverse events (imAEs) is linked to enhanced treatment efficacy and improved outcomes in patients receiving atezolizumab plus bevacizumab (Atez/Bev) for advanced HCC [36]. In alignment with these findings, our investigation revealed that the development of imAEs served as an independent predictor of superior progression-free survival (PFS) and OS in patients undergoing Durvalumab plus Tremelimumab (Dur/Tre) therapy.

At present, no prospectively verified biomarkers are available to anticipate the onset of severe imAEs in the context of Dur/Tre treatment. In this study, we explored potential contributors associated with imAE occurrence; however, multivariate analysis failed to identify any significant predictive variables. Although the peripheral lymphocyte count did not correlate with the emergence of imAEs in our dataset, this hypothesis warrants additional prospective validation in future studies.

Histopathological evaluation using immunohistochemistry on tumor biopsy specimens has been proposed as a method to distinguish patient subgroups likely to respond favorably to immune checkpoint inhibitors (ICI) in advanced HCC [37]. In particular, the density of $CD8^+$ tumor-infiltrating lymphocytes has been reported as a valuable marker for anticipating clinical benefit in Dur/Tre therapy [38]. Nevertheless, routine biopsy acquisition is often impractical in many patients with advanced HCC due to tumor location or medical condition. Moreover,

several individuals initiate Dur/Tre therapy with tumor marker levels within normal limits, suggesting that AFP or DCP alone may not adequately represent treatment efficacy or prognosis across all cases.

In contrast, Δlymphocyte — reflecting the early change in circulating lymphocyte counts — can be easily determined through routine blood testing, rendering it a practical, minimally invasive, and cost-efficient indicator. However, the present research has several constraints. First, it was a single-center retrospective study, inherently subject to potential selection and observational biases. Second, the relatively limited cohort size and short follow-up duration may restrict the generalizability of the findings. Third, immune profiling by flow cytometry to assess specific lymphocyte subsets was not performed. Prior research has indicated that the proportion of $CD3^+CD8^+$ T cells significantly increases following ICI therapy in HCC [39]. Furthermore, the early rise in peripheral $Ki67^+$ $CD8^+$ T-cell populations during STRIDE therapy has been associated with objective response [19]. Therefore, the early elevation of lymphocyte counts observed in our study may reflect expansion of circulating $CD8^+$ T cells during Dur/Tre treatment.

Given the retrospective and non-prospective nature of this work, definitive conclusions regarding the predictive role of Δlymphocyte cannot yet be drawn. Nonetheless, these results may provide a foundation for future hypothesis-driven investigations and should be confirmed in larger, multicenter studies with extended observation periods.

Conclusion

An early elevation in circulating lymphocyte count within two weeks after administering a single priming dose of Tremelimumab may serve as a promising biomarker for anticipating objective treatment response and prolonged progression-free survival in patients with advanced hepatocellular carcinoma treated with the STRIDE regimen.

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