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*Original Article*

# An immune-inflamed tumor microenvironment as defined by CD8 score is associated with favorable oncologic outcomes in hepatocellular carcinoma independent of measures of tumor mutational burden

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**Abstract:** Despite low mutational burden, immune checkpoint inhibitors have demonstrated promising results in a significant minority of hepatocellular carcinoma (HCC) patients with advanced disease. We hypothesized that HCC patients with higher levels of CD8+ T cell infiltration reflect an immune-inflamed cohort which has improved oncologic outcomes. 355 HCC patients with clinical and transcriptome data in the Cancer Genome Atlas (TCGA) and 151 HCC patients from cohort GSE7624 were analyzed. xCell computational algorithm was used to analyze immune cell infiltration in these patients. Each cohort was divided into high and low expression by the highest 2 terciles value. Gene Set Enrichment Analysis was performed to identify enriched gene sets. High CD8 score associated with improved overall survival in both cohorts (both $P < 0.05$). High score correlates with early BCLC stage ($P = 0.035$) but not AJCC stage. High CD8 also correlated with increased IFN-γ response ($p = 0.038$), lymphocyte infiltration ($P < 0.001$), and leukocyte fraction ($P < 0.001$). It was associated with increased polyclonality of T cell ($P < 0.001$) and B cell response ($P = 0.017$). High CD8 score correlated with increased cytolytic activity score ($P < 0.001$) and expression of multiple immune checkpoints including PD-1, PD-L1, CTLA-4 and Lag3 (all $P < 0.001$). There was no correlation to tumor mutational burden and neoantigens. GSEA demonstrated upregulation of several gene sets involved in inflammatory response and IFN-γ response. In conclusion, HCC patients with high CD8 score demonstrated favorable oncologic outcomes, which may be due to immune-mediated tumor cell attack. Furthermore, CD8 score may be a potentially useful biomarker to select patients for immune checkpoint inhibition.

**Keywords:** Hepatocellular carcinoma, CD8 T cell score, T cell immunity, checkpoint blockade, tumor mutational burden

## Introduction

Hepatocellular carcinoma (HCC) is a primary liver malignancy with the second highest lethality of all cancers [1, 2]. In its advanced form prognosis is dismal and even when localized, curative-intent liver-directed therapy is followed by recurrence more often than not. Recently, a novel combination of immune checkpoint inhibition with antiangiogenic therapy led to an unprecedented 30% response rate and a 5% complete response rate in patients with unresectable HCC [3]. Following T-cell immune attack, tumor cells develop 'adaptive immunoresistance', upregulating inhibitory pathways to limit further T cell effector function [4-6]. Antibodies targeting immune checkpoint pathways - called checkpoint blockade (CPB) - dis-

rupt this T cell inhibition, 'releasing the brake' on the tumor-targeted T cell response. CPB is the primary tool for immune activation against a wide range of solid tumors and has led to dramatic, durable responses in clinical scenarios previously considered uniformly fatal [7-9].

Biomarkers of response to CPB include tumor tissue expression of Programmed Death-Ligand 1 (PD-L1) [10-12], immunohistochemical identification of tumor-infiltrating T cells [13, 14], and various measures of tumor mutational burden [15]. A particularly strong correlation exists between CPB response and both quantity and quality of neoantigens [16, 17], which are uniquely capable of strong T cell activation due to their recognition as foreign antigen. Yet for HCC, the tumor mutational burden is low. That nonetheless there is a significant minority of patients who respond to CPB speaks to a cohort of patients with 'hot' immune inflamed tumors with an initiated and active yet suppressed T-cell mediated immune attack. CD8+ T cells are a primary effector of tumor-targeted immunity and one of the major T cell subsets responsible for clinical response to CPB therapy. We have previously demonstrated that RNA expressional measures of T cell immunity are correlated to oncologic outcomes in multiple solid tumor histologies [18-20]. These measures of T cell immunity correlate to T cell effector gene sets and other immune cell subsets as part of a comprehensive evaluation of the tumor environment that informs prognostic significance.

In this manuscript, we identify a cohort of patients for whom a tumor-targeted immune response exists in the absence of high tumor mutational burden, identifying a cohort of HCC patients with favorable overall survival and which may by particularly susceptible to treatment with immune therapies.

## Methods

### *Clinical and transcriptomic data collection for hepatocellular carcinoma patients*

In this study, 355 patients with HCC in The Cancer Genome Atlas (TCGA) [21] cohort were analyzed. Normalized and $\log_2$-transformed gene expression data were obtained from cBio Cancer Genomic Portal. We obtained the pathological grade data for the TCGA tumors using Text Information extraction System (TIES) Cancer Research Network, as described previously [22-25]. Further, in order to validate findings in a second cohort, 115 patients of the GSE76-427 were obtained from the Gene Expression Omnibus (GEO) database (GEO: https://www.ncbi.nlm.nih.gov/geo/). CD8 score association with degree of liver pathology (normal, premalignant, and malignant liver) was assessed in both GSE6764 [26] and GSE89377 [27] cohorts. Probe-level expression values were summarized using mean to obtain gene expression values. Given that the patient data used in this study, TCGA and GEO cohorts, are all de-identified and are in the public domain, Institutional Review Board approval was waived.

### *Tumor immune microenvironment analysis*

xCell algorithm [28] was used to examine whole-tumor gene expression data to score the relative abundance across tumors of 64 types of immune and stromal cells, as we previously described [29-34]. The CD8 T cell of xCell algorithm was used as the CD8 T cell score in this study in the same manner as was done for fibroblast [29] and adipocyte [33] previously.

