Integrated phenotyping of the anti-cancer immune response in HIV-associated hepatocellular carcinoma.

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INTRODUCTION

HCC is a leading cause of mortality in people living with HIV (PLHIV). Previous clinical studies indicate that HIV infection is independently associated with adverse survival in patients with HCC, but the molecular mechanisms behind the relationship has not been fully understood.

The immune-pathogenesis of HIV and HCC both rely on programmed-cell death 1 receptor/ligand (PD-1/PD-L1) pathway, which induces T-cell exhaustion. Currently, PLHIV are excluded from clinical trials of immune checkpoint inhibitors (ICI) , due to the assumption that HIV negatively affects the anti-tumour immunity.

AIM

To verify whether HIV status influences regulation of the anti-tumour immune responses by evaluation of functional characteristics of the T-lymphocyte infiltrate in tumour, peri-tumoural tissue and cirrhosis.

METHOD

From an international, multicentre biorepository of 55 HIV-associated HCC patients from 4 centres in Europe and North America, we evaluated the expression of programmed cell death ligands 1 and 2 (PD-L1/2) in tumour and infiltrating immune cells using a 1% cut-off.
Multiplex immunostaining of CD4, CD8, FoxP3, and PD-1 was used to estimate the cell density of the T-lymphocyte infiltrate (cytotoxic, regulatory and helper T-cell function) in tumoral, peritumoral and background cirrhotic cores (**Table 1**). We explored the relationships between PD-L1/2 expression and the functional characteristics of the T-lymphocyte infiltrate.
Immuno-pathologic features were further correlated with patients' clinic-pathologic data including markers of HIV infection.

Tab 1. Multiplex IHC and types of immune cells

Expression(s)	Type of immune cell
$CD4^+/FoxP3^-/CD8^-/PD\text{-}1^-$	T_H (helper T cell)
$CD4^+/FoxP3^+/CD8^-/PD\text{-}1^-$	Treg (regulatory T cell)
$CD4^-/FoxP3^-/CD8^+/PD\text{-}1^-$	CTL (cytotoxic T cell)
$CD4^-/FoxP3^-/CD8^+/PD\text{-}1^+$	

RESULTS

In total 86% of the patients were of CTP A class and 85% were BCLC stage A. Thirty-one patients (84%) had undetectable HIV viral load, and median blood CD4 cell count was 428 cells/mm^3.

Tab 2. Patient characteristics.

Characteristic	N=55 (%)
Age (median, range), years	52 (41-64)
Gender	
Male	41 (85)
Female	7 (15)
Aetiology of HCC	
Hepatitis B infection	7 (14)
Hepatitis C infection	46 (90)
Hepatitis D infection	2 (4)
Alcohol	6 (12)
Other	3 (6)
Child-Turcotte Pugh Class	
A	44 (86)
B	7 (14)
C	0 (0)
Barcelona Clinic Liver Cancer Stage	
A	40 (85)
B	4 (9)
C	3 (6)
D	0 (0)
AFP (ng/mL) Median (range)	11 (2-6536)
HIV viral load (copies) Median (range)	0 (0-87151)
CD4 count (cells/mm^3) Median (range)	428 (15-908)
Tumour Size (cm) Median (range)	2.5 (1.0-11.0)
Metastasis	
Absent	50 (98)
Present	1 (2)
Portal Vein Thrombosis (PVT)	
Absent	49 (96)
Present	2 (4)
Nodule	
Uninodular	29 (57)
Multinodular	22 (43)

Using a 1% cut-off for positivity, 24/55 cases (52%) were PD-L1 and 13/55 (28%) were PD-L2 positive in tumour tissue cores, demonstrating a 2-fold higher rate of PD-L1 expression compared to the literature (17%, **Ref.1**). PD-L1 expression in tumour was associated with higher intra-tumoural $CD4^+FoxP3^+$ cell density (40.8 vs. 12.3 cells/mm2, p=0.014, **Fig.1**). PD-L1 was frequently co-immunoexpressed in $CD4^+FoxP3^+$ (49.0 vs. 8.2 cells/mm^2, p=0.002) and $CD8^+PD\text{-}1^+$ (40.8 vs. 12.3 cells/mm^2, p=0.016) in tumour-infiltrating lymphocytes (TILs).

