Activated Peripheral Blood Lymphocytes Of Hepatocellular Carcinoma Patients (HCC) Are Associated With Increased Risk Of Early Dermatologic Adverse Effects During Sorafenib Treatment

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INTRODUCTION

Sorafenib-treated patients who develop early dermatologic adverse events (eDAEs) have been shown to have a better outcome(1). However, eDAEs' underlying cellular mechanism, how they are triggered and how they contribute to a better response, are questions that have yet to be elucidated.

Immune checkpoint inhibition (ICI) therapies have revolutionized the landscape of cancer treatments over the last decade. Although initially described for T cells, further research has shown that other populations like Natural Killer (NK) cells also have their own set of checkpoint molecules such as KIRs, NKG2D (2), or the recently described CD96/TIGIT/DNAM-1 axis (3), which can also be expressed on T cells (4).

AIM

To analyze the peripheral blood lymphocyte populations of those sorafenib-treated patients who develop eDAEs in order to find the molecular markers and mechanisms associated with the patients' better outcome.

PATIENTS & METHODS

PBMCs from 52 advanced-staged HCC patients (Table 1) were collected at baseline, 1, 4 and 8 weeks.
B, T and Natural Killer (NK) populations and their expression of the immune markers PD-1, TIM-3, CD69, CXCR6, LAG-3, CD127, CD39, NKG2D, DNAM-1, TIGIT, CD96 plus the transcription factors T-bet and Eomes were analyzed by flow cytometry using baseline and time-dependent models.
Univariate and multivariate time-depend Cox regression models were used to estimate Hazard ratios (HR) and their 95%CI between the patients' immune cells phenotype and the probability of developing eDAEs.

***Table 1**. Summary of patients characteristics*

Patients	n(%) / 52 (100%)
Age (Years), median [IQR]	64 [56 to 72]
Gender (males), n (%)	44 (84.6)
Child-Pugh (non-Cirrhotic* or A / B)	45 (86.5) / 7 (13.5)
BCLC stage (B / C), n (%)	22 (43.3) / 30 (57.7)
Extra-hepatic spread (Yes), n (%)	22 (42.3)
Follow-up (months), median [IQR]	9.6 [3.9 to 19.2]
Treatment time (months), [IQR]	5.1 [2.5 to 9.6]
eDAE (Yes), n (%)	16 (30.8)
Exitus (Yes), n (%)	20 (38.5)

RESULTS

IMMUNE MARKERS PREDICT eDAEs

No association was found between baseline lymphocyte populations and the probability of developing eDAEs. However, the time-dependent analysis showed a significant association between **B cells and higher probability** of eDAEs (Table 2).

When considering different immune checkpoints, **DNAM-1** and **PD-1** expression on T and CD56$^{+bright}$ cells stood out in both baseline and time-dependent models, where both correlated with **decreased probability of developing eDAEs** (Table 2). Other markers like CD69 and CD16 were associated with higher odds (Table 2).

***Table 2**.Summary of the immune populations showing significant correlation (p<0,05) with the probability of developing eDAEs.*

Model	Marker	HR (95% CI)	P-Value
Baseline values	T DNAM-1^{+}	0.92 (0.87 - 0.97)	<0.005
	NK CD56$^{+bright}$ PD-1^{+}	0.47 (0.22-0.99)	<0.05
	*T CD8^{+} CD69^{+}	1.07 (1.00-1.14)	<0.05
Time-dependent values	*B Cell	1.06 (1 - 1.11)	<0.05
	T DNAM-1^{+}	0.93 (0.89 - 0.97)	<0.005
	NK CD56$^{+bright}$ DNAM-1^{+}	0.91 (0.85 - 0.96)	<0.005
	NK CD56$^{+bright}$ PD-1^{+}	0.57 (0.33-0.99)	<0.05
	*T CD4^{+} PD-1^{+}	0.90 (0.82-0.99)	<0.05
	NK CD3^{+} CD16^{+}	1.04 (1.00-1.08)	<0.02
	NK CD3^{+} LAG-3 MFI	1.05 (1.00-1.11)	<0.05
	NK CD3^{+} PD-1 MFI	2.11 (1.12-3.95)	<0.02

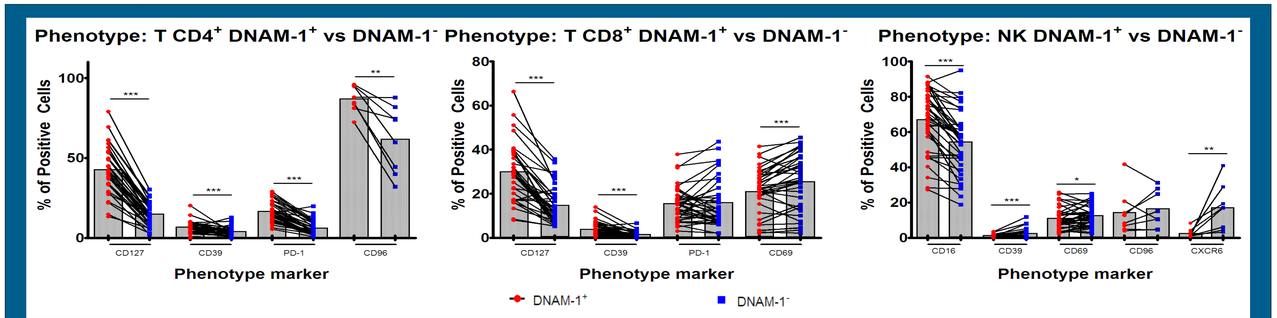
*Variables adjusted for BCLC Stage, Child-Pugh Score and ECOG-PS. HR: Hazard ratio, CI: Confidence Interval, MFI: Mean Fluorescence Intensity. *Adjusted for 1 or 2 co-factors only.*

