#2369 / 81P - Baseline circulating CD4+PD1^{High} T cells as predictors of survival in patients with solid tumors treated with immune-checkpoint inhibitors

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BACKGROUND

PD1 is an inhibitory receptor exposed on the surface of T cells and other immune cells. It is constitutively expressed in case of chronic immune stimulation, including cancer, and is thought to be a potential marker of T cells' exhaustion in this context[1,2]. In non-small cell lung cancer (NSCLC) and after stem cell transplantation, higher levels of CD4+ T cells (TCD4) expressing PD1 (PD1+) correlated with poor survival outcomes[3,4]. Nonetheless, *in vitro* PD1 blockade was found to enhance antitumor immunity and subpopulations of PD1+ TCD4 with higher or lower PD1 levels were identified and associated with differential functional effects and prognosis in follicular lymphoma[5,6]. Immunotherapy with immune-checkpoint inhibitors (ICI), either in monotherapy or combined among them or with chemotherapy, is rapidly becoming a new therapeutic standard in multiple cancers[7]. The most widely adopted ICI are currently represented by anti-PD-L1 or anti-PD1[7]. Unfortunately, there is a current lack of standardized predictive biomarkers. For example PD-L1 detection in tissue, which is usually necessary to select patients candidate for anti PD-L1/PD1 ICI-based therapy[8], is measured by multiple available tests depending on cancer type and ICI[8]. Notably, ICI are extremely expensive and capable of inducing immuno-mediated toxicities, including rare but severe gastroenteritis, hypophysitis, adrenalitis and encephalitis, among others[7,9]. Therefore, optimal selection of patients candidate to ICI is crucial. Considering that peripheral blood sampling and detection of T cell subpopulation is a relatively inexpensive, standardized and easy procedure, we aimed at elucidating the prognostic role of peripheral TCD4PD1+ and TCD4PD1^{High} (TCD4PD1H) in cancer patients treated with ICI-based regimens.

METHODS

Study design

This study is a sub-analysis of the Immuno-blood prospective observational study run at the Hospital Clinic of Barcelona (HCB), aimed at discovering potential circulating blood biomarkers with prognostic and/or predictive value in patients treated with an ICI-based therapy. All patients were treated at HCB Oncology Department. The study was approved by the Ethic Committee of the HCB (IRB: HCB/2017/0371).

Inclusion criteria were: age ≥18 years; to be diagnosed of a solid tumor; to be about to start an ICI-based treatment; to give consent to peripheral blood sampling.

Mandatory peripheral blood samples (BS) were collected at prespecified timepoints (before cycle 1 [C1D1], before cycle 2 [C2D1], evaluation of response). Optional tumor samples before ICI-based therapy were also collected, if available.

Main clinicopathological data were retrieved from medical records and BS at C1D1, C2D1 and progression (CPD) were evaluated with flow cytometry (FC) to assess the proportion of distinct immunologic cell lineages. Tumor responses were carried out by imaging tests (usually CT) requested within the standard care path of each patient, at the discretion of his oncologist and evaluated by RECIST 1.1 criteria[10].

Objectives

Primary objective: assessment of the correlation between the C1D1 proportion of TCD4PD1+ and of the respective subpopulation with high levels of PD1 (TCD4PD1H) with progression-free survival (PFS).

Secondary objectives: assessment of a correlation with overall survival (OS), overall response rates (ORR) and disease control rates (DCR).

Laboratory methods

Blood was processed within 1 hour of being collected. Approximately 34 mL of blood were drawn per time point to perform a complete blood count (1 tube of 10 mL of EDTA) and analyze lymphocyte subpopulations and other peripheral mononuclear cells (PBMC) by FC (2 tubes of 10 mL Heparin Li/Na and a 4 mL EDTA tube).

Blood samples dedicated to the assessment of PBMC were analyzed on the day of collection using standard protocols of the HCB Immunology service for carrying out a complete blood count, and the determination of lymphocyte populations by FC, including CD4+PD1+ and TCD4PD1H. TCD4PD1^{High} cells were defined as the top half log of the CD4 cells expressing PD1.

Statistical analysis

Patient and tumor characteristics were reported using descriptive statistics, proportions were compared with chi square test or Fisher exact test, where appropriate. We performed univariate Cox regressions to detect a correlation between TCD4PD1+ and TCD4PD1H with PFS and OS. When an association was identified with either one or the other, a maximally selected rank statistic (MSRS) method was applied to define the optimal cut-off for selecting patients with low and high levels of the specific T cell subset of interest. High level patients were then compared to low level patients for PFS and OS by the Kaplan-Meier method and significant differences was assessed with the log rank test. Univariate and multivariate Cox regression models were then adopted to define hazard ratios (HR) with 95% confidence intervals (CI). ORR and DCR were also evaluated and an association with better responses for high level vs. low level patients was explored with univariate and multivariate logistic regressions. Two-tailed p<0.05 was considered for statistical significance.

