Research Article
Hepatic and Biliary Cancer

JOURNAL OF HEPATOLOGY

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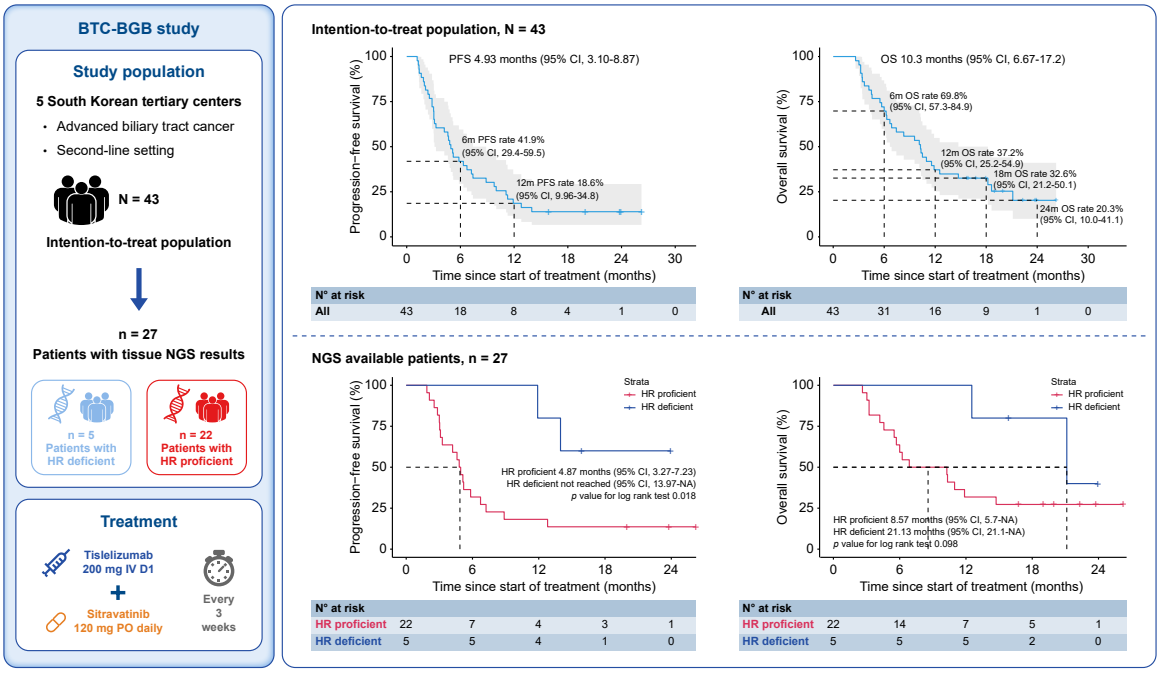
Authors

Jeesun Yoon, Choong-kun Lee, Jin Won Kim, …, Myung Ah Lee, Tae-Yong Kim, Do-Youn Oh

Correspondence

ohdoyoun@snu.ac.kr (D.-Y. Oh).

Graphical abstract



Highlights

- Second-line sitravatinib and tislelizumab combination therapy demonstrated efficacy and acceptable safety in advanced BTC.
- Efficacy was observed regardless of prior exposure to immune checkpoint inhibitors.
- The presence of HRD detected by NGS is a potential biomarker for optimal patient selection.

Impact and Implications

The results of this multi-center, open-label, phase II study suggest that immunotherapy and anti-angiogenic agent combination treatment has a promising efficacy and safety as second-line treatment for patients with advanced biliary tract cancer. Furthermore, the presence of homologous recombination deficiency detected on tumor next-generation sequencing may select patients who have benefit from this combination, which supports further research exploring immunotherapy with anti-angiogenics combination strategy in this setting.

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Open-label, single-arm, phase II trial to investigate the efficacy of sitravatinib plus tislelizumab combination as a second-line treatment for advanced biliary tract cancer

Jeesun Yoon[1], Choong-kun Lee[2], Jin Won Kim[3], Beodeul Kang[4], Se Jun Park[5], Ji-Won Kim[3], Hong Jae Chon[4], Hye Jin Choi[2], Myung Ah Lee[5], Tae-Yong Kim[1], Do-Youn Oh[1,*]

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Background & Aims: Immune checkpoint inhibitors (ICIs) combined with cytotoxic chemotherapy are now the standard first-line treatment for advanced biliary tract cancer (BTC). With this evolving landscape, therapeutic options using ICIs after first-line failure are urgently needed. Anti-angiogenic agents may enhance anti-tumor immunity by increasing tumor antigen presentation and promoting lymphocyte infiltration and migration. We aimed to evaluate the efficacy of sitravatinib plus tislelizumab as second-line therapy for advanced BTC.

Methods: In this open-label, single-arm, phase II trial, patients were enrolled regardless of prior ICI treatment history. The primary endpoint was disease control rate. Key secondary endpoints included objective response rate, progression-free survival, overall survival, safety, and biomarker analyses (NCT04727996).

Results: A total of 43 patients were enrolled. The median follow-up was 10.5 months (95% CI 7.03-15.6). Nine patients had previously received ICI therapy. The disease control rate was 65.1% (95% CI 50.3–78.0), and the objective response rate was 18.6% (95% CI 9.2–32.1). Median progression-free and overall survival were 4.93 months (95% CI 3.10–8.87) and 10.3 months (95% CI 6.67–18.2), respectively. Anti-tumor activity was observed regardless of prior ICI exposure. The most common treatment-related adverse events were associated with sitravatinib and were predominantly grade 1–2. In exploratory analyses, patients with homologous recombination deficiency (HRD), detected by baseline tissue next-generation sequencing (frequency 18.5%), showed better outcomes than those without HRD. Responders displayed higher inflammatory signaling in baseline and on-treatment tumor tissue compared with non-responders.

