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ORIGINAL ARTICLE

Genomic profiling in hepatocellular carcinoma: a real-world retrospective analysis

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Background: Hepatocellular carcinoma (HCC) is a leading cause of cancer-related mortality, with limited molecular characterization due to reliance on radiological diagnosis. This study aims to characterize the genomic landscape of advanced HCC and assess the prognostic and predictive roles of genetic alterations (GAs).

Patients and methods: This study used a de-identified nationwide (USA-based) HCC clinico-genomic database to retrospectively analyze patients with advanced HCC who received systemic therapies and underwent comprehensive genomic profiling via tissue or liquid biopsy. GAs were identified using Foundation Medicine, Inc.'s next-generation sequencing tests. Time to progression (TTP) was assessed using Kaplan—Meier analysis and Cox proportional hazards models.

Results: In total, 370 patients were analyzed. The most frequent GAs in the tissue cohort ($n = 291$) involved *TERT* promoter (*TERT*p, 61.5%), *CTNNB1* (34.0%), and *TP53* (33.0%). Key affected pathways were cell cycle and apoptosis (56%), DNA damage and control (43%), WNT (40.9%), and p53 (38.1%). Viral etiology was significantly associated with alterations in *TERT*p, *CTNNB1*, and the WNT pathway, while non-viral HCC was associated with alterations in the RTK/RAS pathway. TTP analysis revealed a trend toward improved outcomes with atezolizumab + bevacizumab (A + B) compared with tyrosine kinase inhibitors. *TP53,* p.V157F, and p.R249S mutations were associated with significantly shorter TTP. *MYC* seemed to be a negative predictor for A + B versus tyrosine kinase inhibitors, but statistical significance was not reached.

Conclusions: This study highlights the genomic landscape of advanced HCC, identifying cell cycle and apoptosis, DNA damage and control, WNT, and p53 as the key affected pathways. Further research is warranted to confirm such findings.

Key words: HCC, genomic profiling, immunotherapy, atezolizumab, bevacizumab, TKI

INTRODUCTION

The incidence of liver cancer is growing worldwide,[1] ranking as the fourth leading cause of cancer-related deaths.[2] Hepatocellular carcinoma (HCC) is the most common primary liver cancer.[3] In 80%-90% of cases, HCC arises in the context of cirrhosis due to hepatitis B virus (HBV) and hepatitis C virus (HCV) infections, chronic alcohol consumption, or metabolic dysfunction-associated steatotic liver disease.[4,5]

Unfortunately, most patients are diagnosed at advanced stages, when curative treatments are no longer viable.[6]

According to guidelines,[7-9] if immunotherapy is not contraindicated, first-line combinations are atezolizumab + bevacizumab[10,11] or durvalumab + tremelimumab[12-14]; tyrosine kinase inhibitors (TKIs) are recommended as second- and third-line treatments. If immunotherapy is contraindicated, lenvatinib/sorafenib[15] is the first choice.

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Since HCC can also be diagnosed through radiological criteria, unlike other neoplasms where diagnosis relies solely on histological or cytological assessment, there is a lack of data on histological and molecular characterization. Several molecular pathways were reported to be dysregulated, but translating these findings into clinical practice remains challenging, as only 28% of HCCs carry potentially actionable alterations[16] and none can currently be classified as Scale for Clinical Actionability of molecular Targets I-II in this tumor type.[17]

While most genomic studies have focused on early-stage disease,[18,19] the molecular landscape of advanced HCC remains poorly characterized.

This study used the Flatiron Health (FH)-Foundation Medicine, Inc. (FMI) clinico-genomic database (CGDB) to primarily characterize the genomic profile of patients with advanced HCC who received systemic therapies in the United States. Moreover, we sought to assess the prognostic and predictive roles of altered genes and gene pathways.

MATERIALS AND METHODS

Data source

This study relied on the nationwide (USA-based) de-identified FH-FMI HCC CGDB. The de-identified data originated from ∼280 USA cancer clinics (∼800 sites of care). Retrospective longitudinal clinical data were derived from electronic health record data, comprising patient-level structured and unstructured data, curated via technology-enabled abstraction, and were linked to genomic data derived from FMI comprehensive genomic profiling (CGP) tests in the FH-FMI CGDB by de-identified, deterministic matching.[20] Genetic alterations (GAs) were identified via CGP on FMI next-generation sequencing tests.[21-24] More information on FMI tests can be found in the Supplementary Materials and Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2025.105879. The data are de-identified and subject to obligations to prevent re-identification and protect patient confidentiality.

Patient population

Patients were analyzed if they were diagnosed with HCC, had two or more visits in the FH network between 1 January 2011 and 31 March 2024, underwent tissue and/or liquid CGP on a tumor sample within 30 days before or any time after the HCC initial diagnosis, and received systemic therapy. Patients with evidence of treatment from unstructured activity that occurred >30 days before the start of structured activity (i.e. indicative of potentially missing therapy data) were excluded to ensure correct treatment sequencing.

Lines of therapy

Oncologist-defined, rule-based lines of therapies were classified into categories based on input from clinical experts: anti-angiogenic agents, including TKIs atezolizumab + bevacizumab (A + B); other immunotherapy (IO); TKI-IO combinations; experimental treatment; and other treatments (Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2025.105879).

Outcomes

The primary objective of the study was to characterize the genomic landscape of HCC by assessing the frequency and co-occurrence of GAs. Patients were analyzed separately based on different forms of biopsy (tissue versus liquid). Gene mutational status was determined by aggregating all test results, with only known or likely pathogenic variants considered to be positive [mutant (Mut) versus wild-type (WT)]. Genes were manually assigned to signaling pathways, as detailed in Supplementary Table S3, available at https://doi.org/10.1016/j.esmoop.2025.105879.[25-28] A pathway was considered altered in a patient if at least one gene within the pathway had a qualifying GA.

The secondary outcome measurement was time to progression (TTP), which was defined as the time from the start of L1 therapy (index date) to the first progression date >14 days after treatment initiation.[29] Patients without an event were censored at the date of death[30] or the last clinical note. Survival analyses were limited to patients profiled with tissue-based CGP who initiated treatment before 30 September 2023, to ensure a minimum follow-up period of 6 months. Patients profiled with liquid biopsy were excluded due to insufficient sample size.

Statistical analysis

Descriptive statistics included demographics, clinical characteristics, treatment, and testing patterns. To handle missing data, we used the missing indicator method for categorical covariates, adding an extra level for missing values, and applied a pairwise deletion strategy for the exposure, removing incomplete records from specific analyses as needed.

Two-sided Fisher's exact test with false discovery rate correction for multiple testing was used to compare gene frequencies among groups and to assess the co-occurrence and mutual exclusivity of top altered genes.

TTP was estimated using the Kaplan—Meier (KM) method and compared among groups using the log-rank test.

The association between the TTP and individual genomic features was assessed by a series of univariate and multivariate Cox proportional hazards (PH) regression models, adjusting for age, sex, race/ethnicity, albumin/bilirubin grade, prior locoregional therapies, Eastern Cooperative Oncology Group performance status, alpha-fetoprotein, and etiology. The results were expressed as hazard ratios (HRs) and 95% confidence intervals (CIs). The PH assumption was tested using the scaled Schoenfeld residuals. These analyses were restricted to genes and pathways altered in at least 10 cases.

To evaluate the predictive role of molecular characteristics, we performed subgroup and interaction analyses. Univariate Cox PH models were employed to assess the

treatment effect (A + B versus TKIs) in mutational status subgroups (Mut versus WT), whilst the interaction test was performed by inserting a biomarker*treatment interaction term in the regression equations. These analyses were limited to genes/pathways altered in at least five patients per treatment group.

No correction for multiple hypothesis testing was applied for survival analyses, considering their exploratory nature. Values of $P < 0.05$ were considered significant. Statistical analyses were performed using R statistical software version 4.1.2.

Table 1. Characteristics of the study patients

Characteristic	*N* = 370
Sex, *n* (%)	
Male	282 (76.2)
Female	88 (23.8)
Age (years)[a], median (IQR)	66 (60-73)
Self-reported race/ethnicity[b], *n* (%)	
Non-Hispanic white	193 (52.2)
Non-Hispanic other	47 (12.7)
Non-Hispanic black	43 (11.6)
Non-Hispanic unknown race	41 (11.1)
Hispanic or Latin American	27 (7.3)
Non-Hispanic Asian	19 (5.1)
Genomic ancestry[c], *n* (%)	
EUR	243 (65.7)
AFR	51 (13.8)
AMR	48 (13.0)
EAS/SAS	28 (7.6)
Documented history of[d], *n* (%)	
HBV	30 (8.1)
HCV	132 (35.7)
Alcohol abuse	91 (24.6)
Diabetes	144 (38.9)
Obesity	75 (20.3)
HCC etiology[d], *n* (%)	
Viral	156 (42.2)
Non-viral	130 (35.1)
Not documented	84 (22.7)
Stage at initial diagnosis[e], *n* (%)	
I	13 (3.5)
II	27 (7.3)
III	43 (11.6)
IV	106 (28.6)
Not reported	181 (48.9)
Evidence of extrahepatic spread[f], *n* (%)	157 (42.4)
ECOG PS[g], *n* (%)	
0	101 (27.3)
1	145 (39.2)
2+	48 (13.0)
Not reported	76 (20.5)
ALBI grade[g], *n* (%)	
Grade 1	113 (30.5)
Grade 2	151 (40.8)
Grade 3	27 (7.3)
Not reported	79 (21.4)
AFP[g], *n* (%)	
<400 µg/l	126 (34.1)
≥400 µg/l	72 (19.5)
Not reported	172 (46.5)
Documented history of hepatic decompensation within 60 days before the start of systemic therapy, *n* (%)	
Ascites	65 (17.6)
Encephalopathy	11 (3.0)
Previous therapy[h], *n* (%)	
Loco-regional	122 (33.0)
Transplant	17 (4.6)
Resection	58 (15.7)
Year of start of therapy, median (IQR)	2019 (2017-2021)
Follow-up time (months)[i], median (IQR)	11 (6-23)

AFP, alpha-fetoprotein; AFR, African; ALBI, albumin-birulin; AMR, American; EAS, East Asia; ECOG PS, Eastern Cooperative Oncology Group Performance Status; EUR, European; HBV, hepatitis B virus; HCC, hepatocarcinoma cancer; HCV, hepatitis C virus; IQR, interquartile range; SAS, South Asia; SBRT, stereotactic body radiation therapy; TACE, transarterial chemoembolization; TARE, transarterial radioembolization.