### *Gene set enrichment analysis*

To explore signaling pathways enrichment, Gene Set Enrichment Analysis (GSEA) [35] was performed between low and high CD8 T cells score groups using GSEA Java software (https://www.gsea-msigdb.org/gsea/index.jsp version 4.0) with MSigDb Hallmark gene sets [36]. A false discovery rate (FDR) of less than 0.25 was used to deem statistical significance, as recommended by the GSEA.

### *Other statistical analyses*

All analyses and data plotting were performed using R software (https://www.r-project.org/ version 4.0.1, R Project for Statistical Computing) and Microsoft Excel (version 16, Redmond, WA, USA) for Windows. All depicted boxplots are of Tukey type, showing medians and inter-quartile ranges. One-way analysis of variance (ANOVA) or Fisher's exact tests were used to compare group means. The third tertile of the CD8 T cell score was used to divide patients into low and high groups (high = upper 1/3 tertile). Survival among groups was compared using the Kaplan-Meier plot with the log-rank

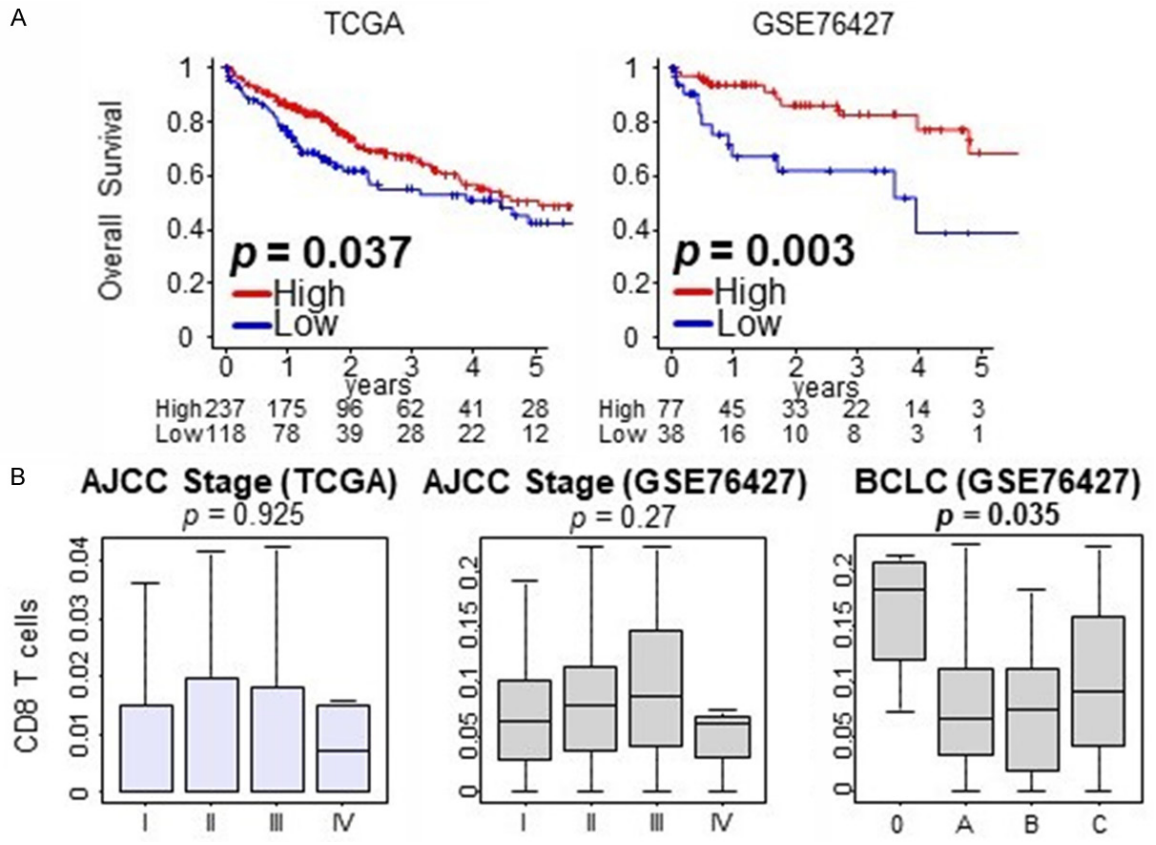


**Figure 1.** A. Correlation of CD8 T Cell score with overall survival in TCGA and GSE76427 patient cohorts in hepatocellular carcinoma. B. Correlation of CD8 T cell score with AJCC and BCLC staging systems.

test. Cox proportional-hazards regression models tested hazard ratios of CD8 T cell score and measures of tumor mutational burden association with overall survival. A *p* value less than 0.05 was considered statistically significant. All experimental protocols were approved by institutional IRB and meet the guidelines of their responsible governmental agency.

## Results

### *CD8 score is associated with overall survival, and with very early stage HCC and pre-malignant liver*

We analyzed gene expression of bulk tumors using the xCell algorithm [28]. The genes that xCell uses for scoring CD8 T cells are listed in Table S1. To establish the clinical relevance of CD8 T-cell infiltration as measured by CD8 score, we first examined the association of the CD8 score with overall survival (OS). After dichotomizing CD8 score to high and low groups using the score's lower tertile value for each cohort (bottom tertile vs top two tertiles), we found a positive association between high CD8 score and OS in both TCGA and GSE764-27 cohorts (**Figure 1A**, both P < 0.05).