Conclusions: Sitravatinib plus tislelizumab demonstrated meaningful efficacy and an acceptable safety profile as second-line therapy for advanced BTC. HRD-based patient selection may provide a promising strategy for optimizing treatment in this setting.

Clinicaltrials.gov Identifier: NCT04727996

Introduction

Biliary tract cancer (BTC) comprises a group of heterogeneous malignancies, including intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, gallbladder cancer, and ampulla of Vater cancer, whose incidence has been increasing worldwide in recent years.[1,2] BTC is typically diagnosed at an advanced stage, with poor prognosis driven by high recurrence rates and suboptimal response to palliative chemotherapy.[1] However, the recent advent of immune checkpoint inhibitors (ICIs) has begun to change the paradigm of systemic treatment for advanced BTC.

The TOPAZ-1 study was the first global, phase III trial evaluating immunotherapy plus chemotherapy as a first-line treatment for advanced BTC.[3] This study evaluated the addition of the anti–programmed cell death ligand 1 (PD-L1) inhibitor durvalumab to gemcitabine–cisplatin as first-line treatment for advanced BTC and demonstrated improvements in overall survival (OS) and other clinical outcomes. This is the first study to demonstrate an improvement in OS since the ABC-02 trial and the first to show the effectiveness of an immunotherapy combination strategy in advanced BTC.[3] In the recent 41-month follow-up analysis, the addition of

* Corresponding author. Address: Department of Internal Medicine, Seoul National University Hospital, Cancer Research Institute, Seoul National University College of Medicine, Seoul, Korea, 101 Daehak-ro, Jongno-gu, Seoul 03080, South Korea; Tel: +82-2-2072-0701, fax: +82-2-762-9662.
E-mail address: ohdoyoun@snu.ac.kr (D.-Y. Oh).
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durvalumab yielded a hazard ratio for OS of 0.74, and the 3-year OS rate was 14.6% in the durvalumab arm compared with 6.9% in the placebo arm.[5] A greater proportion of patients treated with durvalumab achieved long-term survival, defined as survival beyond 30 months after randomization.

The KEYNOTE-966 study[6] evaluated the anti–programmed cell death 1 (PD-1) inhibitor pembrolizumab in combination with gemcitabine–cisplatin and, unlike the TOPAZ-1 study, permitted gemcitabine maintenance therapy after eight cycles of combination treatment. The KEYNOTE-966 study also showed an improvement in OS compared to chemotherapy alone, and recent 3-year follow-up results showed that the efficacy of pembrolizumab combination therapy was maintained, with a 2-year survival rate of 24.9%, higher than the 19.2% observed in the placebo arm.[7] The results of these two studies established an immunotherapy combination as the first-line treatment for advanced BTC, moving away from the traditional cytotoxic chemotherapy combination.

With the emergence of precision medicine, there have been various changes in the subsequent treatment strategies for advanced BTC.[2] Targeted therapy is now applicable for patients with fibroblast growth factor receptor 2 (*FGFR2*) rearrangement, isocitrate dehydrogenase 1 (*IDH1*) mutations, or *HER2 (ERBB2)* overexpression,[8–10] and drug approvals for tumor-agnostic targetable genomic alterations are also actively progressing.[11] However, in clinical practice, only a small number of patients harbor identifiable targetable genomic alterations, which, in most cases, results in the use of cytotoxic chemotherapy combinations, such as FOLFOX, as second-line treatment.[12–14] Introduction of ICI combinations, highlighting the need to develop new second-line options following immunotherapy exposure. To improve the efficacy of subsequent treatment, optimal combinations, and biomarker-based patient selection strategies should be considered.

Anti-angiogenic agents targeting the vascular endothelial growth factor (VEGF)-vascular endothelial growth factor receptor (VEGFR) pathway are among the combination partners clinically proven to improve the effectiveness of immunotherapy.[15,16] VEGF impairs the maturation of antigen-presenting dendritic cells and induces the polarization of tumor-associated macrophages to the immunosuppressive M2 phenotype, thereby inducing an immune-suppressive tumor microenvironment. Anti-angiogenic agents induce intratumoral immunomodulatory effects by inhibiting VEGF. They also induce vascular normalization through VEGF/VEGFR inhibition, facilitating T cell migration to the tumor and subsequent response to immunotherapy.[16] Sitravatinib is an oral tyrosine kinase inhibitor and, a multi-target agent that targets the VEGFR family, c-MET, AXL, and MER. Based on its mode of action, sitravatinib inhibits tumor angiogenesis, and alleviates immunosuppressive effects in the tumor microenvironment by decreasing the levels of myeloid-derived suppressor cells and regulatory T cells.[17] Thus, sitravatinib is being evaluated for its potential to overcome resistance following ICI treatment and for its synergistic effects in combination with ICIs across different tumor types.[18,19]

This study aimed to evaluate a combination strategy of sitravatinib and tislelizumab (a PD-1 inhibitor) as second-line treatment for advanced BTC, to reveal the immunomodulatory effects of combining an immunotherapy with an antiangiogenic agent, and to identify suitable biomarkers for this strategy.

Patients and methods

Study designs and participants

This was a phase II, open-label, single-arm, multicenter study evaluating the efficacy and safety of sitravatinib combined with tislelizumab as second-line therapy for advanced BTC.