Viral etiology was defined as the presence of HCV and/or HBV. Patients with metabolic syndrome or alcohol-related liver disease who tested negative for both HBV and HCV were classified as non-viral.

[a]Age at diagnosis was calculated by subtracting the date of birth from the start date of systemic therapy. Patients with a birth year of 1939 or earlier may have an adjusted birth year in Flatiron datasets due to patient de-identification requirements.

[b]Race and ethnicity were combined following the Surveillance, Epidemiology, and End Results (SEER)'s strategy.[31]

[c]Genomic ancestry for each patient was computed using principal component analysis of single nucleotide polymorphisms trained on data from the 1000 Genomes Project.[32]

[d]Documented evidence of etiology is defined as having any of the following risk factors before HCC diagnosis: hepatitis B, hepatitis C, obesity (excluding increased weight due to ascites), diabetes (including type I or type II diabetes mellitus but excluding pre-diabetes and gestational diabetes), or heavy or binge alcohol use (excluding occasional or social alcohol use). Laboratory results were not used to assess whether a patient had a history of hepatitis.

[e]Stage was defined by the American Joint Committee on Cancer staging system.

[f]Reported metastatic diagnosis date before the initiation of systemic treatment.

[g]ECOG PS/ALBI/AFP was assigned by selecting the score closest to the start date of first-line in a predefined time window [(−30 to +7) days around the start date]. If there are multiple values on the closest date, then the highest score will be selected.

[h]Received pre-first-line therapy is defined as patients having loco-regional treatment (TACE/SBRT/TARE/RFA) or surgery before the start of systemic therapy.

[i]Follow-up time was calculated from the start date of systemic therapy to the date of death or the date of the last confirmed activity.

RESULTS

Patient population

The study included 370 United States patients from the HCC FH-FMI CGDB between 1 January 2011 and 31 March 2024 who received systemic therapy (Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2025.105879). Table 1 summarizes the baseline characteristics, with details of the last procedure performed before systemic treatment provided in Supplementary Table S4, available at https://doi.org/10.1016/j.esmoop.2025.105879.

We used an upset plot to illustrate the distribution and overlap of HCC etiologies (Supplementary Figure S2, available at https://doi.org/10.1016/j.esmoop.2025.105879). Overall, 42.2% of patients were classified as viral (history of HCV and/or HBV) and 35.1% as non-viral, while 22.7% had no documented risk factors.

Treatment pattern

The median number of systemic therapy lines was 2 [interquartile range (IQR): 1-2], with 50.8% of patients receiving 2 lines and 22.4% receiving 3 lines. Treatment patterns are depicted and can be thoroughly inspected in

Supplementary Figure S3 and Table S5, respectively, available at https://doi.org/10.1016/j.esmoop.2025.105879.

Anti-angiogenic agents were the most commonly used treatments in L1 (49.5%, exclusively TKIs) and across all lines (47.3%). Among L1-TKIs, sorafenib was the most frequently used agent (73.8%). IOs were the second most common treatment (38.4% in L1 and 34.0% overall). Of those, A + B was primarily used in L1 (25.7%), while other IOs were more commonly administered in subsequent lines (31.4% in L2 and 19.3% in L3).

CGP testing

A total of 382 samples from 370 patients were sequenced, with 11 patients undergoing repeated next-generation sequencing testing. The samples were distributed as follows: 181 (47.4%) with FoundationOne®CDx, 114 (29.8%) with FoundationOne®, 71 (18.6%) with FoundationOne®Liquid CDx, and 16 (4.2%) with FoundationOne®Liquid. Overall, 291 patients (78.6%) were profiled using tissue-based CGP, while 86 patients (23.2%) underwent liquid-based CGP, with 7 patients included in both cohorts (Supplementary Table S6, available at https://doi.org/10.1016/j.esmoop.2025.105879). The characteristics of the two cohorts are presented in Supplementary Table S7, available at https://doi.org/10.1016/j.esmoop.2025.105879.

In total, 73.0% of patients underwent their first FMI test after starting L1 therapy, although specimen collection occurred before L1 treatment in most cases (72.2%) (Supplementary Table S8, available at https://doi.org/10.1016/j.esmoop.2025.105879). The median time from specimen collection to reporting of results was 1.2 months (IQR: 0.5-5.0 months). Tumor tissue was obtained from biopsy of the primary tumor in 73.9% of cases, and from metastatic sites in 26.1% of cases (Supplementary Figure S4, available at https://doi.org/10.1016/j.esmoop.2025.105879).

Tissue tumor mutational burden and blood TMB were evaluable in 204 and 70 samples, respectively, with 5.9% and 8.6% classified as TMB-high. Microsatellite instability (MSI)-high status was detected in 1 (1.1%) liquid biopsy, while 4 out of 234 MSI-evaluable tissue samples (1.7%) had MSI-intermediate. One-third (31.0%, $n = 27/82$) of blood samples with available tumor fraction had a value >10% (median tumor fraction: 4.8%, IQR: 1.4%, 19.2%). Further details are provided in Supplementary Table S9, available at https://doi.org/10.1016/j.esmoop.2025.105879.

Tissue cohort. In the tissue cohort ($n = 291$), a total of 1056 GAs were detected in 158 different genes, with a median number of 3 altered genes per patient (IQR: 2-5). Figure 1 displays the genomic landscape of the tissue cohort, with additional details provided in Supplementary Table S10, available at https://doi.org/10.1016/j.esmoop.2025.105879.

TERT promoter (*TERT*p) mutations were the most frequent GAs (61.5%), followed by *CTNNB1* (34.0%), *TP53* (33.0%), *MYC* (16.2%), and *ARID1A* (11.7%) (Figure 1A and Supplementary Table S10A, available at https://doi.org/10.1016/j.esmoop.2025.105879). Following *TERT*p, the most common single nucleotide variants were in *TP53* and *CTNNB1* (Supplementary Table S10B, available at https://doi.org/10.1016/j.esmoop.2025.105879). Of note, 2.7% of patients harbored *TP53* mutations p.V157F and p.R249S. When focusing on copy number alterations, deletions were most frequently detected in *CDKN2A* and *CDKN2B,* and amplifications in *MYC*, the *FGF3/FGF4/FGF19/CCND1* locus, and *LYN*.

Analysis of interactions among the most frequently altered genes revealed co-occurrences between *TERT*p mutations and alterations in *CTNNB1* and *MYC* (Figure 1B and Supplementary Table S10C, available at https://doi.org/10.1016/j.esmoop.2025.105879). *CTNNB1* was mutually exclusive with *RB1*, while *TERT*p showed mutual exclusivity with *BAP1*. *CDKN2A* was significantly associated with *CDKN2B* and *LYN* amplifications were enriched in *MYC*-altered tumors. *ARID1A* was significantly associated with *CTNNB1*. Additionally, significant co-occurrence was observed between the FGF genes and *CCND1,* and within the FGF pathway.

Pathway alteration frequencies are shown in Figure 1C and Supplementary Table S10D, available at https://doi.org/10.1016/j.esmoop.2025.105879, while mutation rates of pathway member genes are detailed in Supplementary Table S10E, available at https://doi.org/10.1016/j.esmoop.2025.105879. *TP53* was involved in the most frequently activated pathways, including cell cycle (CC) and apoptosis (56%), DNA damage control (43.0%), and p53 (38.1%). *CTNNB1* was the main mutated gene in the WNT pathway, altered in 40.9% of cases. Interestingly, no pathogenic GAs in *AXIN1* were detected. Other commonly affected pathways were PI3K (28.2%), homologous recombination deficiency (HRD)/BRCAness (27.8%), chromatin modification (26.8%), cyclin (24.7%), RTK/RAS (19.9%), and cMYC (16.2%). Notably, *MYC* was the only altered gene in the cMYC pathway. The NRF2 pathway was altered in 9.3% of patients with alterations in *NFE2L2* (5.5%) and *KEAP1* (4.5%).

Pathway-based co-occurrence analysis found 13 significant co-occurring pairs (Supplementary Figure S5 and Table S10F, available at https://doi.org/10.1016/j.esmoop.2025.105879). Notably, among pathways with different gene sets, FGF/FGFR alterations significantly co-occurred with cyclin alterations.

