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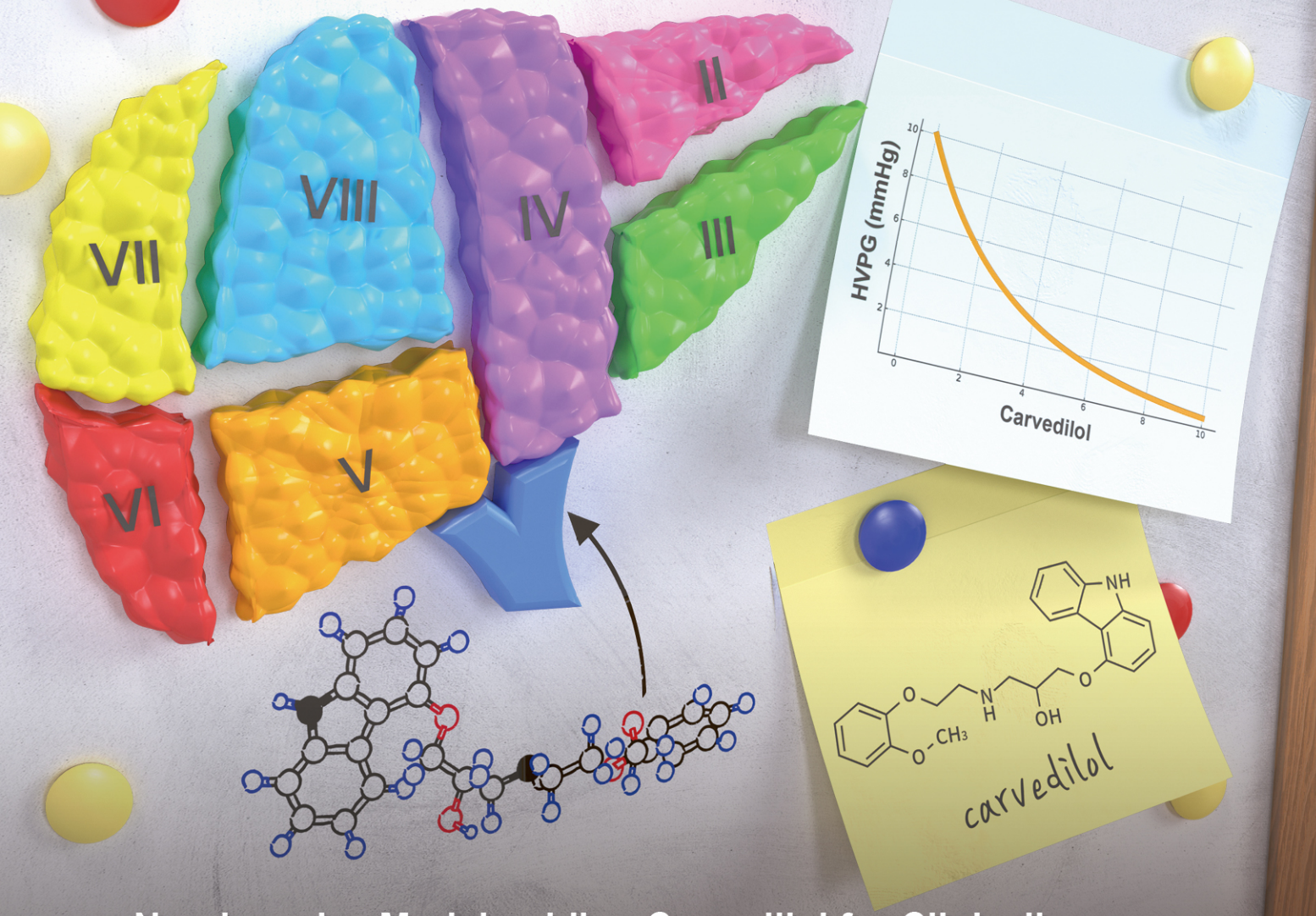


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Editorial

# Personalized approaches to the treatment of hepatocellular carcinoma using immune checkpoint inhibitors: Editorial on "Genomic biomarkers to predict response to atezolizumab plus bevacizumab immunotherapy in hepatocellular carcinoma: Insights from the IMbrave150 trial"

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Most cancers diagnosed in routine clinical practice are characterized by a suppressive tumor immune microenvironment (TME), which enables them to evade anti-tumor immunity. Since immune checkpoint inhibitors (ICIs) aim to reactivate anti-tumor immunity, understanding the characteristics of the TME is crucial for predicting the efficacy of ICI-based treatments.[1]