Eligible participants were adults aged ≥20 years with histologically proven advanced BTC (intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, gallbladder cancer, and ampulla of Vater cancer) that progressed after only one prior line of gemcitabine-based palliative systemic chemotherapy. For the exploratory evaluation, we allowed up to 10 patients to enroll who had used ICI combination regimens as first-line therapy. Participants had at least one measurable lesion based on RECIST version 1.1. Other major inclusion criteria were: ECOG (Eastern Cooperative Oncology Group) performance status of 0 or 1, a life expectancy of ≥16 weeks, and adequate organ and bone marrow function. Exclusion criteria included unacceptable toxicity from prior ICI treatment, defined as: grade ≥3 ICI-related adverse events (AEs) unresponsive to standard management for immune-related AEs (irAEs) requiring ICI discontinuation; grade ≥2 irAEs unresolved after ICI withholding; or grade ≥2 irAEs inadequately controlled with steroids. However, patients with prior endocrine AEs were permitted to enroll if they were stable on appropriate replacement therapy and asymptomatic. Other exclusion criteria included previous autoimmune disease, and uncontrolled intercurrent illness. The full eligibility criteria are provided in the supplementary materials. This trial was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonisation Good Clinical Practice Guidelines. The institutional review boards of all five participating centers approved the study protocol. All participants provided written informed consent prior to screening. The full study protocol is provided in the supplementary materials.

Procedure

All patients were administered sitravatinib 120 mg orally once daily in combination with tislelizumab 200 mg intravenously once every 3 weeks. Each cycle lasted 3 weeks. The treatment continued until disease progression, intolerable toxicity, or patient withdrawal. CT scans for response evaluation were conducted every 6 weeks (2 cycles). A biopsy sample of the tumor tissue was obtained at screening, after two treatment cycles (after 6 weeks), and upon disease progression. Blood samples were collected at baseline, on day 1 of each cycle and upon disease progression (Fig. S1A). After the participants signed an informed consent form, next-generation sequencing (NGS) results were collected from each institution. Local targeted sequencing methods are described in the supplementary methods.

Bulk RNA sequencing was performed on tumor samples obtained at baseline, after 6 weeks, and during disease progression. RNA was isolated from tumor tissue using the QIAamp RNA Mini kit (Qiagen) according to the manufacturer's instructions, RNA quality was assessed with a 4200 TapeStaion System using an RNA Screen Tape (Agilent Technologies), and RNA was quantified using a Qubit (Thermo Fisher Scientific). Total RNA libraries were constructed using the KAPA RNA HyperPrep Kit with RiboErase (Roche, Basel,

Switzerland) according to the manufacturer's instructions. High throughput sequencing was performed as paired-end 150 sequencing runs using a NovaSeq 6000 (Illumina). Raw reads were assembled, and low-quality reads were filtered using Cutadapt (version 3.4). Filtered reads were aligned on a reference genome downloaded from Ensembl (GRCh38 [Homo sapiens] or GRCm39 [Mus musculus]) using STAR (version 2.7.8a) and gene expression per sample was calculated using RSEM (version 1.3.1). Read counts were normalized to the effective library size using the DESeq2 package (version 1.26.0), and a two-sided Wald test was performed to analyze differentially expressed genes according to each condition using the DESeq2 package. Gene set-enrichment analysis was performed in a Hallmark and KEGG gene set from MsigDB (UC San Diego and Broad Institute) using "clusterProfiler" with the following parameters (nPerm = 10000, minGSSize = 10, maxGSSize = 1000, pAdjustMethod = "BH", by = "fgsea") (version 3.18).

Circulating tumor DNA (ctDNA) analysis was performed on plasma samples collected at screening, after two cycles of treatment, and during disease progression. ctDNA was isolated according to the manufacturer's instructions from 2 to 4 ml of plasma using a Maxwell® RSC ctDNA Plasma Kit (Promega, USA) and quantified using a 4200 TapeStation (Agilent Technologies, Santa Clara, CA, USA). DNA was quantified using the Qubit dsDNA High Sensitivity Kit (Thermo Fisher Scientific, USA). Genomic DNA was isolated from peripheral blood mononuclear cells using a Maxwell® RSC Blood DNA Kit (Promega, USA). The DNA NGS library was constructed using the IMBdx NGS DNA Library Prep Kit. Solution-based target enrichment was performed at IMBdx, Inc. (Seoul, South Korea), using the AlphaLiquid® 100 target capture panel. The AlphaLiquid® 100 target panel included 118 cancer-related genes and was designed to cover the entire exon of the genes.

Outcomes

The primary endpoint was disease control rate (DCR), calculated as the percentage of participants with a confirmed complete response, partial response, or stable disease for at least 6 weeks. The secondary endpoints were objective response rate (ORR) (the proportion of participants who achieved a confirmed complete response and partial response), progression-free survival (PFS) (time from first dose to disease progression or death by any cause), OS (time from first dose to death by any cause), and safety. Responses were assessed by the investigator according to RECIST version 1.1. Safety and tolerability were assessed using CTCAE version 5.0 in all patients who received at least one treatment dose of tislelizumab or sitravatinib. The exploratory endpoints included evaluating baseline immune parameters and their modulation by the sitravatinib–tislelizumab combination in peripheral blood and tumor tissue samples.

Statistical analysis

We hypothesized that the sitravatinib–tislelizumab combination would achieve a DCR of 50% in the second-line setting, compared with a null hypothesis of 25%, based on historical data from a meta-analysis of second-line treatments in advanced BTC.[20] Using a one-sided alpha level of 0.05, 90% power, and 10% attrition rate, 43 patients were needed.

Survival was analyzed using Kaplan–Meier curves and is reported as median PFS and OS with 95% CIs. Descriptive statistics were used for all the variables. Continuous variables are shown as medians (IQR). Categorical variables are presented as n (%). The Cox proportional hazard model was used to evaluate the effect of patients' characteristics on survival. In exploratory analysis, Fisher's exact test and Pearson's χ^2 test were used to compare nominal variables between the two groups, and the Kruskal-Wallis H test was used to compare changes in continuous data between the two groups. The log-rank test was used to compare survival. A nominal p value of less than 0.05 was considered statistically significant. Analyses were performed using the R software (version 4.2.0). This study was registered at ClinicalTrials.gov, NCT04727996 (active).