RESULTS

At the time of this analysis, a first cohort was already enrolled (Cohort 1), while an independent validation cohort (Cohort 2) is currently under recruitment. Here we present cohort 1 results, that includes sixty-nine patients who had C1D1 detectable levels of TCD4PD1+ and TCD4PD1H in blood. Patients characteristics are reported in **Table 1**. The median proportion of TCD4PD1+ was 13.9% (interquartile range[IQR]: 9.3–17.4%) and of TCD4PD1H was 0.7% (IQR: 0.3-1.3%). The median follow-up was: 36.4 months (95%CI: 28.7 - NE). A significant association with OS, but not PFS was observed only for TCD4PD1H, as continuous variable (HR: 1.15, 95%CI: 1.00 – 1.31, p=0.048).

A cut-off of 1.1% was identified with the MSRS method (**Figure 1**) and defined two prognostically significant subgroups of patients, consisting in 19 cases with TCD4PD1H% above vs. 50 below the threshold (TCD4PD1H$_A$ vs. TCD4PD1H$_B$, respectively).

TCD4PD1H$_B$ vs. TCD4PD1H$_A$ presented with better PFS (median PFS [mPFS]: 2.30 [95%CI: 1.64 - 6.12] vs. 2.24 [95%CI: 1.38 - 3.68], HR: 0.50, 95%CI: 0.28 - 0.89, p=0.018), OS (mOS: 13.12 [95%CI: 7.66 - 21.81] vs. 4.67 [95%CI: 2.93 - 14.67], HR: 0.42, 95%CI: 0.24 - 0.74, p=0.002) (**Figure 2**) and ORR (20.0% vs 0.0%, χ^2 p=0.035) (**Figure 3**). After adjusting for age, sex, ECOG, cancer type, treatment line, treatment type, visceral status, number of metastases, ICI type and immune-naive status, TCD4PD1H$_B$ still showed better association with PFS (adjusted HR [aHR]: 0.52, 95%CI: 0.28 – 0.98, p=0.041) and OS (aHR: 0.39, 95%CI: 0.21 - 0.72, p=0.003) than TCD4PD1H$_A$.

Table 1. Population characteristics

Demographics		Low Basal CD4+PD1+		High Basal CD4+PD1+		Overall Population		P
		N	%	N	%	N	%	
		50	72.5	19	27.5	69	100.0	
Age	≥65	21	42.0	9	47.4	30	43.5	
	<65	29	58.0	10	52.6	39	56.5	0.897
	Total	50	100.0	19	100.0	69	100.0	
Gender	Female	18	36.0	6	31.6	24	34.8	
	Male	32	64.0	13	68.4	45	65.2	0.951
	Total	50	100.0	19	100.0	69	100.0	
ECOG basal	0 - 1	44	88.0	16	84.2	60	87.0	
	2 - 3	6	12.0	3	15.8	9	13.0	0.986
	Total	50	100.0	19	100.0	69	100.0	
Tumor type	Lung Cancer	11	22.0	7	36.8	18	26.1	
	Prostate Cancer	4	8.0	0	0.0	4	5.8	
	Renal Cancer	3	6.0	1	5.3	4	5.8	
	Suprarenal Cancer	0	0.0	1	5.3	1	1.4	
	Urothelial Cancer	5	10.0	1	5.3	6	8.7	
	Breast Cancer	7	14.0	1	5.3	8	11.6	
	Colorectal cancer	7	14.0	4	21.1	11	15.9	
	Head & Neck Cancer	5	10.0	2	10.5	7	10.1	
	Melanoma	4	8.0	0	0.0	4	5.8	0.356
	Esofagous Cancer	0	0.0	1	5.3	1	1.4	
	Gastric cancer	1	2.0	0	0.0	1	1.4	
	Ginecologic cancer	0	0.0	1	5.3	1	1.4	
	Sarcoma	1	2.0	0	0.0	1	1.4	
	CNS	1	2.0	0	0.0	1	1.4	
	Thymic Carcinoma	1	2.0	0	0.0	1	1.4	
	Total	50	100.0	19	100.0	69	100.0	
Visceral	Yes	37	74.0	4	21.1	41	59.4	
	No	13	26.0	15	78.9	28	40.6	**<0.001**
	Total	50	100.0	19	100.0	69	100.0	
Number of Metastases	<3	9	18.0	4	21.1	13	18.8	
	≥3	41	82.0	15	78.9	56	81.2	0.956
	Total	50	100.0	19	100.0	69	100.0	
ICI Treatment Line	1-2L	26	52.0	9	47.4	35	50.7	
	≥3L	24	48.0	10	52.6	34	49.3	0.941
	Total	50	100.0	19	100.0	69	100.0	
Immune-naive	Yes	47	94.0	16	84.2	63	91.3	
	No	3	6.0	3	15.8	6	8.7	0.417
	Total	50	100.0	19	100.0	69	100.0	
Treatment schedule	Monotherapy	30	60.0	13	68.4	43	62.3	
	Combination	20	40.0	6	31.6	26	37.7	0.714
	Total	50	100.0	19	100.0	69	100.0	
ICI Class	Anti-PD1/PDL1-based	47	94.0	16	84.2	63	91.3	
	Other	3	6.0	3	15.8	6	8.7	0.417
	Total	50	100.0	19	100.0	69	100.0	
Best response	PD	25	50.0	12	63.2	37	53.6	
	SD	15	30.0	7	36.8	22	31.9	
	PR	8	16.0	0	0.0	8	11.6	0.217
	CR	2	4.0	0	0.0	2	2.9	
	Total	50	100.0	19	100.0	69	100.0	
ORR	Yes	10	20.0	0	0.0	10	14.5	
	No	40	80.0	19	100.0	59	85.5	**0.035**
	Total	50	100.0	19	100.0	69	100.0	
DCR	Yes	25	50.0	7	36.8	32	46.4	
	No	25	50.0	12	63.2	37	53.6	0.478
	Total	50	100.0	19	100.0	69	100.0	