Upon evaluating CD8 score (as a continuous variable) association with HCC stage - either American Joint Committee on Cancer staging (AJCC, available for both cohorts) or Barcelona-Clinic Liver Cancer staging (available for GSE-76427 cohort), CD8 score has a statistically significant association with very early HCC (BCLC stage 0, P = 0.035) but not with AJCC staging in neither cohort (**Figure 1B**).

To evaluate an association between a potential loss of immune surveillance as measured by CD8 score with development of a malignant phenotype, we compared CD8 score along a spectrum of pre-malignant histologic change (chronic liver disease/cirrhosis, dysplasia and HCC). CD8 score is associated with pre-malignant cirrhotic livers (**Figure 2**) in both GSE6764 and GSE89377 cohorts.

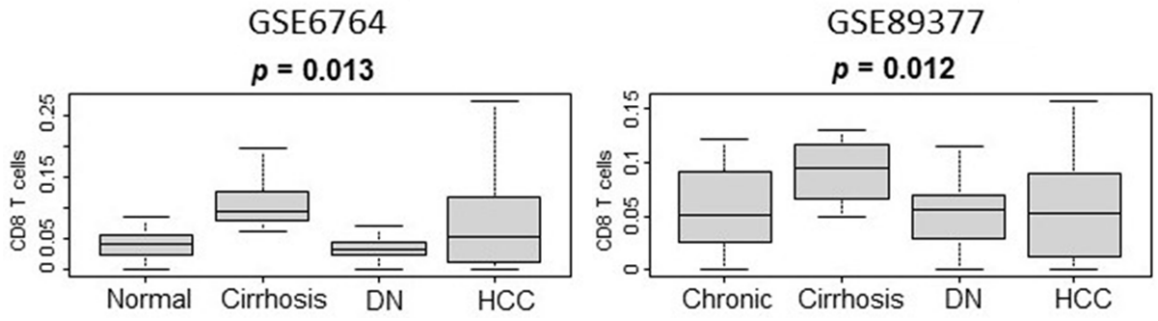


**Figure 2.** Correlation of CD8 T cell score with liver histology along a spectrum of pre-malignant change including chronic liver disease, cirrhosis, dysplasia (DN) and hepatocellular carcinoma (HCC) in patient cohorts GSE67674 and GSE89377.

*CD8 score is associated with measurements of elevated immune activity*

We next analyzed the extent to which CD8 score reflects a favorable anti-tumor inflamed tumor environment. We evaluated immune activity by multiple established methods of measuring immune response at the transcription level, to include cell populations, immune-related scores, and gene set enrichment analysis.

Analyzing immune cell subsets, high CD8 score is associated with CD4+ memory T cells ($P < 0.001$ in both TCGA and GSE76427 cohorts), dendritic cells ($P < 0.001$ TCGA, $P = 0.002$ GSE76427), B cells ($P < 0.001$ TCGA, $P = 0.005$ GSE76427), regulatory T cells ($P < 0.001$ TCGA, $P = 0.019$ GSE76427), Th2 helper T cells ($P = 0.005$ TCGA, $P < 0.001$ GSE76427), and M1 macrophages ($P = 0.069$ TCGA, $P < 0.001$, GSE76427). **Figure 3A** depicts cell subsets typically associated with a pro-cancer environment whereas **Figure 3B** depicts cell subsets typically representing an anti-cancer environment.

Gene set enrichment analysis (GSEA) of MSigDb Hallmark gene sets demonstrated high CD8 score significantly enriched for effector immunity gene sets depicting inflammatory response, IFN-gamma response, and allograft rejection gene sets in both cohorts (**Figure 4A**; normalized enrichment score (NES) = 1.66, NES = 1.58, and NES = 1.54; false discovery rate (FDR) = 0.03, FDR = 0.19, and FDR = 0.03 in TCGA, NES = 1.56, NES = 1.66, NES = 1.72, FDR = 0.03, FDR ≤ 0.01, FDR < 0.01 in GSE76427, respectively). The TGF-β [37, 38] and Wnt/β-catenin [39, 40] signaling pathways contribute to HCC carcinogenesis and also inhibit T cell immune activation [41-44]; we did not find a consistent association with CD8 score in both cohorts (**Figure 4A**).

Next, we investigated the relationship between CD8 score and several tumor immune-related features in the TCGA cohort as previously quantified by Thorsson et al. [45]. HCC tumors with a high CD8 score had significantly increased levels of IFN-gamma response ($P = 0.038$), lymphocyte infiltration ($P < 0.001$), and leukocyte fraction ($P < 0.001$). Antigen-specific T-cell receptors (TCRs) and B-cell receptors (BCRs) serve as determinants of tumor antigen recognition. A high TCR and BCR repertoire polyclonality- or richness - may reflect a more robust anti-tumor immune cell response, with multiple different T cell and B cell clones recognizing tumor antigen and undergoing expansion. We found that CD8 score is associated with polyclonality of the T cell and B cell immune response, as measured by T cell receptor (TCR, $P < 0.001$) and B cell receptor (BCR, $P = 0.017$) richness (**Figure 4B**). Furthermore, CD8 score is positively associated with cytolytic activity score (CYT) - reflecting those genes involved in CD8+ T cell-mediated cytotoxicity/tumor cell killing ($P < 0.001$, **Figure 4C**). These data together suggest that the CD8 score reflects an anti-tumor immune microenvironment that potentially is the biological basis for the positive association with favorable oncologic outcomes.