Results

In total, 50 participants were enrolled in this trial between April 12, 2021, and April 15, 2022, and seven were excluded during screening. Baseline participants' characteristics are shown in Table 1. The participants' median age was 64 years (range, 41.1-78.8). Twenty-five participants had an ECOG performance status of 1 (58.1%). The majority of the primary tumors were intrahepatic cholangiocarcinoma (51.2%), and 24 (55.8%) participants underwent curative intent surgery before recurrence. Nine (20.9%) participants had previously received ICI treatment as first-line therapy, and most of them used durvalumab. Patients with prior immunotherapy had a longer duration of first-line systemic treatment compared with those without prior immunotherapy (Table S1). The most common sites of metastasis were the liver (n = 40 [93%]) and the lymph nodes (n = 27 [63%]).

At the data cut-off on July 31, 2023, the median follow-up was 10.5 months (95% CI 7.03–15.6). Four patients discontinued: two withdrew consent, and two were withdrawn per investigator decision (one due to decreased performance status from multiple embolic infarctions, and one due to seizure from newly identified brain metastasis). Three patients (7.0%) remained on study treatment without disease progression, 35 patients discontinued due to radiological disease progression, and one patient discontinued due to unacceptable toxicity (Fig. S1B). In the intention-to-treat population, the DCR was 65.1% (95% CI 50.3–78.0). Eight (18.6%) of the 43 patients achieved an objective response (two [22.2%] of nine in the ICI-treated group and six [17.6%] of 34 in the ICI-naïve group; Fig. 1A,B, Table S2). The median duration of response was 8.15 months (95% CI 5.83-NA). The median time to response was 3.35 months (range 1.23-7.47). A significantly shorter time to response was observed in patients with prior immunotherapy compared to patients without prior immunotherapy (1.30 months [range 1.23-1.37] *vs.* 4.02 months [range 1.40-7.47], $p = 0.004$).

Median PFS was 4.93 months (95% CI 3.10-8.87; Fig. 1C), with a 6-month PFS rate of 41.9% (95% CI 29.4-59.5) (Table S2). Median OS was 10.3 months (95% CI 6.67-18.2; Fig. 1D), with a 12-month OS rate of 37.2% (95% CI 25.2-54.9), and 24-month OS rate of 20.3% (95% CI 10.0-41.1) (Table S2). Similar clinical efficacy was observed regardless of prior use of ICI therapy (Fig. S2A and B, Table S2). No

Table 1. Baseline characteristics.

	Participants (N = 43)
Age (Median)	64.1 (41.4-78.8)
Sex	
Male	22 (51.2%)
Female	21 (48.8%)
Eastern Cooperative Oncology Group performance status	
0	18 (41.9%)
1	25 (58.1%)
History of viral hepatitis	
HBV	6 (14.0%)
No	37 (86.0%)
Primary tumor type	
Intrahepatic cholangiocarcinoma	22 (51.2%)
Extrahepatic cholangiocarcinoma	7 (16.3%)
Gallbladder cancer	12 (27.9%)
Ampulla of Vater cancer	2 (4.6%)
Pathological differentiation	
Well differentiated	1 (2.3%)
Moderately differentiated	17 (39.5%)
Poorly differentiated	9 (20.9%)
Others*	16 (37.2%)
History of surgery	
Yes	24 (55.8%)
No	19 (44.2%)
Site of metastatic lesion	
Liver	40 (93.0%)
Lymph node	27 (62.8%)
Lung	10 (23.3%)
Bone	8 (18.6%)
Peritoneum	14 (32.6%)
Prior first-line chemotherapy	
Gemcitabine/cisplatin	9 (20.9%)
Gemcitabine/cisplatin/Nab-paclitaxel	21 (48.9%)
Acelarin(NUC-1031)/cisplatin	3 (7.0%)
Gemcitabine	1 (2.3%)
Gemcitabine/cisplatin + durvalumab	4 (9.3%)
Gemcitabine/cisplatin + durvalumab + tremelimumab	3 (7.0%)
Gemcitabine/cisplatin + bintrafusp alfa (M7824)	2 (4.6%)
Median cycles of first-line chemotherapy	9.0 (2.0-38.0)
Progression-free survival of first-line treatment	7.8 months (95% CI 5.23-10.60)

Data shown as n (%) or median (IQR).
*Others include unknown, n = 14, and squamous cell carcinoma, n = 2.

differences were observed in PFS or OS when stratified according to baseline clinical characteristics (Fig. S3).

The median number of treatment cycles was 4.0 (IQR 2.0-9.0). Treatment-related adverse events (TRAEs) occurred in 38 (88.4%) of 43 participants (Table 2). Commonly reported TRAEs were related to sitravatinib, including palmar-plantar erythrodysesthesia (60.5%) and hypertension (34.9%). Grade 4 AEs occurred in two participants, one of whom developed grade 4 hypertension and discontinued the study because of uncontrolled hypertension. Twenty-one (48.8%) participants required one or more sitravatinib dose reductions because of AEs. The most common irAE was hypothyroidism (32.6%). Four patients developed grade 3 irAEs (two AST/ALT elevations and two increased lipases), all of which resolved after the dose delay without steroid use. No TRAEs led to tislelizumab discontinuation. No TRAEs resulted in death.