Correlation with etiology. Associations between GAs and etiology are shown in Figure 2 and Supplementary Table S11, available at https://doi.org/10.1016/j.esmoop.2025.105879. Generally, mutational load, expressed as the number of altered genes per patient, appeared higher in viral cases than in non-viral cases (median: 4 versus 3, $P = 0.053$), although no differences in tissue mutational burden were observed ($P = 0.18$).

Viral-related HCC were significantly enriched in alterations of *TERT*p (viral versus non-viral: 75.4% versus 53.9%, $P = 0.010$), *CTNNB1* (50.0% versus 23.5%, $P = 0.001$), and WNT pathway (53.4% versus 31.4%, $P = 0.020$). In contrast, RTK/RAS alterations were more frequent in non-viral HCC (13.6% versus 29.4%, $P = 0.042$). While not reaching strict statistical significance after adjustment, *TP53* alterations were more common in viral HCC (44.1% versus 28.4%).

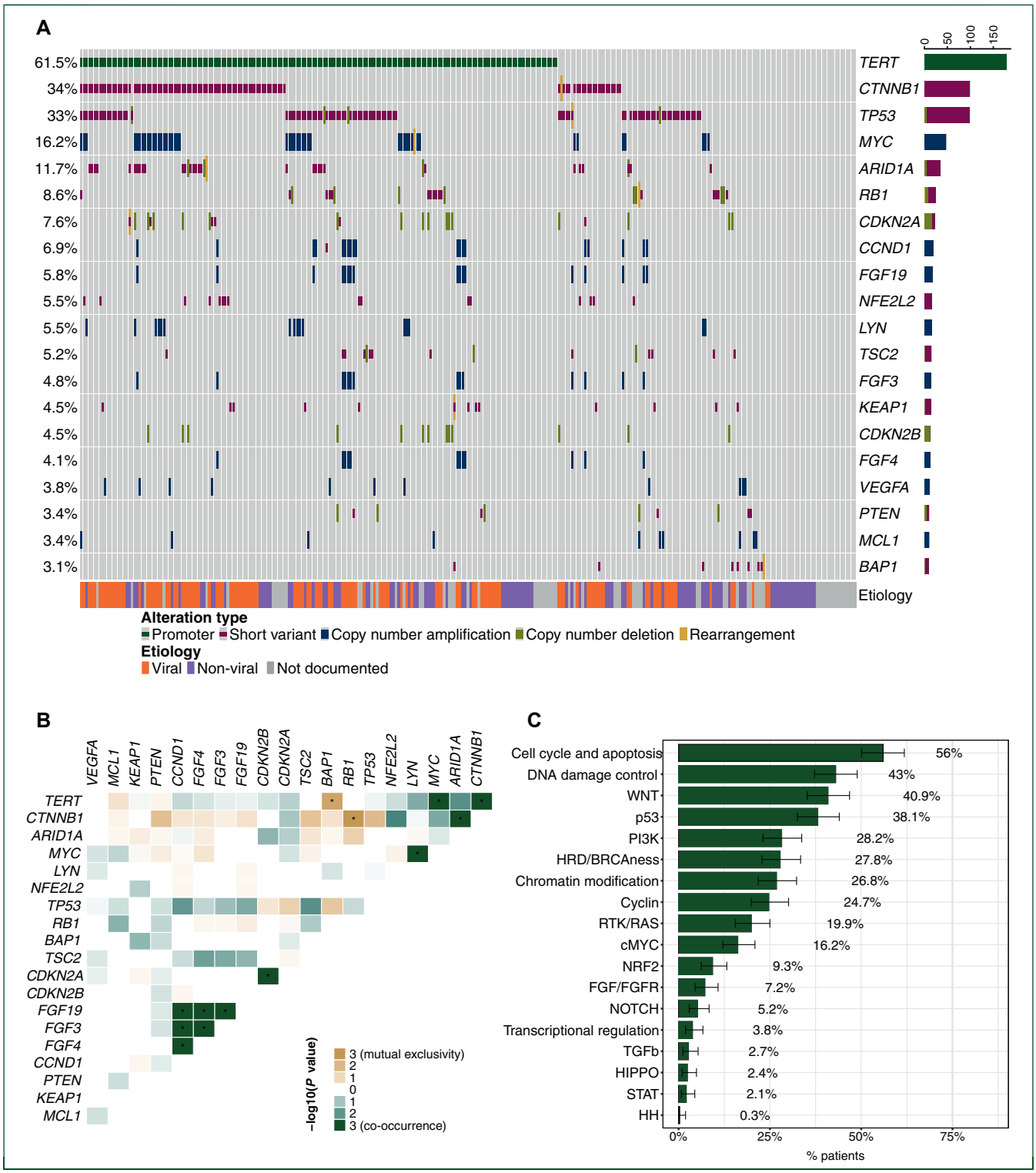


Figure 1. Genomic landscape of HCC—tissue cohort ($n = 291$). (A) Oncoplot of prevalent genetic alterations (>3%). (B) Co-occurrence and mutual exclusivity of top-altered genes.
*Adjusted $P < 0.05$. (C) Distribution of altered signaling pathways.

Conversely, alterations in transcriptional regulation were observed exclusively in non-viral patients (0.0% versus 5.9%). When excluding patients with mixed etiologies and alcohol-related liver disorders, similar trends were observed, with the exception of *TSC2*, whose mutation rate seemed higher in patients with metabolic disease (Supplementary Figure S6, available at https://doi.org/10.1016/j.esmoop.2025.105879).

Liquid cohort. A total of 389 GAs were detected in circulating tumor DNA samples of 86 patients. The median number of altered genes per patient was 3 (IQR: 2-5). The

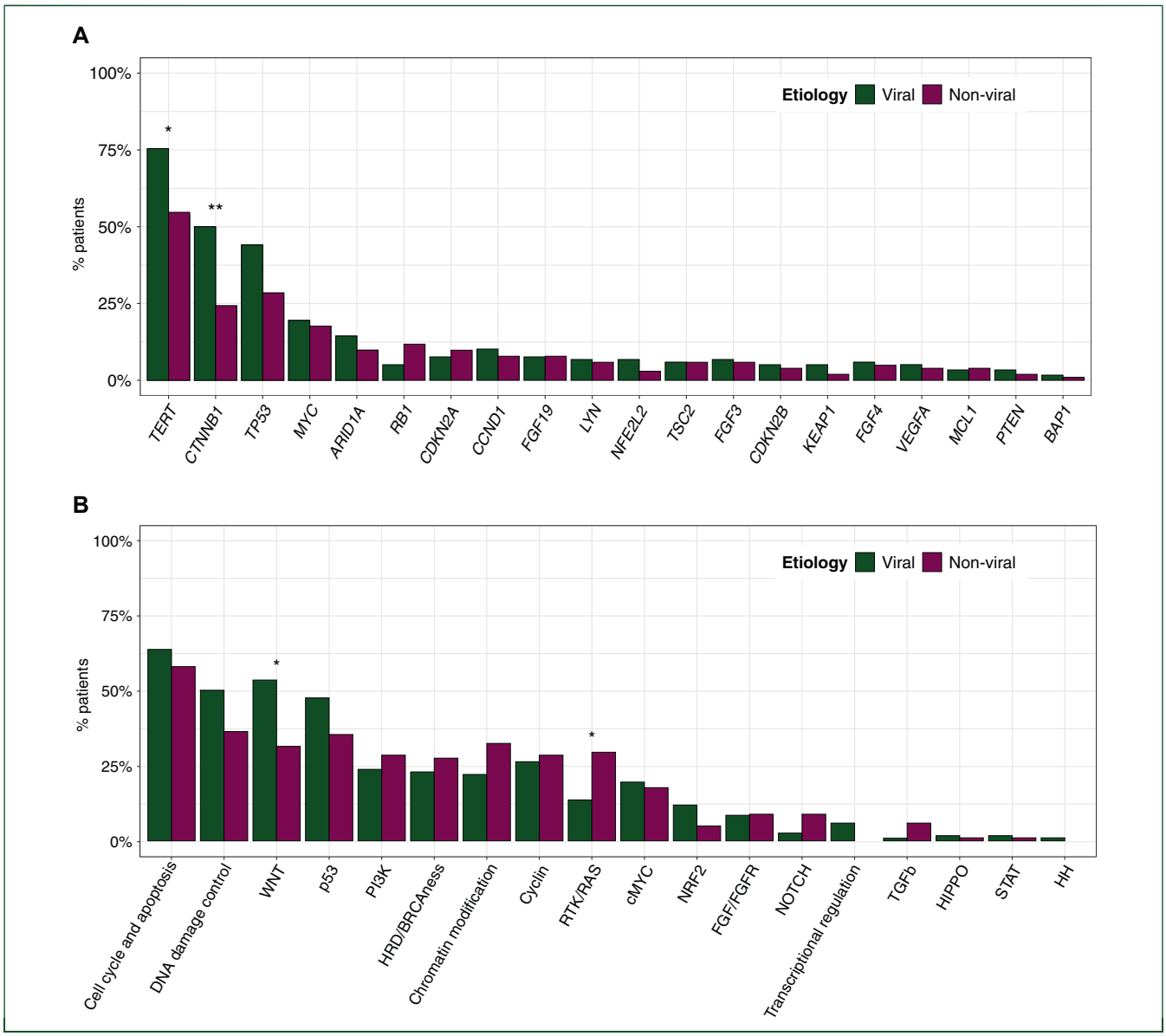


Figure 2. Association of genetic features and etiology. Association of genes (A) and pathways (B) with etiology: viral ($n = 118$) vs. non-viral ($n = 102$). Patients with undocumented etiology were excluded from the analysis. The Y-axis indicates the percentage of patients with the altered genes/pathway across the X-axis for groups under study. *Adjusted P value < 0.05, **Adjusted P value < 0.01.

molecular profile of the liquid cohort is shown in Figure 3 and Supplementary Table S12, available at https://doi.org/10.1016/j.esmoop.2025.105879.

