that the HR for OS was slightly lower among patients with high AFP in KEYNOTE-240 (pembrolizumab vs placebo)[65] and CheckMate 459 (nivolumab vs sorafenib),[135] while the contrary was observed in IMbrave150 (atezolizumab with bevacizumab vs placebo).[16 70] Furthermore, objective remissions occur irrespective of AFP levels after nivolumab or pembrolizumab monotherapies, or the combination of ipilimumab and nivolumab.

A number of features of the tumor microenvironment have been associated with HCC prognosis, including overall lymphocyte infiltration, density of Tregs, and tumor-associated macrophages (TAMs), especially if M2-polarized. In melanoma, the presence of conventional type 1 dendritic cells seems critical to promote a T and NK cell infiltrate and for the action of ICIs.[136] In HCC animal models, β-catenin-mutations in HCC (which are present in around 25% of human HCCs) result in a paucity of intratumoral conventional type 1 dendritic cells,[137] and it has been proposed that β-catenin defects may be used to identify patients with disease that will fail to respond to PD-1 blockade.[138] This feature awaits investigation in clinical trials. Soluble factors also modulate the immune response against HCC. For example, transforming growth factor beta (TGF-β) downregulates antitumor responses through a variety of different mechanisms, and high levels of the cytokine shape the response to pembrolizumab.[139]

Pretreatment tumor infiltration by T cells and their activity status are key to determine response to ICIs in various cancers. In advanced HCC, $CD4^+$ and $CD8^+$ T cell infiltration showed weak correlations with survival after second-line treatment with PD-1 inhibitors in CheckMate 040.[140] In the trial, deep antitumor responses were observed regardless of PD-L1 expression after nivolumab treatment, although the response rate was higher among patients with at least 1% of tumor cells expressing PD-L1.[140]

On the other hand, PD-L1 expression in tumor or stromal immune cells was higher among responders to pembrolizumab, but remissions also occurred in the absence of expression in both cell types.[65] In CheckMate 459, median OS after nivolumab and sorafenib was 16.1 months versus 8.6 months among patients that had tumor PD-L1 expression ≥1% (HR 0.80), and 16.7 months versus 15.2 months among those that had tumor PD-L1 expression <1% (HR 0.84).[135] Interestingly, macrophage infiltration, including M2-polarized TAMs, was not associated with clinical outcomes after nivolumab treatment. A meta-analysis including 894 patients across nine trials of ICIs in advanced HCC found a positive association between PD-L1 expression and response to therapy—especially for single-agent anti-PD-1. Strikingly, in the analysis, PD-L1 expression status had minimal association with response to therapy for patients being treated with anti-CTLA-4-containing combinations.[141] Analytical heterogeneity in PD-L1 expression is substantial, however, and may contribute to the performance of this test as a predictive biomarker.[142]

Several inflammatory gene signatures are correlated with higher response rate and improved OS after nivolumab treatment.[140] Interestingly, the most complex transcriptomic classifications of inflammatory HCC including a large number of genes[143] were not identified as predictive of response in this analysis, suggesting that short gene signatures may be more relevant for clinical development. Regarding ICI combinations, objective remissions occurred with ipilimumab plus nivolumab irrespective of PD-L1 expression in tumor cells.[144] An early burst of $Ki67^+CD8^+$ cells in the peripheral blood was also seen in one of the randomized expansion cohorts for Study 22, which evaluated combinations of durvalumab and tremilimumab at different dosing regimens,[145] hinting that cytotoxic T cell proliferation after therapy may predict response. Altogether, though, it seems unlikely that a single biomarker could be used to inform clinical decisions in a timely fashion. However, it is probable that composed and integrative multifactorial indexes might help identify patient subsets who are likely to benefit, further underscoring the importance of obtaining pretreatment tumor biopsies for future translational studies.

Pembrolizumab is FDA-approved for two tissue-agnostic indications based on tumor-intrinsic characteristics. Approval for pembrolizumab for the treatment of microsatellite-high (MSI-H) or mismatch repair deficient (dMMR) tumors was based on a pooled ORR of 39.6% (95% CI 31.7% to 47.9%), with a 7% CR rate among 149 patients with 15 different tumor types in five single-arm multi-cohort multicenter trials: KEYNOTE-016,[146] KEYNOTE-164,[147] KEYNOTE-012[148] KEYNOTE-028,[149] and KEYNOTE-158.[150] Approval for pembrolizumab for non-MSI-H/dMMR tumors with high mutation burden (TMB-H)—defined as ≥10 mutations per megabase (mut/Mb) as assayed by the FoundationOne CDx companion diagnostic—was based on KEYNOTE-158.[151] No patients with HCC were included in the cohorts upon which the tissue-agnostic indications for pembrolizumab were approved, however.

TMB correlates with the number of neoantigens and response to ICIs in tumors with >20 somatic mut/Mb, such as melanoma.[152 153] However, HCC is infrequently MSI-H/dMMR or TMB-H. One study that performed comprehensive genomic profiling of 755 patients with advanced HCC found a median TMB of 4 mut/Mb and that only six tumors (0.8%) were TMB-H. Furthermore, out of 542 cases assessed, only one (0.2%) was MSI-H.[154] Another analysis found a rate for MSI-H as low as 6%.[155]

Markers of systemic inflammation like neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) have shown a strong prognostic impact in HCC across tumor stages. Lower NLR has been associated with better outcomes after sorafenib,[156 157] and similar trends are emerging from trials of ICIs. In CheckMate 040, patients progressing on nivolumab had a higher NLR and PLR than patients who had disease control as the best overall response.[140] Consistent with this observation,