Twenty-seven (62.7%) participants had baseline tumor NGS collected prior to study treatment. The most frequently mutated genes were *TP53* (48%), *CDKN2A* (30%), *KRAS* (22%), and *ARID1A* (15%; Fig. 2A). Two patients had a rearrangement in *FGFR2*, and two patients had mutations in *IDH1*. During the study period, FGFR2-and IDH1-targeted therapies were not approved and reimbursed in South Korea. Therefore, these participants were included in this study. One patient with an *FGFR2* rearrangement received erdafitinib after end of this study through another trial. In this study, homologous recombination repair deficiencies (HRD) were defined as deletion, frameshift, nonsense, and multiple missense mutations in the following 20 genes involved in the homologous recombination repair pathway; *ATM, ATR, BAP1, BARD1, BRCA1, BRCA2, BRIP1, CDK12, CHEK1, CHEK2, FANCL, MRE11, NBN, PALB2, RAD51, RAD51B, RAD51C, RAD51D, RAD54L*, and *XRCC2*. Five (18.5%) of the 27 participants had genomic alterations defined as HRDs. Interestingly, patients with HRDs tended to have a relatively higher response rate than those without HRDs (Fig. 2B). For PFS in patients with HRDs *vs*. those without HRDs, the hazard ratio was 0.20 (95% CI 0.04-0.86; $p = 0.031$; Fig. 2C). OS showed a similar trend to PFS but did not reach statistical significance (hazard ratio 0.30; 95% CI 0.07-1.35; $p = 0.118$; Fig. 2D).

Baseline ctDNA data was available for 35 patients (81.4%). Similar to the tissue NGS results, the most frequently mutated genes were *TP53* (74%), *KRAS* (23%), and *ARID1A* (22%). The same gene variants were identified in one of the two *FGFR* rearrangements and one of the two *IDH1* mutations identified by tissue NGS (Fig. S4A). When defining HRD using the same criteria as for tissue NGS, eight patients (22.8%) were identified as having HRD. Among these, four patients were also identified as having HRD by tissue NGS, demonstrating high concordance between the two tests with a kappa coefficient of 0.808. Consistent with tissue NGS findings, patients with HRD detected by ctDNA had a higher ORR than those without HRD, although the difference did not reach statistical significance (Fig. S4B). In addition, numerically longer PFS and OS were observed in patients with HRD (Fig. S4C and D). Patients with HRD had a relatively higher tumor mutation burden (TMB) level than those without (Fig. S4E; 14.15 mutations/Mb [range 6.29-72.35] *vs*. 9.44 mutations/Mb [0.00-37.75], $p = 0.197$). Of the 28 patients for whom pre- and post-treatment ctDNA analyses were available, tumor ctDNA variant allele frequencies in the plasma decreased after two treatment cycles in 24 patients (85.7%). In an exploratory analysis, lower ctDNA variant allele frequencies were observed after two cycles in the responders compared to non-responders ($p < 0.001$; Fig. S5A). Additionally, responders showed a statistically significant reduction in ctDNA variant allele frequency compared with screening levels, whereas this trend was not observed in non-responders (Fig. S5B and C).

Bulk RNA sequencing data were collected from 44 samples (baseline, n = 30, on-treatment, n = 12; end of treatment, n = 2). The interferon-γ and inflammatory response pathways were activated in the on-treatment samples compared with the screening samples (Fig. S6A–C). The angiogenesis pathway was suppressed in the on-treatment sample according to the mode of action of sitravatinib (Fig. S6B and D). Comparing the screening and on-treatment samples assessed as PD immediately at the first response assessment, inflammatory signaling pathways were suppressed (Fig. S7A–D), unlike previous comparisons. Moreover, when comparing the samples at baseline in the responders and non-responders, the