The most frequently altered genes were *TERT*p (58.1%), *TP53* (50.0%), *CTNNB1* (44.2%), *DNMT3A* (41.4%), *TET2* (17.1%), *ASXL1* (15.7%), and *ARID1A* (15.7%). No significant interaction was found in this cohort; we only observed the tendency of *NFE2L2* and *PIK3CA* to co-occur.

Clonal hematopoiesis (CH) and clonal hematopoiesis of indeterminate potential are age-related processes characterized by the accumulation of somatic mutations in blood cells. To account for the background noise introduced by CH in plasma cell-free DNA analysis, we compared the gene frequencies between liquid (FoundationOne®Liquid CDx) and tissue biopsies, stratified by age (Supplementary Figure S7, available at https://doi.org/10.1016/j.esmoop.2025.105879). Except for CH-related genes (*DNMT3A*, *TET2*, *ASXL1*, and *ATM*), no significant differences in alteration frequency were found between test types. Intriguingly, *DNMT3A* and *ASXL1* variants were detected more frequently in liquid biopsy samples compared with tissue specimens (liquid versus tissue: 23.1% versus 2.5% for *DNMT3A*; 17.9% versus 1.5% for *ASXL1*; $P < 0.01$), even among patients younger than 70 years of age.

TTP

Clinical outcomes were explored for 286 (98.3%) patients in the tissue cohort with index date on or before 30 September 2023. Overall, the median TTP was 5.1 months

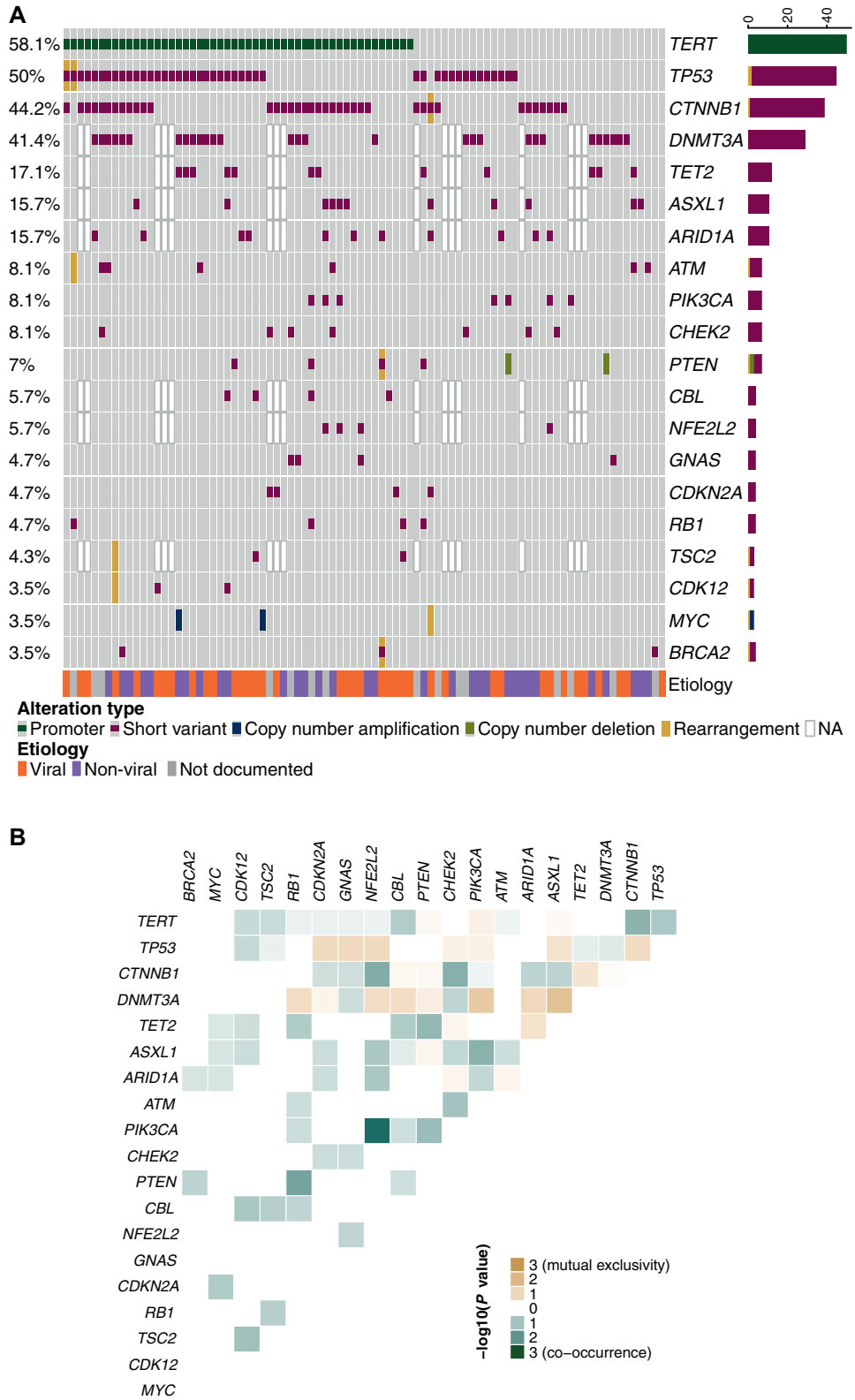
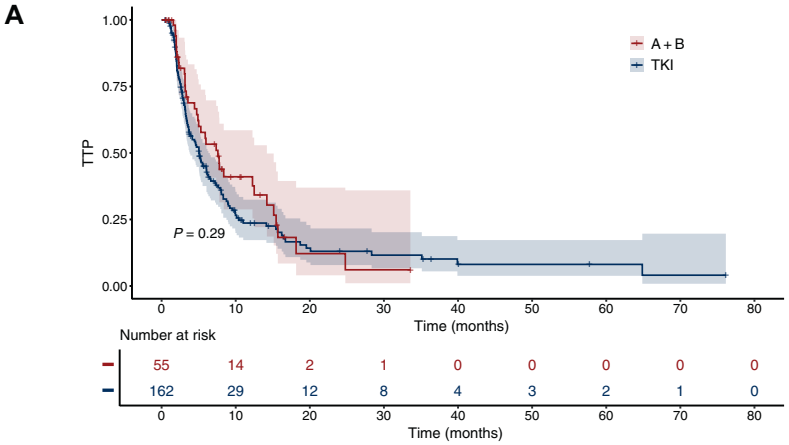


Figure 3. Genomic landscape of HCC—liquid cohort ($n = 86$). (A) Oncoplot of prevalent genetic alterations (>3%). (B) Co-occurrence and mutual exclusivity of top-altered genes.
*Adjusted $P < 0.05$.