Yim et al.[2] developed an immune signature score (ISS) based on mRNA expression to stratify patients with hepatocellular carcinoma (HCC) treated with atezolizumab plus bevacizumab (Atezo/Bev). Among patients classified as high responders by the ISS, those treated with the Atezo/Bev combination exhibited improved overall survival (OS), progression-free survival, and a better objective response rate, such outcomes not observed in patients treated with sorafenib. To create a practical tool, the ISS was refined to include the expression profile of 10 key genes (ISS10). A significant correlation was found between ISS10 and the response to Atezo/Bev as well as nivolumab and ipilimumab (Nivo/Ipi) treatment. The ISS10 high subtype exhibited a more favorable TME, characterized by higher proportions of anti-tumor macrophages and activated T-cells.

Several transcriptomics-based approaches have been conducted to classify HCC according to the TME and to identify molecular signatures for predicting responsiveness to ICIs. HCCs are generally classified into two main TME classes: one characterized by abundant immune cells within the tumor (inflamed class, 25–40% of HCC cases) and the other by a paucity of immune-related cells (non-in-

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flamed class, 60–75%). A subset of inflamed HCCs (15–20%) shows particularly high infiltration of $CD8^+$ cytotoxic T lymphocytes (CTLs), with low levels of immunosuppressive cells, such as regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs). This subclass has been referred to as "immune active," "immunogenic," "cytolytic activity," or "immune-activated". Inflamed HCCs are also characterized by high expression of molecules involved in antigen presentation (e.g., Human Leukocyte Antigen), activation of dendritic cells (DCs), infiltration of M1 macrophages, high expression of chemokines that attract immune effector cells (e.g., CCL4, CCL5, CXCL10, CXCL11), and enhanced interferon-γ (IFN-γ) and programmed cell death 1 (PD-1) signaling.[3-7] Additionally, the presence of tertiary lymphoid structures, which are thought to be involved in the establishment and maintenance of acquired immunity, is more frequently observed.[3,8] These findings strongly suggest that this subclass is characterized by robust antigen presentation, immune cell recruitment, and subsequent anti-tumor immune responses. The TME profile that contributes to the efficacy of Atezo/Bev combination therapy reportedly includes a high intratumoral density of $CD8^+$ CTLs, high expression of effector T cell-related molecules and PD-L1, and low proportions of Tregs,[9] consistent with the features of the "immune active" subclass.

Conversely, a subclass within non-inflamed HCCs shows sparse tumor-infiltrating lymphocytes (TILs) and a high frequency of β-catenin mutations.[3,7] This subclass, often referred to as "immune excluded" or "immune desert," exhibits activation of the WNT/β-catenin signaling pathway and accounts for approximately 20–30% of HCCs. These tumors demonstrate low expression of molecules involved in antigen presentation and of cytokines/chemokines that activate immune effecter cells. Molecules related to IFN-γ signaling and immune checkpoint pathways are also expressed at low levels. This subclass is reportedly associated with high expression of hepatocyte nuclear factor 4A, which is involved in hepatocyte differentiation, along with nuclear accumulation of β-catenin. Thus, this subclass may exhibit features of differentiated HCC with activation of WNT/β-catenin signaling. While this type of HCC shows a relatively favorable prognosis, it is believed to have low responsiveness to immunotherapies due to the paucity of anti-tumor immune effector cells.[9,10] However, despite genomic alterations in the WNT/β-catenin signaling pathway being proposed as a cause for sparse DCs and TILs,[11] analyses of samples from large prospective trial cohorts do not support the role of genes involved in this pathway as biomarkers for non-responders. Another study indicate that baseline gene expression signatures related to differentiation correlate with more favorable outcomes, whereas activation of signaling pathways associated with aggressive disease is linked with poorer outcomes on ICIs.[12] This evidence suggests that a prolonged anti-tumor immune response may well induce the production of various immunosuppressive cytokines, chemokines, growth factors, and metabolites, which attract immunosuppressive cells into tumor tissues and eventually establish a solid immunosuppressive TME.