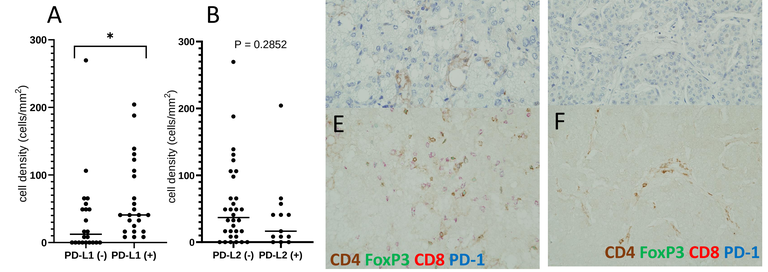

Fig. 1. (A-B) Histograms of $CD4^+FoxP3^+$ cell density and PD-L1/2 expression. (C-F) Representative sections of PD-L1 and multiplex IHC

Peripheral blood CD4 cell count was positively correlated with $CD4^+FoxP3^-$ cell density in tumour and peritumour but not in cirrhotic tissue cores (Spearman r=0.38, 0.45, 0.34, p=0.032, 0.009, 0.053, respectively, **Fig. 2**). However, we found no correlation between blood CD4 count and $CD4^+FoxP3^+$ cell density in any of the sampled areas.

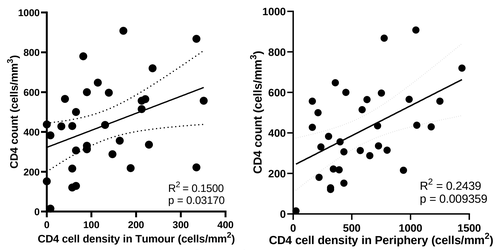

Fig.2. Scatter plot of blood CD4 count and $CD4^+FOXP3^-$ density in tumour (above right) and peritumour (above left)

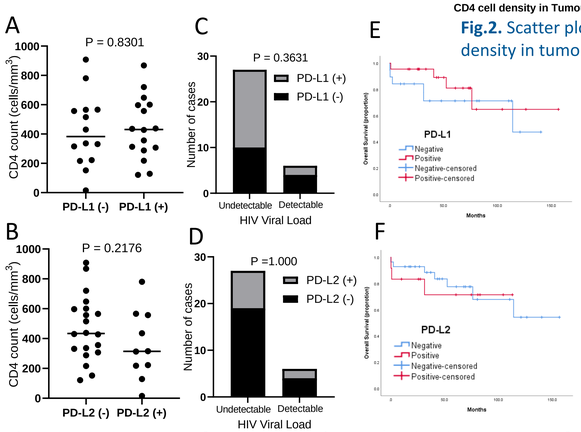

Fig. 3 (A-B) Histograms of CD4 count and PD-L1/L2. (C-D) Proportions of PD-L1/L2 positive. (E-F) Kaplan-Meier curves of overall survival.

PD-L1/L2 expression was not associated with parameters reflective of severity of HIV infection including peripheral blood CD4 count (p=0.830, 0.218, respectively, **Fig. 3A&B**) or HIV viral load (p=0.363, 1.00, respectively, **Fig. 3C&D**).
PD-L1 and PD-L2 expression were independent of key clinicopathologic features of HCC including CTP, BCLC stage, AFP levels, and presence of PVT.
We found no association between PD-L1 or PD-L2 and overall survival (log-rank test p=0.41, 0.75, respectively, **Fig. 3E&F**).

CONCLUSIONS

- PD-L1 expression in HCC cells is a driver of cancer-related immune suppression.
- The positive relationship between peripheral blood CD4 count and $CD4^+FoxP3^-$ T-cell density in tumour & peritumoural tissue indicates a surrogate role for CD4 count as a marker of an effective anti-tumour immune response by identification of T_H cell-rich tumours.
- The contexture of microenvironment was not influenced by biomarkers of severity of HIV infection, suggesting HIV-associated HCC to be potentially responsive to immunotherapy.

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