*Tumor mutation CD8 T cell score does not correlate to tumor mutation burden and mutation burden does not correlate with oncologic outcomes in HCC*

Inflamed tumor environments such as those represented by high CD8 scores are commonly associated with high tumor mutational burden

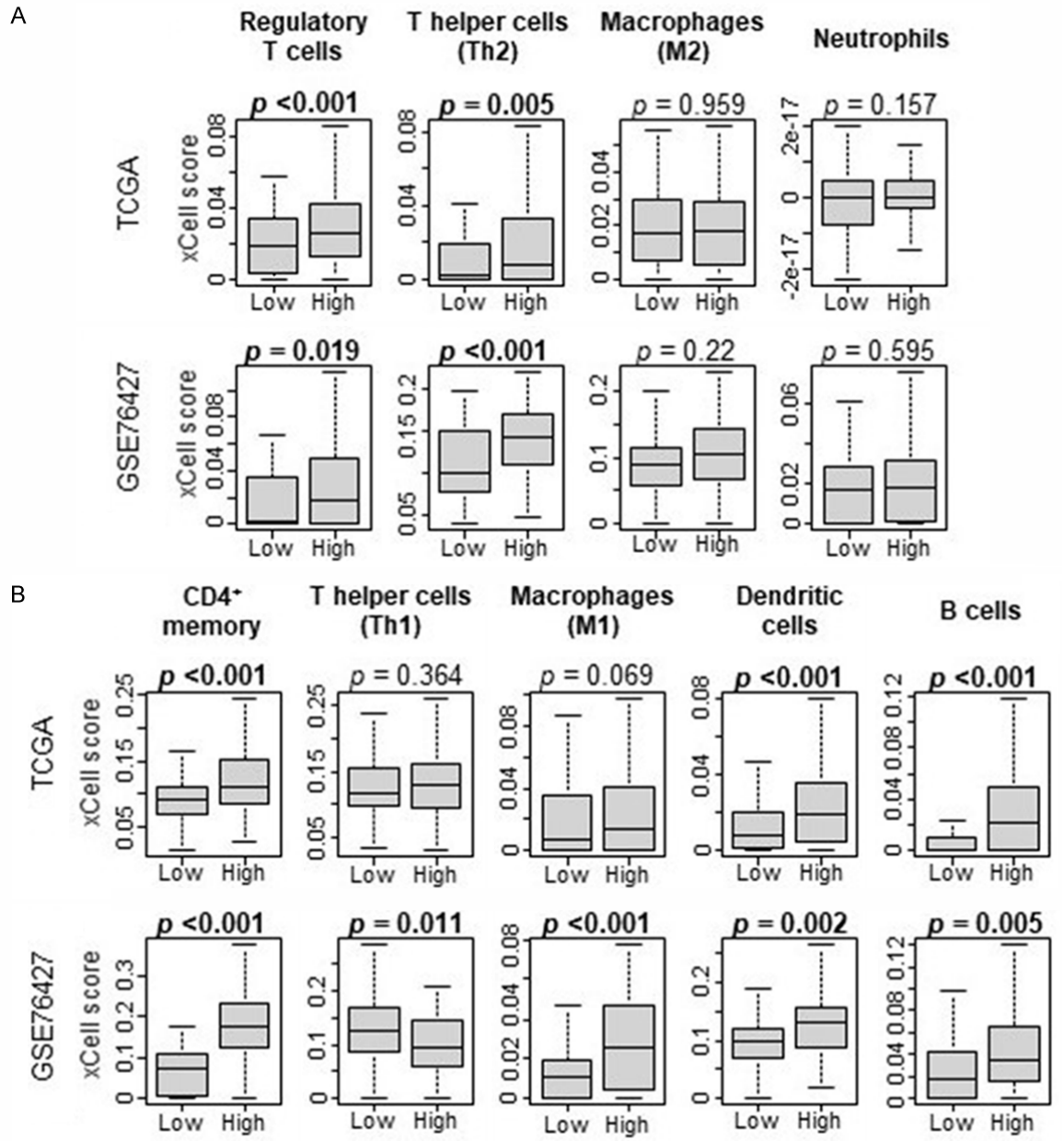


**Figure 3.** A. Correlation of CD8 xCell score with pro-tumorigenic immune cell populations. B. Correlation of CD8 xCell score with anti-tumorigenic immune cell populations.

(TMB) [15]. Nonsilent (or non-synonymous) mutations in tumor coding regions can generate immunogenic neoantigens recognized by T cells as foreign peptides [16, 17]. Insertion and deletion (Indel) mutations create novel open reading frames and as such generate more highly immunogenic peptides than single nucleotide variant (SNV) type mutations [46]. Measurements of DNA damage (intratumor heterogeneity, DNA chromosomal copy number alterations (CNA), and deficiencies in homologous recombination DNA damage repair proteins as measured by the HRD score) can represent predisposition to form immunogenic mutations [47-49]. We assessed correlation of CD8 score to measurements of genomic instability (**Figure 5A**), and measurements of mutational rate (total non-silent mutation rate, as well as neoantigens arising from either Indel or SNV mutations, **Figure 5B**) and did not find any association. High microsatellite mutation rate, or microsatellite instability, also often associates with an inflamed tumor environment and response to checkpoint blockade immunotherapy [50]. We also correlated CD8 score to MSI sensor score, which is a measure of mutation