https://doi.org/10.1016/j.esmoop.2025.105879



B

	Frequency (*N* = 286)	Median TTP (95% CI) WT	Median TTP (95% CI) Mut	Univariate analysis cHR (95% CI)	Univariate analysis *P*	Multivariate analysis aHR (95% CI)	Multivariate analysis *P*
TERT	176 (61.5%)	5.3 (4.1-7.8)	5.0 (4.0-7.4)	0.82 (0.61-1.08)	0.161	0.75 (0.55-1.03)	0.075
CTNNB1	97 (33.9%)	5.3 (4.6-7.3)	5.1 (3.6-8.2)	0.87 (0.64-1.17)	0.362	0.86 (0.62-1.20)	0.374
TP53	94 (32.9%)	5.2 (4.6-7.7)	4.7 (3.1-7.6)	1.23 (0.91-1.65)	0.175	1.29 (0.93-1.79)	0.125
MYC	47 (16.4%)	5.1 (4.1-6.1)	8.2 (3.6-28)	0.74 (0.48-1.12)	0.151	0.74 (0.47-1.16)	0.185
ARID1A	34 (11.9%)	5.1 (4.6-6.2)	6.6 (3.0to—)	0.67 (0.40-1.10)	0.110	0.74 (0.44-1.25)	0.267
RB1	25 (8.7%)	5.0 (4.0-6.3)	8.1 (5.1-25)	0.87 (0.53-1.43)	0.589	0.76 (0.45-1.29)	0.317
CDKN2A	22 (7.7%)	5.1 (4.2-6.2)	7.3 (3.7to—)	0.76 (0.43-1.34)	0.341	0.77 (0.43-1.38)	0.373
CCND1	19 (6.6%)	5.3 (4.6-7.2)	3.1 (2.3to—)	1.06 (0.61-1.83)	0.834	1.09 (0.61-1.92)	0.776
FGF19	16 (5.6%)	5.3 (4.6-6.6)	4.0 (2.8to—)	0.96 (0.53-1.73)	0.893	1.00 (0.54-1.86)	0.998
LYN	16 (5.6%)	5.0 (4.1-6.0)	8.9 (8.2to—)	0.60 (0.31-1.18)	0.137	0.64 (0.32-1.28)	0.206
NFE2L2	16 (5.6%)	5.2 (4.4-6.5)	5.1 (3.6to—)	0.92 (0.50-1.70)	0.792	0.98 (0.50-1.89)	0.942
TSC2	15 (5.2%)	5.2 (4.4-6.5)	4.6 (2.3to—)	1.48 (0.75-2.89)	0.255	1.59 (0.79-3.21)	0.196
CDKN2B	13 (4.5%)	5.1 (4.4-6.1)	16 (7.3to—)	0.48 (0.20-1.17)	0.105	0.44 (0.18-1.10)	0.078
FGF3	13 (4.5%)	5.2 (4.6-6.5)	4.0 (2.8to—)	0.88 (0.45-1.73)	0.721	0.87 (0.43-1.77)	0.709
KEAP1	13 (4.5%)	5.2 (4.6-6.5)	3.4 (3.0to—)	1.20 (0.64-2.28)	0.568	1.32 (0.65-2.70)	0.443
FGF4	11 (3.8%)	5.3 (4.6-6.6)	3.6 (2.8to—)	1.21 (0.62-2.36)	0.584	1.28 (0.64-2.55)	0.492
VEGFA	11 (3.8%)	5.1 (4.1-6.3)	7.8 (5.2to—)	0.83 (0.39-1.77)	0.629	0.84 (0.38-1.87)	0.663
MCL1	10 (3.5%)	5.2 (4.6-6.3)	3.8 (3.2to—)	0.97 (0.48-1.97)	0.929	0.91 (0.44-1.90)	0.801
PTEN	10 (3.5%)	5.2 (4.6-6.5)	3.9 (2.2to—)	0.90 (0.37-2.21)	0.816	1.18 (0.46-3.01)	0.735
Cell Cycle_Apoptosis	161 (56.3%)	5.5 (3.8-9.0)	5.1 (4.2-7.2)	1.09 (0.83-1.45)	0.529	1.08 (0.80-1.47)	0.602
DNA Damage Control	123 (43.0%)	5.5 (4.7-7.9)	4.6 (3.2-7.6)	1.30 (0.98-1.72)	0.072	1.35 (1.00-1.83)	0.050
WNT	117 (40.9%)	5.9 (4.6-7.7)	5.0 (3.6-6.6)	0.93 (0.70-1.23)	0.603	0.93 (0.67-1.27)	0.630
p53	109 (38.1%)	5.2 (4.6-7.3)	5.1 (3.2-7.6)	1.19 (0.89-1.58)	0.248	1.23 (0.90-1.68)	0.202
HRD/BRCAness	81 (28.3%)	5.2 (4.6-6.3)	5.1 (3.2-10)	0.97 (0.70-1.33)	0.834	1.01 (0.72-1.41)	0.975
PI3K	80 (28.0%)	5.1 (4.4-7.3)	5.2 (3.6-8.1)	1.05 (0.77-1.43)	0.766	1.09 (0.79-1.51)	0.600
Chromatin Modification	77 (26.9%)	5.0 (3.8-6.0)	7.2 (4.4-15)	0.73 (0.52-1.03)	0.072	0.76 (0.53-1.09)	0.135
Cyclin	71 (24.8%)	5.0 (3.6-6.5)	6.0 (4.6-8.7)	0.86 (0.62-1.20)	0.382	0.84 (0.60-1.18)	0.308
RTK/RAS	56 (19.6%)	5.3 (4.6-7.6)	5.0 (3.5-7.7)	1.18 (0.84-1.67)	0.340	1.27 (0.88-1.83)	0.208
NRF2	27 (9.4%)	5.2 (4.4-6.5)	4.6 (3.2-15)	0.97 (0.60-1.56)	0.905	1.02 (0.61-1.71)	0.930
FGF/FGFR	20 (7.0%)	5.3 (4.7-7.2)	4.0 (2.8-18)	1.09 (0.64-1.85)	0.753	1.08 (0.62-1.89)	0.777
NOTCH	14 (4.9%)	5.1 (4.2-6.5)	6.0 (6.0to—)	0.65 (0.33-1.26)	0.201	0.53 (0.26-1.09)	0.084
Transcriptional Regulation	10 (3.5%)	5.3 (4.6-7.2)	4.4 (1.9to—)	1.96 (0.86-4.44)	0.108	2.04 (0.85-4.86)	0.109
TMB-H[a]	11 (5.6%)	5.3 (4.6-7.7)	7.9 (3.4to—)	0.94 (0.38-2.30)	0.888	1.05 (0.40-2.73)	0.920

C

	TKI (*N* = 162) *n*	TKI (*N* = 162) Median TTP (95% CI)	A + B (*N* = 55) *n*	A + B (*N* = 55) Median TTP (95% CI)	cHR (95% CI)	*P*-int
TERT						
WT	58	6.0 (3.8-8.3)	23	7.8 (4.7to—)	0.90 (0.51-1.60)	0,699
Mut	104	5.0 (3.4-6.5)	32	7.4 (4.4to—)	0.75 (0.45-1.25)	
CTNNB1						
WT	108	5.0 (3.6-7.3)	35	7.8 (5.3to—)	0.75 (0.46-1.23)	0,512
Mut	54	5.1 (3.5-9.5)	20	4.4 (3.2to—)	0.95 (0.52-1.73)	
TP53						
WT	108	5.2 (4.1-8.1)	34	6.0 (3.6-18)	0.82 (0.51-1.33)	0,907
Mut	54	3.6 (3.0-7.2)	21	7.8 (5.3to—)	0.79 (0.43-1.48)	
MYC						
WT	139	5.0 (3.6-6.0)	45	7.8 (5.3-15)	0.75 (0.49-1.14)	0,287
Mut	23	7.3 (3.3to—)	10	4.4 (3.3to—)	1.39 (0.53-3.66)	
ARID1A						
WT	142	5.2 (4.1-7.2)	50	7.6 (5.0-15)	0.82 (0.56-1.22)	0,834
Mut	20	3.0 (2.7to—)	5	4.4 (3.3to—)	0.56 (0.12-2.5)	
RB1						
WT	149	5.0 (3.6-6.3)	46	7.6 (4.7-15)	0.79 (0.53-1.19)	0,739
Mut	13	7.1 (5.1to—)	9	8.4 (5.0to—)	1.11 (0.37-3.34)	
CDKN2A						
WT	153	5.1 (3.7-6.3)	48	7.6 (5.0-14)	0.82 (0.55-1.22)	0,894
Mut	9	6.3 (2.6to—)	7	4.4 (3.6to—)	0.93 (0.26-3.32)	
Cell Cycle_Apoptosis						
WT	74	5.0 (3.6-8.9)	20	15 (3.2to—)	0.72 (0.38-1.34)	0,521
Mut	88	5.1 (3.2-7.2)	35	7.4 (4.9-12)	0.89 (0.55-1.43)	
DNA Damage Control						
WT	90	5.5 (4.4-8.1)	29	5.9 (3.6to—)	0.88 (0.52-1.49)	0,657
Mut	72	3.7 (3.1-7.2)	26	7.8 (7.4-16)	0.75 (0.43-1.3)	
WNT						
WT	99	5.0 (3.6-8.1)	32	7.8 (5.9to—)	0.75 (0.44-1.27)	0,556
Mut	63	5.1 (3.5-6.6)	23	5.3 (3.3-16)	0.9 (0.52-1.58)	
p53						
WT	101	5.2 (4.1-7.9)	29	5.9 (3.6to—)	0.84 (0.51-1.4)	0,803
Mut	61	3.7 (3.0-7.6)	26	7.8 (5.3to—)	0.79 (0.44-1.4)	
HRD/BRCAness						
WT	111	5.1 (3.8-6.5)	41	7.6 (5.0-15)	0.80 (0.52-1.24)	0,957
Mut	51	5.1 (3.1-9.0)	14	4.9 (3.3to—)	0.83 (0.37-1.89)	
PI3K						
WT	123	5.1 (3.6-7.2)	37	7.8 (4.7-25)	0.71 (0.45-1.13)	0,251
Mut	39	5.1 (3.0-11)	18	6.8 (3.3to—)	1.10 (0.55-2.21)	
Chromatin Modification						
WT	119	5.1 (3.7-6.3)	38	7.8 (3.2-15)	0.86 (0.55-1.34)	0,664
Mut	43	5.2 (3.2-14)	17	7.6 (4.9to—)	0.73 (0.35-1.5)	
Cyclin						
WT	128	5.0 (3.6-6.5)	36	7.4 (3.3-15)	0.84 (0.54-1.31)	0,761
Mut	34	6.0 (3.2-14)	19	7.8 (5.3to—)	0.80 (0.38-1.67)	
RTK/RAS						
WT	134	5.1 (3.8-7.3)	43	7.8 (4.9-15)	0.76 (0.49-1.17)	0,514
Mut	28	5.2 (3.3-14)	12	6.2 (2.3to—)	1.02 (0.46-2.27)	

Figure 4. Analysis of TTP. (A) KM plot of TTP: A+B vs. TKIs. (B) Prognostic associations of genomic features and TTP—univariate and multivariate analyses. (C) Predictive associations of genomic features and TTP—univariate analyses. Violation of proportional hazard assumption for *ARID1A*, *RB1*, and *LYN* (in univariate analysis).

A + B, atezolizumab + bevacizumab; aHR, adjusted hazard ratio; cHR, crude hazard ratio; CI, confidence interval; Mut, mutant; *P*-int, *P*-interaction; TKI, tyrosine kinase inhibitors; TMB-H, tumor mutational burden high; TTP, time to progression; WT, wild-type.