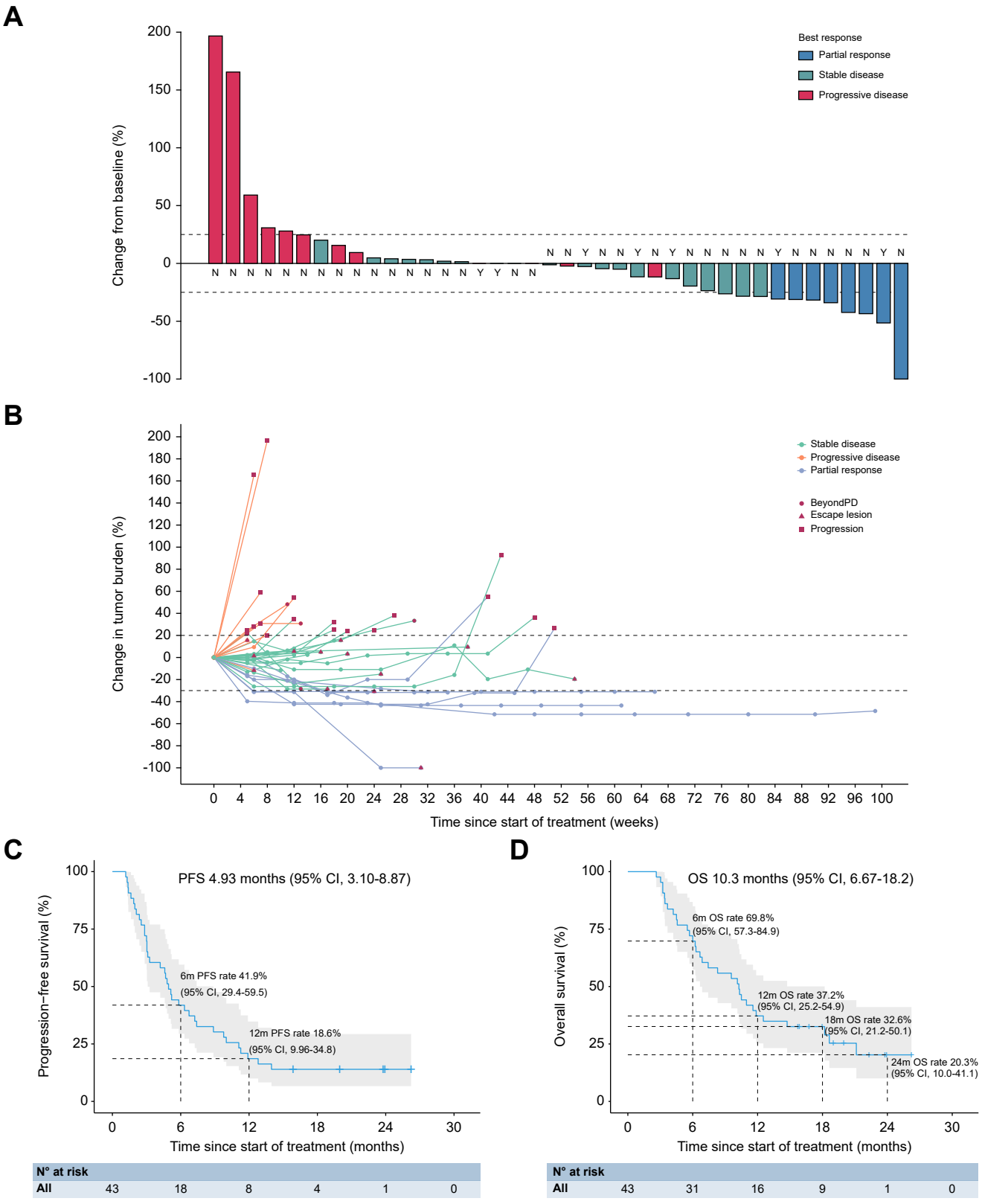

Fig. 1. Clinical outcomes in participants treated with sitravatinib and tislelizumab as second-line therapy. (A) Maximum percentage change from baseline in the size of target lesions with the best responses based on RECIST version 1.1 and history of prior systemic treatment with ICIs in all participants. (B) Percentage change from baseline in the total number of target lesions over time. The dotted lines represent a 20% tumor increase and 30% tumor reduction from baseline. (C) Progression-free survival in all patients over time. (D) Overall survival in all patients over time. ICIs, immune checkpoint inhibitors; OS, overall survival; PFS, progression-free survival. (This figure appears in color on the web.)

Table 2. Treatment-related adverse events.

	Any grade	Grade 3	Grade 4
Hematological			
Thrombocytopenia	6 (13.9%)	1 (2.3%)	1 (2.3%)
Anemia	3 (7.0%)	2 (4.7%)	0
Neutropenia	1 (2.3%)	1 (2.3%)	0
Non-hematological			
Palmar-plantar erythrodysesthesia	26 (60.5%)	0	0
Hypertension	15 (34.9%)	4 (9.3%)	1 (2.3%)
Stomatitis	8 (18.6%)	0	0
General weakness	8 (18.6%)	3 (7.0%)	0
Fever	7 (16.3%)	0	0
Anorexia	5 (11.6%)	1 (2.3%)	0
Proteinuria	5 (11.6%)	0	0
Hoarseness	5 (11.6%)	0	0
Duodenal bleeding	1 (2.3%)	1 (2.3%)	0
Immune-related			
Hypothyroidism	14 (32.6%)	0	0
AST/ALT increased	7 (16.3%)	2 (4.7%)	0
Diarrhea	5 (11.6%)	0	0
Skin rash	5 (11.6%)	0	0
Lipase increased	2 (4.7%)	2 (4.7%)	0
Pancreatitis	1 (2.3%)	1 (2.3%)	0

ALT, alanine aminotransferase; AST, aspartate aminotransferase. Data shown as n (%) of all participants (N = 43). Any grade treatment-related adverse events with an incidence of more than 10% or any treatment-related adverse events that were grade 3 or worse are shown.

inflammatory response pathway was activated in responders at screening (Fig. 3A,B). Baseline *CD274* gene expression, encoding PD-L1, between responders and non-responders showed a log2-fold change of 1.217 with a false discovery rate (FDR)-adjusted *p* value of 0.615, indicating that the difference in PD-L1 expression was not statistically significant between responders and non-responders (Fig. S8A). Notably, cytosolic DNA sensing, mismatch repair, and homologous recombination pathways were relatively enriched in responders at baseline (Fig. 3C and S8B). Additionally, responders showed a higher inflammatory signal on-treatment than non-responders (Fig. 3D–F).

Discussion

This multi-center, single-arm, phase II study to investigate the second-line sitravatinib and tislelizumab combination showed promising clinical activity in patients with advanced BTC refractory to gemcitabine-based chemotherapy. The primary endpoint of DCR was met with a DCR of 65.1% (95% CI 50.3-78.0). Although no direct comparison can be made, the clinical outcomes of the sitravatinib–tislelizumab combination (ORR 18.6%, PFS 4.93 months, OS 10.3 months) are encouraging when considered alongside reported DCRs for standard second-line cytotoxic chemotherapy (33% with FOLFOX; 65% with liposomal irinotecan plus 5-FU/LV).[12,21] Furthermore, the sitravatinib–tislelizumab combination demonstrated greater efficacy in patients with NGS-detected HRD-related genetic alterations than in those without, with an ORR of 60.0%, PFS not reached, and OS of 21.13 months.

Several studies have demonstrated the applicability of immunotherapy as a second-line treatment for advanced BTC. ICI monotherapy demonstrated ORRs of 3-6% and PFS of 2-5 months depending on the agent.[22–24] For dual ICI combinations with different mechanisms, the durvalumab and tremelimumab combination showed an ORR of 10.8% and PFS of 1.6 months,[24] and the nivolumab and ipilimumab combination showed an ORR of 27% and a PFS of 2.9 months.[25]

Anti-angiogenic agents are clinically proven combination partners that improve the efficacy of immunotherapy. Few studies have been conducted in second- or later-line settings for advanced BTC. The LEAP-005 study investigated the efficacy and safety of lenvatinib/pembrolizumab combination therapy for various tumor types. In the BTC cohort, ORR was 10.0% (95% CI 2-26) and PFS was 6.1 months (95% CI 2.1-6.4).[26] A study of avelumab in combination with regorafenib showed similar clinical activity (ORR 13.8%, and PFS 2.5 months),[27] while the combination of rivoceranib and camrelizumab achieved an ORR of 19% (95% CI 7–40), PFS of 4.4 months (95% CI 2.4–6.3), and OS of 13.1 months (95% CI 8.1–18.2).[28] These studies all included patients who had failed a gemcitabine-based chemotherapy combination,[4] and excluded patients with prior ICI exposure. To the best of our knowledge, this is the first study to evaluate the combination of an ICI and anti-angiogenic agent as a second-line treatment, including patients who received immunotherapy combinations as the first-line treatment for advanced BTC. Sitravatinib and tislelizumab combination therapy was effective regardless of prior immunotherapy exposure (Fig. S2). Rather, a trend towards numerically longer clinical outcomes was observed in patients with prior ICI exposure than in those without (Table S1).