While numerous studies have reported associations between the TME and the efficacy of ICIs in HCC, there are currently no established biomarkers that can reliably predict clinical response in real-world practice. The ICI-containing treatment regimens approved for HCC include durvalumab monotherapy (anti-PD-L1 antibody), the combination of durvalumab and tremelimumab (Durva/Treme: anti-PD-L1 and anti-CTLA-4 antibody), and Atezo/Bev combination therapy (anti-PD-L1 and anti-VEGF-A antibody). The first two are considered pure ICI treatments, while the Atezo/Bev combination is defined as a combination of an ICI and an anti-VEGF molecular-targeted agent. Regarding the exploration of biomarkers for combination of ICI and an anti-VEGF molecular-targeted agent, analyses of 358 HCC patients enrolled in the phase 1 (GO30140) and phase 3 (IMbrave150) trials of the Atezo/Bev combination therapy have been reported (Table 1). Pre-existing immunity, defined by high PD-L1 expression, high expression of effector T cell (Teff)-related genes (CXCL9, PRF1, GZMB), and high

**Abbreviations:**

Atezo/Bev, atezolizumab and bevacizumab; CCL, C-C chemokine ligand; CTLA-4, cytotoxic T lymphocyte antigen-4; CTLs, cytotoxic T lymphocytes; CXCL, C-X-C motif chemokine ligand; DCs, dendritic cells; Durva/Treme, durvalumab and tremelimumab combination therapy; HCC, hepatocellular carcinoma; ICIs, immune checkpoint inhibitors; IFN-γ, interferon-γ; ISS, immune signature score; MDSCs, myeloid-derived suppressor cells; Nivo/Ipi, nivolumab and ipilimumab; OS, overall survival; PD-1, programmed cell death 1; PD-L1, programmed cell death ligand 1; PFS, progression-free survival; Teff, effector T cell; TGF, transforming growth factor; TILs, tumor-infiltrating lymphocytes; TME, tumor immune microenvironment; Tregs, regulatory T cells; VEGF, vascular endothelial growth factor


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**Table 1.** Clinical trial reported for ICI-based therapy and exploratory biomarker study

| Type | ICI and anti-VEGF combination | | Pure ICI | | | |
|---|---|---|---|---|---|---|
| Trial | IMbrave 150 & GO30140 | HIMALAYA | Checkmate 459 | Checkmate 040 | Checkmate 040 | KEYNOTE-224 |
| Phase | Phase III and phase I | Phase III | Phase III | Phase I/II | Phase I/II | Phase II |
| Agents | Atezo/Bev combination | Durva/Treme combination | Nivolumab | Nivolumab | Nivo/Ipi combination | pembrolizumab |
| Design | Atezo/Bev vs. sorafenib | Durva/Treme vs. durvalumab vs. sorafenib | Nivolumab vs. sorafenib | Nivolumab | Nivo/Ipi (for 2nd line therapy) | pembrolizumab (n=52) |
| Biomarker | Treg/Teff ratio, PD-L1 expression, $CD8^+$ TIL, Teff gene expression signature, Atezo/Bev response signature. Myeloid inflammation signature | PD-L1 (tumor area) | PD-L1 (tumor cell) | PD-L1 (tumor cell), T-cell infiltration, inflammatory gene signature, NLR and PLR | PD-L1 (tumor cell) | PD-L1 (CPS and TPS) |
| Observation | Teff gene expression signature, Atezo/Bev response signature, myeloid inflammation signature, high PD-L1 expression, and dense $CD8^+$ TIL is associated with improved mPFS. A high ratio of Tregs to Teff cells is associated with poorer clinical benefits. | No correlation with response. | No correlation with OS[a] Baseline expression of inflammation-associated genes shows benefit in nivolumab treatment[14] | PD-L1 in tumor cell ≥ 1%, $CD3^+$ and $CD8^+$ T-cell infiltration, inflammatory gene signature, and low NLR and PLR are associated with improved OS. | Both patients with PD-L1-posivtive and -negative HCC responded to treatment. | PD-L1 CPS is associated with response. |
| Reference | 8 | [b] | 14, [a] | 13 | [C] | 16 |

ICIs, immune checkpoint inhibitors; VEGF, vascular endothelial growth factor; Atezo/Bev, atezolizumab and bevacizumab; Durva/Treme, durvalumab and tremelimumab; Nivo/Ipi, nivolumab and ipilimumab; Treg, regulatory T cells; Teff, effector T cell; PD-L1, programmed cell death ligand 1; TIL, tumor infiltrating lymphocyte; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio; CPS, combined positive score; TPS, tumor proportion score; PFS, progression-free survival; OS, overall survival.

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[a]Yau T, Park JW, Finn RS, Cheng AL, Mathurin P, Edeline J, et al. Nivolumab versus sorafenib in advanced hepatocellular carcinoma (CheckMate 459): a randomised, multicentre, open-label, phase 3 trial. Lancet Oncol 2022;23:77-90.

[b]Abou-Alfa GK, Lau G, Kudo M, Chan SL, Kelley RK, Furuse J, et al. Tremelimumab plus durvalumab in unresectable hepatocellular carcinoma. NEJM Evid 2022;1:EVIDoa2100070.