[a]Analysis conducted on 196 patients with available TMB.

(IQR: 4.4, 6.3). We compared patients treated with A + B versus those receiving TKIs. Baseline characteristics of these patients are reported in Supplementary Table S13, available at https://doi.org/10.1016/j.esmoop.2025.105879. KM analysis indicated a trend toward longer TTP in patients treated with A + B compared with those receiving TKIs [median TTP (95% CI) 7.6 (4.9-14) months versus 5.1 (3.8-6.3) months; aHR (95% CI) 0.75 (0.50-1.13)] (Figure 4A). Although the difference was not statistically significant, a separation between the curves is evident in the first year, before the substantial decrease in sample sizes.

The association of TTP with genomic features for the entire population, regardless of treatment, is reported in Figure 4B, with KM plots presented in Supplementary Figure S8, available at https://doi.org/10.1016/j.esmoop.2025.105879. Alterations in *TERT*p, *CDKN2B*, *MYC*, chromatin modification, and NOTCH appeared to have a positive effect on TTP, but statistical significance was lacking. Conversely, a trend showing worse TTP was identified in patients with *TP53* (or p53), *TSC2*, DNA damage control, RTK/RAS, and transcriptional regulation alterations. Specifically, *TP53* variants p.V157F and p.R249S were associated with significantly shorter TTP compared with WT patients (aHR 2.58, $P = 0.046$). When the predictive role of GAs was analyzed (Figure 4C), only *MYC* seemed to be a negative predictor for A + B versus TKIs (Supplementary Figure S9, available at https://doi.org/10.1016/j.esmoop.2025.105879), with a negative impact of A + B in Mut patients (HR 1.39, $P = 0.5$) and a positive impact in WT patients (HR 0.75, $P = 0.2$). The interaction test, however, did not reach statistical significance.

DISCUSSION

At present, available genomic data mainly derive from early-stage resected HCC collected in The Cancer Genome Atlas Program,[33] whereas studies on advanced disease remain limited.[34,35] Our study provides further biological insight in this setting.

The most frequent GAs in the tissue cohort involved *TERT*p (61.5%), *CTNNB1* (34.0%), *TP53* (33.0%), *MYC* (16.2%), *ARID1A* (11.7%), and *RB1* (8.6%) genes. In the liquid cohort the most commonly altered genes were *TERT*p (58.1%), *TP53* (50.0%), *CTNNB1* (44.2%), and *DNMT3A* (41.4%). Except for CH-related genes (*DNMT3A*, *TET2*, *ASXL1*, and *ATM*), no significant differences in alteration frequency were found between test types. The same six genes of our tissue cohort were the most commonly mutated in another study on advanced HCC[36] with similar rates, particularly for the top three. Other analyses on HCC not specific for advanced disease[16,19,37-39] found that *TERT*p mutations were the most common (30%-61%), followed by *CTNNB1* (20%-40%), and *TP53* (24%-50%).

In addition to *TERT*p, which remains the most frequently altered gene (61.5% and 58.1% in our tissue and liquid cohort, respectively), we found that the most commonly altered pathways were CC/apoptosis (56%), DNA damage control (43%), WNT (41%), p53 (38%), PI3K (28%), HRD/BRCAness (28%), chromatin modification (27%), and cyclin (25%).

Overall, compared with published literature,[33] we may speculate that the mutational profile of HCC remains relatively stable as the disease progresses through its stages.

Despite heterogeneity among studies, our findings are consistent with the literature. In fact, an exome sequencing analysis on HCC tumors (which could not detect alterations in *TERT*p) found that the most frequently altered pathways were WNT/β-catenin (54%), PI3K-AKT-mTOR (51%), TP53/CC (49%), and MAPK (43%).[16] Two analyses on patients with HCC found that the most commonly altered pathways were CC, WNT, NOTCH, p53, chromatin remodeling, and PI3K, with co-occurring CC and p53 pathways.[40,41]

We observed a high prevalence of alterations in the HRD/BRCAness (28%) and chromatin modification (27%) pathways, which warrants further investigation, as drugs targeting these pathways—PARP inhibitors (PARPi) and epigenetic drugs—are already available. It should be noted, however, that our definition of HRD/BRCAness pathway was broad, and, to date, the efficacy of PARPi has been established for a narrower subset of genes.[42]

Although the most frequent mutations in HCC currently lack effective targeted therapies, ongoing clinical trials investigating drugs targeting WNT/β-catenin, *TERT*, and p53-pathway offer promising opportunities.[43] In this context, CGP plays a crucial role, as it enables a deeper understanding of HCC molecular mechanisms and supports future efforts to identify predictive biomarkers for both current and emerging therapies.

Co-occurrence and mutual exclusivity patterns were consistent with those reported in the wide cBioPortal for Cancer Genomics.[44]

The literature confirmed that alterations in *CTNNB1* were almost mutually exclusive with *TP53* alterations[38,45] and associated with *TERT*p alterations.[16,38,45] *In vivo* and *in vitro* studies suggest that the co-expression of the latter two genes promotes hepatocarcinogenesis and that *TERT* regulates WNT/β-catenin signaling,[46] thus possibly influencing prognosis, response to immunotherapy, and future therapeutic strategies.

CTNNB1 alterations are often associated with less aggressive tumor growth and a lower metastasis rate compared with other GAs, such as *TP53* alterations.[47] The co-occurrence of *TERT*p + *CTNNB1,* however, could promote tumorigenesis and autonomous tumor growth, thereby influencing HCC progression.[38]

In our study, viral etiology was significantly associated with alterations in *TERT*p and *CTNNB1*, and we found a trend toward an association with *TP53* alterations. In the literature, *TERT*p alterations were positively associated with HCV etiology[39] and negatively associated with HBV etiology.[37] In our study population, HCV history was more frequent than HBV history (36% versus 8%, respectively). Alterations in *CTNNB1* were reported at a lower frequency in patients with HBV and at higher frequency in those with

alcohol- and HCV-related HCCs.[48] *TP53* mutations have been reported to be associated with HBV etiology.[19] In addition, *in vitro* findings suggest that both HBV and HCV may be involved in the etiology of *TP53* mutations.[48]

WNT alterations were significantly associated with viral etiology, whilst RTK/RAS alterations were significantly more frequent in non-viral HCC. The most frequently altered gene in the WNT pathway, *CTNNB1,* was nevertheless reported as an additive event in the development of HCC, irrespective of etiological background,[48] but alterations in the WNT pathway could occur due to other mechanisms.[49]

Interestingly, the frequency of *TSC2* alterations seemed higher in patients with pure metabolic disease, while no differences were detected between viral and non-viral HCC.

In clinical practice, stratifying HCC by etiology is challenging due to overlapping causes, resulting in a wide spectrum of disease profiles. Moreover, in recent years, globally the etiology of HCC has changed[50] due to the increase in non-viral and metabolic dysfunction-associated steatotic liver disease forms and the decrease in viral forms.[50-52] In our dataset, the prevalence of viral forms was still greater than non-viral forms (42.2% versus 35.1%). Covering 13 years, our study reflects an outdated epidemiological landscape, with recent prevalence offset by early 2010s data.

TP53 alterations are associated with lower disease-free survival and therapy resistance,[39,45] and we also identified a trend toward worse TTP in carriers.

Furthermore, 2.7% of tissue cohort patients harbored p.V157F and p.R249S point mutations of *TP53*—associated with poor prognosis[39,53,54]—and showed significantly shorter TTP than WT patients.

In this study, *TERT*p alterations showed a trend toward improved TTP, contrasting with two advanced HCC analyses that found a negative predictive role.[55,56]

MYC amplifications have been repeatedly associated with large undifferentiated liver tumors, poor prognosis, metastasis, and HCC recurrence,[39] whereas we found a trend toward a positive effect on TTP.

Interestingly, alterations in chromatin modification and NOTCH pathways showed a trend toward better prognostic significance in our study. Conversely, alterations in DNA damage control, p53, RTK/RAS, and transcriptional regulation pathways had a trend toward the association with worse TTP.

In other tumors, HRD/BRCAness alterations are associated with a high mutational burden and high genomic instability, which enhance immunogenicity.[57] These features are generally associated with responsiveness to immune checkpoint inhibitors[57] and to enhanced sensitivity to synthetic lethality strategies. Given the high prevalence of HRD/BRCAness alterations observed in our study, we would have expected these alterations to have a significant predictive role in the response to treatment with A + B. Our findings did not support this expectation, possibly due to the limited numerosity of the cohort; nonetheless, they open up to new putative synthetic lethality strategies in an up-front selected advanced HCC population.

In our study cohort, TKIs were the most commonly used treatments, while IOs, particularly A + B, were left as the second most common treatment category. This aligns with expectations, as the recommendation to use A + B as a first-line treatment was introduced only in the most recent guidelines,[46] based on findings from the IMbrave150 phase III clinical trial,[11] which demonstrated that A + B resulted in better overall and progression-free survival outcomes than sorafenib, whilst our analysis covers the period between 1 January 2011 and 31 March 2024. Before 2022, and for the majority of the observation period in our study, the recommended first-line treatments were sorafenib and lenvatinib.