Legend. ORR: overall response rates; DCR: disease control rates; PD: progressive disease; SD: stable disease; PR: partial response; CR: complete response; ICI: immune-checkpoint inhibitor; CNS: central nervous system.

Figure 1. Optimal cut-off for TCD4PD1H based on overall survival

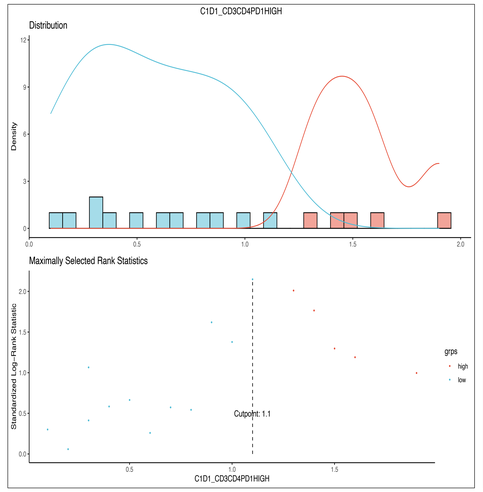
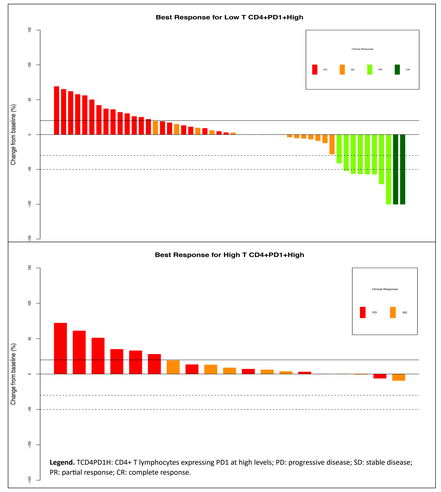
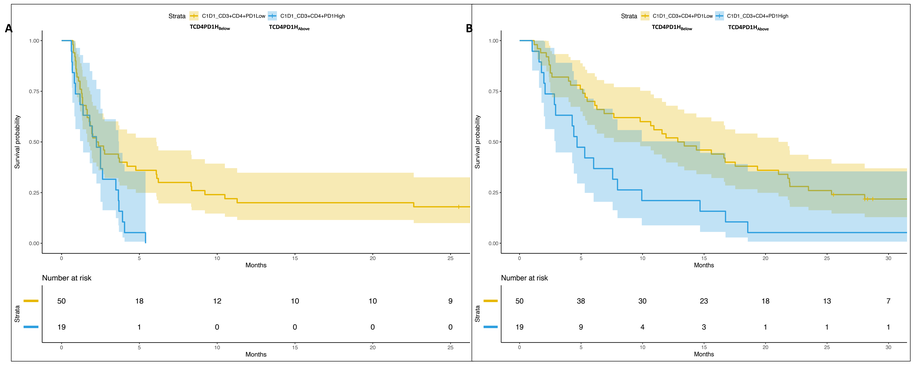


Figure 3. Best responses according to TCD4PD1H levels



Legend. TCD4PD1H: CD4+ T lymphocytes expressing PD1 at high levels; PD: progressive disease; SD: stable disease; PR: partial response; CR: complete response.

Figure 2. Kaplan-Meier curves with 95% confidence intervals of progression-free survival (A) and overall survival (B) according to TCD4PD1H levels



CONCLUSIONS

Our results preliminarily show that low levels of peripheral CD4+ T lymphocytes expressing high levels of PD1 in their surface membrane might be associated with improved PFS, OS and ORR in ICI-treated patients with solid tumors, independently from cancer type, metastatic patterns, main patients and treatment characteristics. These results are coherent with previous data observed in NSCLC[3].

The optimal cut-off to identify prognostic low and high levels of such T lymphocyte subpopulation seem to be 1.1%.

These results require further validation in an independent cohort, whose recruitment is currently ongoing. Sub-analysis focused on anti-PD1/antiPD-L1 and in non-NSCLC tumors will be also carried out.

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DISCLOSURES

JGC declares participation in multiple clinical trials and meetings as speaker (advisory role) and financial sponsor support for meeting attendance.

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