Sitravatinib is a selective tyrosine kinase inhibitor targeting VEGFR and TAM (TYRO3, AXL, MerTK) that modulates the tumor microenvironment to enhance anti-tumor immunity.[17] In a preclinical study, angiogenesis-related genes were downregulated in sitravatinib-treated tumors, while inflammatory genes were upregulated in sitravatinib-treated tumors.[17] Also, AXL/MERTK signaling blockade sensitized tumor cells to anti-PD-1 treatment in murine cholangiocarcinoma.[29] Based on this mode of action, a study of the nivolumab/sitravatinib combination in immunotherapy-experienced patients with lung cancer reported encouraging clinical efficacy, with an ORR of 15.3%, including 2 complete responses and 17 partial responses.[18] The duration of response was 11 months,

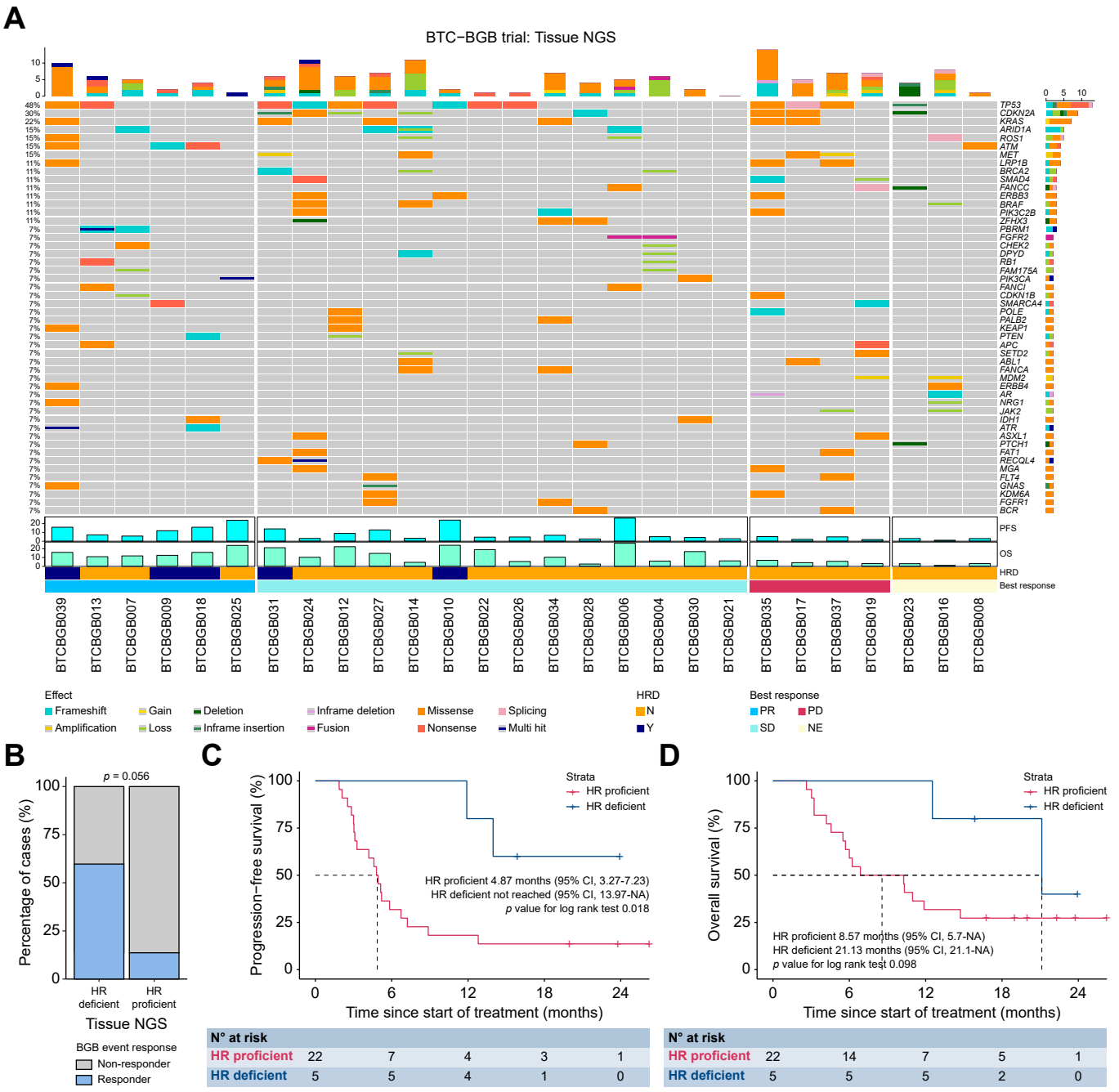

Fig. 2. Overview of the genomic spectrum and association between gene alterations and clinical outcomes. (A) Pre-treatment genomic alterations from 27 patients. The mutations and CNVs are categorized into types of genetic changes in different colors. (B) Patients with HRD had relatively better objective response rate than those without (60% *vs*. 13.6%, $p = 0.056$ [Fisher's exact test]) (C) Progression-free survival according to the presence of HRD on tumor NGS ($p = 0.018$ by log-rank test). (D) Overall survival according to the presence of HRD on tumor NGS ($p = 0.098$ by log-rank test). CNVs, copy number variations; HRD, homologous recombination deficiency; NE, not evaluable; NGS, next-generation sequencing; PD, progressive disease; PR, partial response; SD, stable disease. (This figure appears in color on the web.)

demonstrating that patients previously treated with ICIs could be re-challenged to ICIs with sitravatinib. Our study also reported encouraging clinical activity with the addition of sitravatinib in patients previously exposed to ICIs for advanced BTC. Exploratory analyses demonstrated that the inflammatory response was upregulated with sitravatinib compared to baseline, and that this upregulation was significantly higher in responders than in non-responders.