[c]Yau T, Kang YK, Kim TY, El-Khoueiry AB, Santoro A, Sangro B, et al. Efficacy and safety of nivolumab plus ipilimumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib: The CheckMate 040 Randomized Clinical Trial. JAMA Oncol 2020;6:e204564.

$CD8^+$ TIL density, was also associated with favorable outcomes, like those observed in pure ICI therapy. On the other hand, a high ratio of Tregs to effector T cells and the expression of oncofetal proteins were associated with poorer clinical benefits.[9,13] Interestingly, when comparing the outcomes of Atezo/Bev combination with atezolizumab monotherapy, improved outcomes with Atezo/Bev were observed in patients with increased infiltration of Tregs and myeloid cells, as well as high expression of VEGF receptor and high tumor vessel density.[9] Therefore, HCC patients with higher levels of Treg and myeloid cell proliferation, and/or angiogenesis in tumor, which reflect features observed in a subset of non-inflamed tumors, may benefit from VEGF-targeted ICI combination therapy, a benefit not observed with pure ICI therapies. From this perspective, VEGF-targeted therapies may play a role in modulating immunosuppressive cells and contributing to the conversion of an immunosuppressive TME into an immune-active one. Furthermore, in a real-world setting, the Atezo/Bev combination therapy has been reported to be more effective in HCC patients with high neutrophil-to-lymphocyte ratio (NLR).[13]

In contrast, exploration of biomarkers for pure ICI treatments, nivolumab monotherapy, using samples before treatment have shown that PD-L1 expression, an inflammatory gene expression signature, and the low NLR and platelet-to-lymphocyte ratio in peripheral blood are associated with improved survival outcomes and response rates, according to data from the phase 1/2 CheckMate 040 clinical trial (Table 1).[14] A part of these findings aligns with data from the phase 3 CheckMate 459 study, which reported an association between baseline expression of inflammation-associated genes and benefit from nivolumab compared to sorafenib using transcriptomic analysis.[15] Another analysis found a correlation between clinical responses and an increase in $CD8^+$ TILs, specifically with an increase in two effector T-cell clusters, in HCC patients who received perioperative pure ICI therapy (Nivo/Ipi) followed by surgical resection.[16] A phase 2 study (KEYNOTE-224) using the anti-PD-1 antibody pembrolizumab as second-line therapy also showed a trend towards higher ORR in patients with high PD-L1 expression in tumor tissue compared to those with low PD-L1 expression (Table 1).[17] In a single-arm study involving HCC patients who received second-line pembrolizumab, responders exhibited elevated levels of intratumoral CTLs on pretreatment biopsy samples.[18] Peripheral blood mononuclear cells from pre-treatment and post-treatment samples were also analyzed using single-cell RNA sequencing, demonstrating that patients with a response or stable disease had elevated circulating cytotoxic $CD8^+$ T-cell levels, whereas those with progressive disease had an increased number of both $CD14^+$ and $CD16^+$ monocytes and activation of neutrophil-associated signaling pathways. A clinical trial involving tremelimumab monotherapy, an anti-CTLA-4 antibody, in hepatitis C-related HCC patients, also demonstrated that changes in serum IFN-γ levels at day 60 of treatment were associated with an anti-tumor response.[19]

Although the long-term follow-up of an expanded cohort from CheckMate 040 for the combination of Nivo/Ipi failed to show a clear relationship between tumor PD-L1 expression or $CD8^+$ TILs and OS, likely due to the effects of subsequent treatments altering the TME,[20] many reports commonly pointed out that features indicative of pre-existing immunity, including high PD-L1 expression, Teff cell gene signatures, and dense $CD8^+$ TILs, enrich the benefit from pure ICI treatment in HCC patients. However, current predictive biomarkers leave considerable room for improvement. Especially, although anti-VEGF therapy has the potential to improve the immunosuppressive TME by regulating Tregs and MDSCs, further large cohort study is required to determine the role of anti-VEGF agents in non-inflamed HCCs.

Yim et al. reported a practical tool, ISS10, for predicting outcomes of ICI-based therapy. To achieve substantial improvements, future biomarker studies should likely require multimodal integration of data from pathology, medical image, and health records, as well as omics data. Information regarding tumor heterogeneity, clonal structure, and neoantigen expression patterns should also be considered.[21] Given the complexity of TME analysis, the application of deep learning and generative learning technologies will be valuable. Such efforts will be crucial for refining patient stratification and personalizing treatment approaches to enhance the effectiveness of ICI-based regimens.

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## Conflicts of Interest

The author has no conflicts to disclose.

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