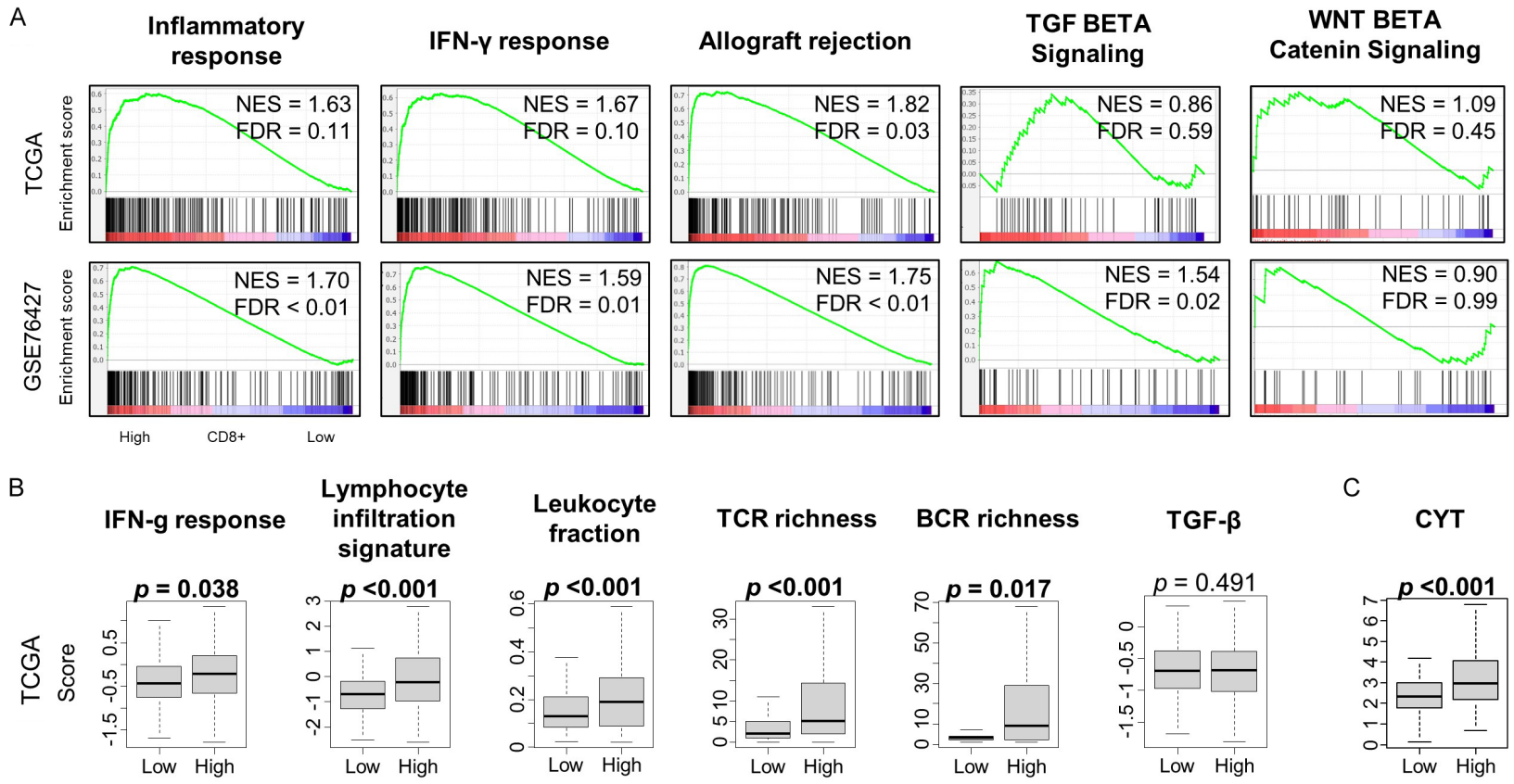


**Figure 4.** A. Gene Set Enrichment Analysis of MSigDb Hallmark gene sets involved in immune signalling and correlation with CD8 score; NES - Normalized Enrichment Score, FDR - False Discovery Rate. B. CD8 T Cell score correlation with tumor immune-related factors in the TCGA cohort. C. Cytolytic Activity Score (CYT) and correlation with CD8 T Cell score.