DNAM-1 DISTINGUISHES FUNCTIONALLY DIFFERENT LYMPHOCYTES

Due to its correlation with eDAEs and the minor knowledge of DNAM-1 role in T cells; we compared the phenotype of T and NK DNAM-1^{+} vs. DNAM-1^{-} cells. **T DNAM-1^{+} cells**, particularly CD4^{+} ones, had higher expression of **immune exhaustion associated markers like CD39 or PD-1**, and of memory markers like CD127 (Fig. 1). CD96 was also highly expressed in this population compared to the DNAM-1^{-} counterparts. On the other hand, **NK DNAM-1^{+} cells** expressed more **co-stimulatory markers such as CD16 and CD69**, while expressing less CD39 and no PD-1 compared to DNAM-1^{-} counterparts (Fig. 1). **CD96**, described as an NK immune checkpoint, had **lower expression on NK cells than on T cells**.

In addition, **CXCR6**, a chemokine receptor associated with liver infiltration and residency, was **only found on DNAM-1^{-} NK cells** (Fig. 1).

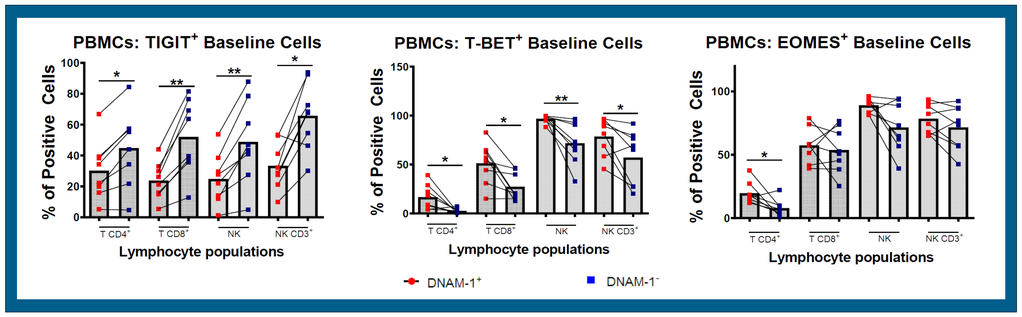
***Figure 1**. Phenotypic differences between DNAM-1+ and DNAM-1- T CD4+ and CD8+ cells and NK cells.*



**: p<0,05, **: p<0,01, ***: p<0,005. n=52.*

TIGIT, the third member of the DNAM-1/CD96 group, had higher expression on all DNAM-1^{-} cell types compared to DNAM-1^{+} counterparts (Fig. 2). On the other hand, the key transcription factor **T-bet** was found more expressed on all **DNAM-1^{+} cells** (Fig. 2), and no difference was found for **Eomes**, also considered a key transcription factor for lymphocytes maturation (Fig. 2).

***Figure 2**. Baseline expression of TIGIT, T-bet and Eomes across the different lymphocyte populations subdivided in DNAM-1^{+} and DNAM-1^{-} pairs.*



**: p<0,05, **: p<0,01, ***: p<0,005. n=8.*

CONCLUSIONS

Advanced-HCC patients immunity is tightly correlated with the probability of developing eDAEs. Population-wise, only **B cells** showed an association. However, function markers such as **PD-1, DNAM-1, CD39, CD69 and CD16** were **predictive** either at **baseline** or due to their **change over time**, suggesting that both the immune background of the patients and their response to the treatment are equally responsible of developing eDAEs. These results raise the chance of using different immune markers to anticipate the patients evolution.

We further analyzed **DNAM-1** associated phenotype, and found a duality between T and NK cells, where **DNAM-1^{+} T cells** expressed more immune **exhaustion markers** while **DNAM-1^{+} NK** cells expressed more **co-stimulatory** ones. Moreover, **CD96**, despite being widely studied as an NK function regulator, had **higher expression on T cells than on NK cells**. Altogether, these results suggest that the CD96/DNAM-1 axis could be playing an important role in HCC immune response as regulators of both T and NK cells.

To our knowledge, this is the first work studying the mechanisms involved in sorafenib-associated eDAEs; and the first work describing the relevance of DNAM-1/CD96 on T cells of advanced-HCC patients.

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