The median TTP in our cohort was 5.1 months, with a trend toward longer TTP in patients treated with A + B compared with those receiving TKIs (7.6 months versus 5.1 months, respectively). Both data confirm the literature findings, both in experimental settings[11,58] and real-world studies.[59-62] The HR for A + B versus TKIs was 0.75, which, although not statistically significant, is broadly consistent with the HR reported in the IMbrave150 trial (0.65).[11]

We found a trend of *MYC* alterations being negative predictors for A + B versus TKIs, with a negative impact of A + B in Mut patients and a positive impact in WT patients. The small sample size prevented us from reaching statistical significance, but our findings were consistent with an analysis performed on patients involved in two clinical trials.[36] *MYC* regulates the expression of many genes involved in cell proliferation, metabolism, and immune response.[63] HCCs with *MYC* alterations are often characterized by an immunosuppressive microenvironment with reduced T-cell infiltration, low PD-L1 expression, which could reduce the efficacy of atezolizumab (anti-PD-L1), and increased angiogenic activity, which could influence the response to bevacizumab (anti-vascular endothelial growth factor).[63]

Alterations in the WNT/β-catenin pathway have been proposed as predictive biomarkers for immunotherapy, but evidence to date remains inconsistent.[36,64-67] In our cohort, patients with wild-type *CTNNB1* appeared to derive greater benefit from A + B compared with TKIs, despite the difference not reaching statistical significance. These findings align with previous reports[36] and highlight the need for further studies to clarify the predictive value of β-catenin alterations.

Both tissue and liquid specimens were considered in this analysis. Generally, circulating tumor DNA genomic profiling has shown, among other liquid biopsy tests, the most significant potential as regards as prognostic and predictive role.[68] Tissue samples have to be analyzed for diagnostic purposes, however. Due to the absence of important differences between tissue and liquid biopsies, we suggest that the latter may be an integrative strategy in case tissue specimen is insufficient to perform biomarker characterizations for research purposes.

This study has several limitations. The population primarily consists of patients treated at community oncology

practices within the FH network who underwent CGP, which may limit the generalizability of the results. Due to the retrospective design and data source, key clinical information (e.g. Child-Pugh score) was unavailable and there were important missing data in patients' characteristics. OS analysis involving the CGDB is prone to left truncation bias,[69] as patients who died before meeting inclusion criteria (e.g. receiving CGP testing) are excluded. Addressing this bias typically requires risk set adjustment, which assumes independent left truncation—a condition that was violated in this case.[70] Landmark analysis was considered but would have significantly reduced the sample size. In contrast, TTP is less affected by this bias, as progression events—even those occurring before genomic testing—do not preclude patient inclusion in the CGDB. For these reasons, we reported TTP rather than OS. A hierarchical algorithm was used to classify patients as viral and non-viral HCC, limiting the analysis of single etiologies due to small subgroup sizes. Additionally, the number of patients in our study allowed to infer on the prognostic or predictive role of a limited number of genomic features. The relatively small sample size also reduced statistical power, resulting in higher degree of uncertainty and limiting the applicability of the results. Larger studies are needed to validate our findings. The use of CGP panels rather than whole-genome sequencing limited the analysis to specific genomic regions, potentially leading to an underestimation of pathway alterations and a reduction in the accuracy of TTP analysis. Lastly, high heterogeneity was present, as biopsy sampling always reflects only a portion of the neoplasm.

Conclusions

In conclusion, this study provides critical insights into the genetic landscape of advanced HCC, identifying key genomic alterations and affected pathways, and their interplay with tumor etiology, and clinical outcomes. Our findings suggest a relatively conserved mutational profile throughout disease progression, and that a different genetic landscape differentiates viral from non-viral HCC. Finally, the emerging potential role of *MYC* as a negative predictor for A + B versus TKIs warrants further investigation.

In other types of cancer, such as non-small-cell lung and cholangiocarcinoma, research about biomarkers has dramatically changed the fate of patients harboring specific alterations, which were hit by targeted therapy. CGP analysis in HCC will be hopefully useful for the same purpose, but at present heterogeneity in tumor biology and liver function represents a significant hurdle in the development of new drugs.[3] To date, the most common alterations in HCC remain untargetable.[17]

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DATA SHARING

The data that support the findings of this study were originated by and are the property of Flatiron Health, Inc. and Foundation Medicine, Inc., which has restrictions prohibiting the authors from making the data set publicly available. Requests for data sharing by license or by permission for the specific purpose of replicating results in this manuscript can be submitted to PublicationsDataaccess@flatiron.com and cgdb-fmi@flatiron.com.

REFERENCES

1. Llovet JM, Kelley RK, Villanueva A, et al. Hepatocellular carcinoma. *Nat Rev Dis Primers*. 2021;7(1):6.
2. World Health Organization. Cancer today. Available at https://gco.iarc.who.int/today/. Accessed November 19, 2024.
3. Yang H, Liu Y, Zhang N, Tao F, Yin G. Therapeutic advances in hepatocellular carcinoma: an update from the 2024 ASCO annual meeting. *Front Oncol*. 2024;14:1453412.
4. Campani C, Zucman-Rossi J, Nault JC. Genetics of hepatocellular carcinoma: from tumor to circulating DNA. *Cancers*. 2023;15(3):817.
5. Rinella ME, Lazarus JV, Ratziu V, et al. A multisociety Delphi consensus statement on new fatty liver disease nomenclature. *J Hepatol*. 2023;79(6):1542-1556.
6. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA Cancer J Clin*. 2022;72(1):7-33.
7. Vogel A, Chan SL, Dawson LA, et al. Hepatocellular carcinoma: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Ann Oncol*. 2025;36(5):491-506.
8. Reig M, Forner A, Rimola J, et al. BCLC strategy for prognosis prediction and treatment recommendation: the 2022 update. *J Hepatol*. 2022;76(3):681-693.
9. Sangro B, Argemi J, Ronot M, et al. EASL Clinical Practice Guidelines on the management of hepatocellular carcinoma. *J Hepatol*. 2025;82(2):315-374.
10. Cheng AL, Qin S, Ikeda M, et al. Updated efficacy and safety data from IMbrave150: atezolizumab plus bevacizumab vs. sorafenib for unresectable hepatocellular carcinoma. *J Hepatol*. 2022;76(4):862-873.
11. Finn RS, Qin S, Ikeda M, et al. Atezolizumab plus bevacizumab in unresectable hepatocellular carcinoma. *N Engl J Med*. 2020;382(20):1894-1905.
12. Abou-Alfa GK, Lau G, Kudo M, et al. Tremelimumab plus durvalumab in unresectable hepatocellular carcinoma. *NEJM Evid*. 2022;1(8):EVIDoa2100070.
13. Sangro B, Chan SL, Kelley RK, et al. Four-year overall survival update from the phase III HIMALAYA study of tremelimumab plus durvalumab in unresectable hepatocellular carcinoma. *Ann Oncol*. 2024;35(5):448-457.
14. Rimassa L, Chan SL, Sangro B, et al. 947MO Five-year overall survival (OS) and OS by tumour response measures from the phase III HIMALAYA study of tremelimumab plus durvalumab in unresectable hepatocellular carcinoma (uHCC). *Ann Oncol*. 2024;35:S656.
15. Kudo M, Finn RS, Qin S, et al. Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomised phase 3 non-inferiority trial. *Lancet*. 2018;391(10126):1163-1173.
16. Schulze K, Imbeaud S, Letouzé E, et al. Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. *Nat Genet*. 2015;47(5):505-511.
17. Song M, Cheng H, Zou H, et al. Genomic profiling informs therapies and prognosis for patients with hepatocellular carcinoma in clinical practice. *BMC Cancer*. 2024;24(1):673.
18. Zucman-Rossi J, Villanueva A, Nault JC, Llovet JM. Genetic landscape and biomarkers of hepatocellular carcinoma. *Gastroenterology*. 2015;149(5):1226-1239.e4.
19. Schulze K, Nault JC, Villanueva A. Genetic profiling of hepatocellular carcinoma using next-generation sequencing. *J Hepatol*. 2016;65(5):1031-1042.
20. Singal G, Miller PG, Agarwala V, et al. Association of patient characteristics and tumor genomics with clinical outcomes among patients with non-small cell lung cancer using a clinicogenomic database. *J Am Med Assoc*. 2019;321(14):1391-1399.
21. Milbury CA, Creeden J, Yip WK, et al. Clinical and analytical validation of FoundationOne®CDx, a comprehensive genomic profiling assay for solid tumors. *PLoS One*. 2022;17(3):e0264138.
22. Frampton GM, Fichtenholtz A, Otto GA, et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. *Nat Biotechnol*. 2013;31(11):1023-1031.
23. Clark TA, Chung JH, Kennedy M, et al. Analytical validation of a hybrid capture—based next-generation sequencing clinical assay for genomic profiling of cell-free circulating tumor DNA. *J Mol Diagn*. 2018;20(5):686-702.
24. Woodhouse R, Li M, Hughes J, et al. Clinical and analytical validation of FoundationOne Liquid CDx, a novel 324-Gene cfDNA-based comprehensive genomic profiling assay for cancers of solid tumor origin. *PLoS One*. 2020;15(9):e0237802.
25. Rimini M, Macarulla T, Burgio V, et al. Gene mutational profile of BRCAness and clinical implication in predicting response to platinum-based chemotherapy in patients with intrahepatic cholangiocarcinoma. *Eur J Cancer*. 2022;171:232-241.
26. Rimini M, Loi E, Fabregat-Franco C, et al. Next-generation sequencing analysis of cholangiocarcinoma identifies distinct IDH1-mutated clusters. *Eur J Cancer*. 2022;175:299-310.
27. Moretto R, Elliott A, Zhang J, et al. Homologous recombination deficiency alterations in colorectal cancer: clinical, molecular, and prognostic implications. *J Natl Cancer Inst*. 2022;114(2):271-279.
28. Jardim DLF, Millis SZ, Ross JS, Lippman S, Ali SM, Kurzrock R. Comprehensive landscape of cyclin pathway gene alterations and co-occurrence with FGF/FGFR aberrations across urinary tract tumors. *Oncologist*. 2023;28(2):e82-e91.
29. Griffith SD, Miksad RA, Calkins G, et al. Characterizing the feasibility and performance of real-world tumor progression end points and their association with overall survival in a large advanced non-small-cell lung cancer data set. *JCO Clin Cancer Inform*. 2019;3:1-13.
30. Curtis MD, Griffith SD, Tucker M, et al. Development and validation of a high-quality composite real-world mortality endpoint. *Health Serv Res*. 2018;53(6):4460-4476.
31. National Cancer Institute. SEER documentation - race and Hispanic ethnicity changes. SEER. Available at https://seer.cancer.gov/seerstat/variables/seer/race_ethnicity/index.html. Accessed July 3, 2024.
32. Carrot-Zhang J, Chambwe N, Damrauer JS, et al. Comprehensive analysis of genetic ancestry and its molecular correlates in cancer. *Cancer Cell*. 2020;37(5):639-654.e6.
33. National Cancer InstituteNational Human Genome Research Institute. The Cancer Genome Atlas Program (TCGA) - NCI. Published May 13, 2022. Available at https://www.cancer.gov/ccg/research/genome-sequencing/tcga. Accessed January 28, 2025.
34. Nault J, Martin Y, Caruso S, et al. Clinical impact of genomic diversity from early to advanced hepatocellular carcinoma. *Hepatology*. 2020;71(1):164-182.
35. Terashima T, Yamashita T, Arai K, et al. Comprehensive genomic profiling for advanced hepatocellular carcinoma in clinical practice. *Hepatol Int*. 2024;19(1):212-221.
36. Zhu AX, Abbas AR, De Galarreta MR, et al. Molecular correlates of clinical response and resistance to atezolizumab in combination with bevacizumab in advanced hepatocellular carcinoma. *Nat Med*. 2022;28(8):1599-1611.
37. Ally A, Balasundaram M, Carlsen R, et al. Comprehensive and integrative genomic characterization of hepatocellular carcinoma. *Cell*. 2017;169(7):1327-1341.e23.