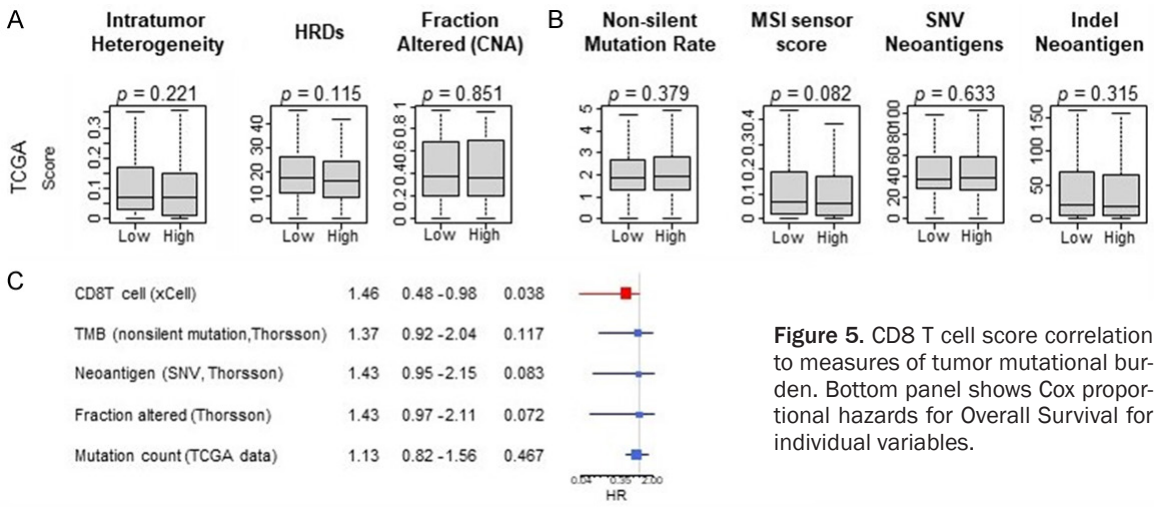


**Figure 5.** CD8 T cell score correlation to measures of tumor mutational burden. Bottom panel shows Cox proportional hazards for Overall Survival for individual variables.

rate in DNA microsatellite regions, and again found no correlation (**Figure 5B**).

Furthermore, tumor mutational burden, number of neoantigens, copy number alterations, and mutation count did not associate with OS (**Figure 5C**). This represents a novel framework within which to classify HCC, identifying an immune inflamed yet mutation poor patient subset with favorable oncologic outcomes.

*CD8 score is associated with expression of multiple immune checkpoints*

We hypothesized that an immune inflamed tumor environment as defined by CD8 score would trigger immune checkpoint receptor and ligand overexpression, a mechanism of adaptive immune resistance that serves to limit the anti-tumor immune response [4-6]. High CD8 score was associated with expression of well characterized checkpoint molecules (PD-1, $r = 0.467$, $P < 0.01$; PD-L1, $r = 0.281$, $P < 0.01$; CTLA-4, $r = 0.512$, $P < 0.01$; LAG3, $r = 0.454$, $P < 0.01$; BTLA, $r = 0.475$, $P < 0.01$; TIM3, $r = 0.275$, $P < 0.01$; **Figure 6**). Association with immune checkpoint expression reinforces the presence of an immune activated tumor microenvironment. Since PD-L1 predicts checkpoint blockade treatment response in other malignancies [10-12], high CD8 score may represent a testable biomarker to select HCC patients for immune checkpoint blockade.

## Discussion

Hepatocellular carcinoma is a primary liver malignancy with the second highest lethality of all cancers [1, 2]. In its advanced form prognosis is dismal and even when localized, curative-intent liver-directed therapy is followed by recurrence more often than not. Recently, a novel combination of immune checkpoint inhibition with antiangiogenic therapy led to an unprecedented 30% response rate and a 5% complete response rate in patients with unresectable HCC [3]. Following T-cell immune attack, tumor cells develop 'adaptive immunoresistance', upregulating inhibitory pathways to limit further T cell effector function [4-6]. Antibodies targeting immune checkpoint pathways disrupt this T cell inhibition, 'releasing the brake' on the tumor-targeted T cell response. Checkpoint blockade (CPB) is the primary tool for immune activation against a wide range of solid tumors and has led to dramatic, durable responses in clinical scenarios previously considered uniformly fatal [7-9]. A particularly strong correlation exists between CPB response and the presence of neoantigens, which are uniquely capable of strong T cell activation due to their recognition as foreign antigen [16, 17]. Yet for HCC, the tumor mutational burden is low. That nonetheless there is a significant minority of patients who respond to CPB speaks to a mechanism of T-cell mediated immune attack with specificity to antigens other than neoantigens and calls for a readily obtainable biomarker to identify those patients who would stand to benefit from CPB therapy so that we may better select patients for therapy.

In this manuscript, we characterize a cohort of patients by a high CD8 T cell RNA expression signature reflecting a primary component of the anti-tumor T cell mediated immune response - CD8+ T cells. That these patients associate with improved overall survival is perhaps

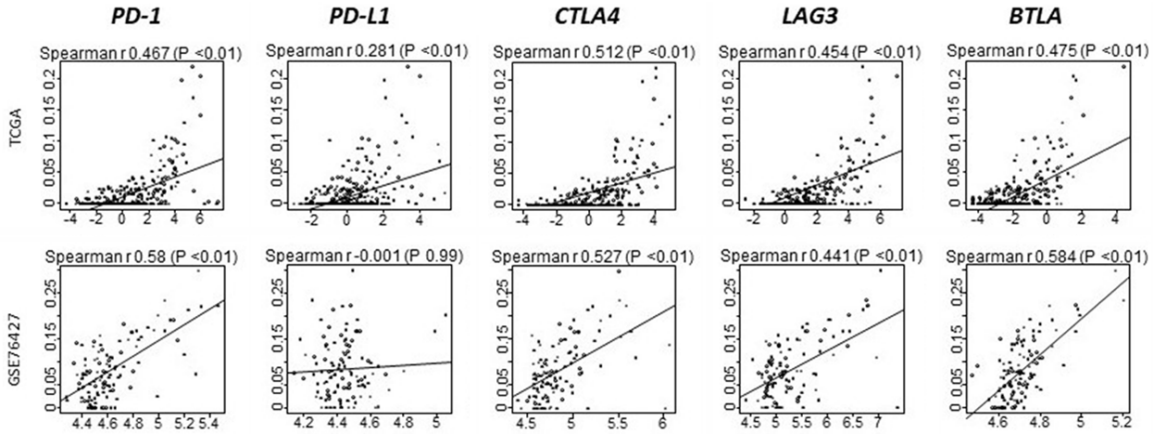


**Figure 6.** CD8 T Cell score correlation with concentration of immune checkpoint markers.

due to a relatively favorable immune-mediated tumor cell attack. We demonstrate that these immune inflamed tumors can be characterized by anti-tumor immune cell subsets, gene set enrichment for effector T cell immunity and expression signatures consistent with activated T- and B-cell mediated immunity, and a corresponding upregulation of inhibitory checkpoint pathways potentially reflecting an adaptive tumor response to an active cancer-specific immunity. Other studies including at our institution have concluded a positive association of HCC survival with other measurements of anti-tumor immune response, including Th1 cytokine producing CD8+ T cells in peripheral blood detected by flow cytometry [51], and immune inflamed gene sets [20, 52]. We extend these observations employing the CD8 T cell score to represent a primary immune cell subset responsible for immune attack and confirm an absence of correlation to multiple measures of tumor mutational burden and neoantigen loads.

That there may be an immune inflamed cohort of patients responsive to immune checkpoint blockade with low mutational burden is a conclusion made by Jaffee and colleagues, identifying such a cohort by immunohistochemistry-based detection of PD-L1 expression within tumor tissue [53]. While we do not have data on treatment response or protein level detection - which is the major limitation of our RNA level expression based analysis - we extend these findings and demonstrate an alternative identifying biomarker of a specifically HCC cohort of patients, a histology typically characterized by an overall low tumor mutational burden yet with a significant minority of patients attaining clinical response to CPB. A significant limitation of our study is that we do not have information on treatments given and treatment response, including any immunotherapy. A next step to be performed in the context of a clinical trial or institutional review would be to test the association of CD8 score with immunotherapy treatment response to assess the value of this marker either alone or in combination with other accepted markers of treatment response (ie. PD-L1 score, tumor mutational burden, and tumor infiltrating lymphocytes [54]).

Other studies identified a CD8 T cell exclusion expression signature correlating to dense fibrosis and HCC disease progression, a signature that may be reversed by blocking TGF-beta signaling [55, 56]. While we similarly detected a decrease in CD8 T cell activity as defined by CD8 T cell score as disease state progressed along the cirrhotic/pre-neoplastic to cancer spectrum, we did not find an association with TGF-beta signaling gene set or Wnt/B-catenin pathway, which is another signaling pathway whose activation has been identified in HCC as critical for both carcinogenesis and T cell immune inhibition [39, 42, 44]. This may reflect as of yet unidentified oncogenic signaling pathways underlying a state of relative T cell dysfunction.

More studies are needed to explain the mechanisms by which HCC patients generate tumor-targeted immunity despite a neoantigen-poor substrate. Immunity to self-antigens - also designated tumor-associated antigens - are a potential target of CD8+ T cell immunity. Such T cell populations have been detected in a multitude of cancer patients across histologies, including for HCC [57]. Whether these T cells merely reflect a correlation and are ultimately

in a state of tolerance given the multiple protective mechanisms limiting self-antigen specific T cell responses has not been studied. The wide breadth of research extensively characterizing the role of neoantigens as the target of high-affinity anti-tumor immune responses and as the target of CPB activated T cell activity serves as an example of how self-antigen specific responses can be studied in cohorts with low tumor mutational burden such as HCC.

## Conclusion

In conclusion, a significant fraction of HCC tumor are 'hot' and can respond to immune therapies. We propose CD8 score to identify this immune inflamed population which can be characterized by improved overall survival, infiltration by anti-tumor immune cell subsets, gene set enrichment for effector T cell immunity, expression signatures consistent with activated T- and B-cell mediated immunity, and a corresponding upregulation of inhibitory checkpoint pathways potentially reflecting an adaptive tumor response to an active cancer-specific immunity. CD8 score may be a potentially useful biomarker to select patients for CPB therapy.

## Disclosure of conflict of interest

None.

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**Table S1.** Symbols and names of genes that constitute CD8 T cell score