38. Lee JS. The mutational landscape of hepatocellular carcinoma. *Clin Mol Hepatol*. 2015;21(3):220.
39. Nia A, Dhanasekaran R. Genomic landscape of HCC. *Curr Hepatol Rep*. 2020;19(4):448.
40. Zhang Y, Liu Z, Li J, et al. Oncogenic pathways refine a new perspective on the classification of hepatocellular carcinoma. *Cell Signal*. 2023;111:110890.
41. Guichard C, Amaddeo G, Imbeaud S, et al. Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma. *Nat Genet*. 2012;44(6):694-698.
42. Incorvaia L, Perez A, Marchetti C, et al. Theranostic biomarkers and PARP-inhibitors effectiveness in patients with non-BRCA associated homologous recombination deficient tumors: still looking through a dirty glass window? *Cancer Treat Rev*. 2023;121:102650.
43. Zheng J, Wang S, Xia L, et al. Hepatocellular carcinoma: signaling pathways and therapeutic advances. *Signal Transduct Target Ther*. 2025;10(1):35.
44. Memorial Sloan Kettering Cancer Center, Dana Farber Cancer Institute, Princess Margaret Cancer Centre in Toronto, et al. cBioPortal for Cancer Genomics. Available at https://www.cbioportal.org/. Accessed February 5, 2025.
45. Yim SY, Lee JS. An overview of the genomic characterization of hepatocellular carcinoma. *J Hepatocell Carcinoma*. 2021;8:1077.
46. Kotiyal S, Evason KJ. Exploring the interplay of telomerase reverse transcriptase and β-catenin in hepatocellular carcinoma. *Cancers*. 2021;13(16):4202.
47. Wang Z, Sheng YY, Gao XM, et al. β-catenin mutation is correlated with a favorable prognosis in patients with hepatocellular carcinoma. *Mol Clin Oncol*. 2015;3(4):936-940.
48. Niu ZS, Niu XJ, Wang WH. Genetic alterations in hepatocellular carcinoma: an update. *World J Gastroenterol*. 2016;22(41):9069.
49. Javanmard D, Najafi M, Babaei MR, et al. Investigation of CTNNB1 gene mutations and expression in hepatocellular carcinoma and cirrhosis in association with hepatitis B virus infection. *Infect Agent Cancer*. 2020;15:37.
50. Koshy A. Evolving global etiology of hepatocellular carcinoma (HCC): insights and trends for 2024. *J Clin Exp Hepatol*. 2025;15(1):102406.
51. Ben Khaled N, Mörtl B, Beier D, et al. Changing treatment landscape associated with improved survival in advanced hepatocellular carcinoma: a nationwide, population-based study. *Eur J Cancer*. 2023;192:113248.
52. Tran S, Zou B, Kam L, et al. Updates in characteristics and survival rates of hepatocellular carcinoma in a nationwide cohort of real-world US patients, 2003—2021. *J Hepatocell Carcinoma*. 2023;10:2147-2158.
53. Villanueva A, Hoshida Y. Depicting the role of TP53 in hepatocellular carcinoma progression. *J Hepatol*. 2011;55(3):724-725.
54. Woo HG, Wang XW, Budhu A, et al. Association of TP53 mutations with stem cell-like gene expression and survival of patients with hepatocellular carcinoma. *Gastroenterology*. 2011;140(3):1063-1070.
55. Hirai M, Kinugasa H, Nouso K, et al. Prediction of the prognosis of advanced hepatocellular carcinoma by TERT promoter mutations in circulating tumor DNA. *J Gastroenterol Hepatol*. 2021;36(4):1118-1125.
56. Chang J, Fowler KJ, Tamayo P, Burgoyne A. Responses to first-line systemic therapies in patients with hepatocellular carcinoma with TERT promoter mutations. *J Clin Oncol*. 2023;41(4 Suppl):589.
57. Przybytkowski E, Davis T, Hosny A, et al. An immune-centric exploration of BRCA1 and BRCA2 germline mutation related breast and ovarian cancers. *BMC Cancer*. 2020;20:197.
58. Llovet JM, Ricci S, Mazzaferro V, et al. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med*. 2008;359(4):378-390.
59. Fulgenzi CAM, Cheon J, D'Alessio A, et al. Reproducible safety and efficacy of atezolizumab plus bevacizumab for HCC in clinical practice: results of the AB-real study. *Eur J Cancer*. 2022;175:204-213.
60. Mahn R, Glüer OA, Sadeghlar F, et al. First-line treatment for advanced hepatocellular carcinoma: a three-armed real-world comparison. *J Hepatocell Carcinoma*. 2024;11:81-94.
61. Brown TJ, Mamtani R, Gimotty PA, Karasic TB, Yang YX. Outcomes of hepatocellular carcinoma by etiology with first-line atezolizumab and bevacizumab: a real-world analysis. *J Cancer Res Clin Oncol*. 2023;149(6):2345-2354.
62. Singal AG, Shamas N, Secrest MH, Tan Mahrus S, Li D. Emerging real-world treatment patterns for unresectable hepatocellular carcinoma (uHCC) patients following approval of atezolizumab plus bevacizumab (A+B) in the United States (US). In: Proceedings of the AASLD Annual Meeting. November 4—8, 2022; Washington, DC, USA.
63. OMIM. MYC protooncogene, bHLH transcription factor; MYC. Availableat, https://omim.org/entry/190080?search=MYC&highlight=myc. Accessed April 16, 2024.
64. Khasawneh B, Abdelrahim S, Esamil A, Al-Najjar E, Abdelrahim M. Abstract A037: CTNNB1 mutations in hepatocellular carcinoma: prognostic significance and superior survival outcomes in patients treated with immunotherapy. *Cancer Res*. 2025;85(suppl 5):A037.
65. Chen L, Zhou Q, Liu J, Zhang W. CTNNB1 Alternation is a potential biomarker for immunotherapy prognosis in patients with hepatocellular carcinoma. *Front Immunol*. 2021;12:759565.
66. Hong JY, Cho HJ, Sa JK, et al. Hepatocellular carcinoma patients with high circulating cytotoxic T cells and intra-tumoral immune signature benefit from pembrolizumab: results from a single-arm phase 2 trial. *Genome Med*. 2022;14(1):1.
67. Shah PM, Peersen A, Jatoi A, et al. Prognostication of ß-catenin (CTNNB1) in patients with HCC treated with first line immunotherapy. *J Clin Oncol*. 2024;42:e15146.
68. Moldogazieva NT, Zavadskiy SP, Terentiev AA. Genomic landscape of liquid biopsy for hepatocellular carcinoma personalized medicine. *Cancer Genomics Proteomics*. 2021;18(suppl 3):369-383.
69. Agarwala V, Khozin S, Singal G, et al. Real-world evidence in support of precision medicine: clinico-genomic cancer data as a case study. *Health Aff*. 2018;37(5):765-772.
70. Sondhi A, Humblet O, Swaminathan A. Quantifying bias from dependent left truncation in survival analyses of real world data. *medRxiv*. 2021. https://doi.org/10.1101/2021.08.02.21261492