| Gene Symbol | Gene Name |
|---|---|
| *AAK1* | AP2 associated kinase 1 |
| *APBB1* | amyloid beta precursor protein binding family B member 1 |
| *ARHGEF1* | Rho guanine nucleotide exchange factor 1 |
| *BTN2A1* | butyrophilin, subfamily 2, member A1 |
| *C7orf26* | chromosome 7 open reading frame 26 |
| *CA6* | carbonic anhydrase 6 |
| *CASP8* | caspase 8 |
| *CBY1* | chibby family member 1, beta catenin antagonist |
| *CCDC25* | coiled-coil domain containing 25 |
| *CCDC53* | coiled-coil domain containing 53 |
| *CCR7* | C-C motif chemokine receptor 7 |
| *CD160* | CD160 molecule |
| *CD27* | CD27 molecule |
| *CD3D* | CD3d molecule |
| *CD7* | CD7 molecule |
| *CD8A* | CD8a molecule |
| *CD8B* | CD8b molecule |
| *CD96* | CD96 molecule |
| *CEPT1* | choline/ethanolamine phosphotransferase 1 |
| *CIAPIN1* | cytokine induced apoptosis inhibitor 1 |
| *CLUAP1* | clusterin associated protein 1 |
| *COG2* | component of oligomeric golgi complex 2 |
| *COPZ1* | coatomer protein complex subunit zeta 1 |
| *CRTAM* | cytotoxic and regulatory T cell molecule |
| *CTSW* | cathepsin W |
| *CX3CR1* | C-X3-C motif chemokine receptor 1 |
| *DHX15* | DEAH-box helicase 15 |
| *DIDO1* | death inducer-obliterator 1 |
| *DNAJB1* | DnaJ heat shock protein family (Hsp40) member B1 |
| *DPP8* | dipeptidyl peptidase 8 |
| *DSC1* | desmocollin 1 |
| *EEF1D* | eukaryotic translation elongation factor 1 delta |
| *EML3* | EMAP like 3 |
| *FAM134C* | family with sequence similarity 134, member C |
| *FBXW4* | F-box and WD repeat domain containing 4 |
| *FKTN* | fukutin |
| *FNBP4* | formin binding protein 4 |
| *FTO* | FTO alpha-ketoglutarate dependent dioxygenase |
| *GGNBP2* | gametogenetin binding protein 2 |
| *GIMAP4* | GTPase, IMAP family member 4 |
| *GJC2* | gap junction protein gamma 2 |
| *GZMH* | granzyme H |
| *GZMK* | granzyme K |
| *GZMM* | granzyme M |
| *HNRNPA0* | heterogeneous nuclear ribonucleoprotein A0 |
| *HNRNPL* | heterogeneous nuclear ribonucleoprotein L |
| *IL16* | interleukin 16 |
| *IPCEF1* | interaction protein for cytohesin exchange factors 1 |
| *IRF3* | interferon regulatory factor 3 |
| *KLHL3* | kelch like family member 3 |
| *KLRB1* | killer cell lectin like receptor B1 |
| *KLRG1* | killer cell lectin like receptor G1 |
| *KRT2* | keratin 2 |
| *LAIR2* | leukocyte-associated immunoglobulin-like receptor 2 |
| *LSM14A* | LSM14A mRNA processing body assembly factor |
| *LY9* | lymphocyte antigen 9 |
| *MED17* | mediator complex subunit 17 |

| | |
|---|---|
| *MKRN2* | makorin ring finger protein 2 |
| *MMP19* | matrix metallopeptidase 19 |
| *MSL3* | male-specific lethal 3 homolog |
| *MTRF1* | mitochondrial translation release factor 1 |
| *MYOM1* | myomesin 1 |
| *NAA16* | N(alpha)-acetyltransferase 16, NatA auxiliary subunit |
| *NDFIP1* | Nedd4 family interacting protein 1 |
| *NDUFS2* | NADH:ubiquinone oxidoreductase core subunit S2 |
| *NFKB1* | nuclear factor kappa B subunit 1 |
| *NKRF* | NFKB repressing factor |
| *NPAT* | nuclear protein, coactivator of histone transcription |
| *NPRL2* | NPR2 like, GATOR1 complex subunit |
| *PCNT* | pericentrin |
| *PFN2* | profilin 2 |
| *PLCG1* | phospholipase C gamma 1 |
| *PLXDC1* | plexin domain containing 1 |
| *POLR3E* | RNA polymerase III subunit E |
| *POP5* | POP5 homolog, ribonuclease P/MRP subunit |
| *PRL* | prolactin |
| *PRMT2* | protein arginine methyltransferase 2 |
| *PRPF4B* | pre-mRNA processing factor 4B |
| *PSD* | pleckstrin and Sec7 domain containing |
| *PTGDR* | prostaglandin D2 receptor |
| *PTPN4* | protein tyrosine phosphatase non-receptor type 4 |
| *PURA* | purine rich element binding protein A |
| *RAPGEF6* | Rap guanine nucleotide exchange factor 6 |
| *RASA2* | RAS p21 protein activator 2 |
| *RBL2* | RB transcriptional corepressor like 2 |
| *RBM34* | RNA binding motif protein 34 |
| *RING1* | ring finger protein 1 |
| *RNF113A* | ring finger protein 113A |
| *RPL37A* | ribosomal protein L37a |
| *RWDD3* | RWD domain containing 3 |
| *S100B* | S100 calcium binding protein B |
| *SDAD1* | SDA1 domain containing 1 |
| *SDCCAG3* | serologically defined colon cancer antigen 3 |
| *SFPQ* | splicing factor proline and glutamine rich |
| *SHANK1* | SH3 and multiple ankyrin repeat domains 1 |
| *SIRPG* | signal-regulatory protein gamma |
| *SLC1A7* | solute carrier family 1 member 7 |
| *SSTR3* | somatostatin receptor 3 |
| *TBCC* | tubulin folding cofactor C |
| *TMEM41B* | transmembrane protein 41B |
| *TOMM7* | translocase of outer mitochondrial membrane 7 |
| *TRAF3IP3* | TRAF3 interacting protein 3 |
| *TSPAN32* | tetraspanin 32 |
| *TTN* | titin |
| *UBE2Q1* | ubiquitin conjugating enzyme E2 Q1 |
| *UBQLN2* | ubiquilin 2 |
| *USP47* | ubiquitin specific peptidase 47 |
| *UTP20* | UTP20 small subunit processome component |
| *WDR82* | WD repeat domain 82 |
| *YLPM1* | YLP motif containing 1 |
| *ZBTB11* | zinc finger and BTB domain containing 11 |
| *ZC3HAV1* | zinc finger CCCH-type containing, antiviral 1 |
| *ZNF154* | zinc finger protein 154 |
| *ZNF200* | zinc finger protein 200 |
| *ZNF611* | zinc finger protein 611 |
| *ZNF639* | zinc finger protein 639 |