Biomarkers that predict response to immunotherapy have not been established for advanced BTC. In both the TOPAZ-1 and KEYNOTE-966 studies, tumor PD-L1 expression had no predictive role for benefit from the immunotherapy plus cytotoxic chemotherapy combination.[3,6] The REGOMUNE study testing the avelumab/regorafenib combination observed no statistically significant association between tumor PD-L1 expression and the efficacy of the ICI plus anti-angiogenic

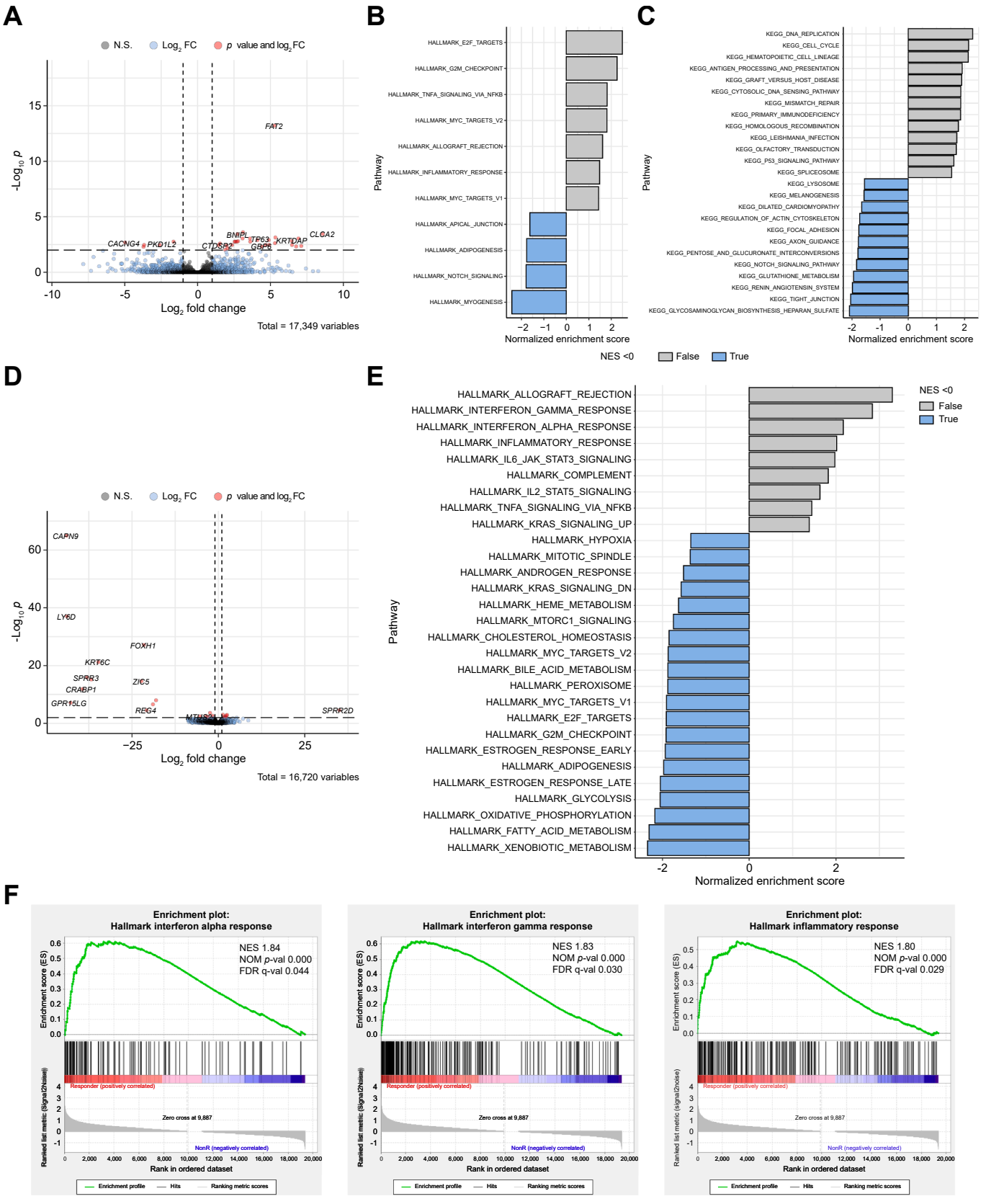

Fig. 3. Identification of differentially expressed genes in responder and non-responders. (A) Volcano plot of DESeq2 results comparing responders and non-responders in baseline tumor tissue. Genes with adjusted *p* values (*p*adj) <0.01 were considered significant. (B) Bar plot showing enriched Hallmark pathways with FDR <0.05 from GSEA comparing responders and non-responders in baseline tumor tissue. (C) Bar plot showing enriched KEGG pathways with FDR <0.05 from GSEA comparing responders and non-responders in baseline tumor tissue. (D) Volcano plot of DESeq2 results between responders and non-responders in on-treatment tumor tissue. (E) Bar plot showing enriched Hallmark pathways with FDR <0.05 from GSEA comparing responders and non-responders in on-treatment tumor tissue. (F) Enrichment plots of representative gene sets in on-treatment tumor tissue. FDR, false discovery rate; GSEA, gene set-enrichment analysis; NES, normalized enrichment score. (This figure appears in color on the web.)

inhibitor combination.[27] In our RNA sequencing data, expression of *CD274* (encoding PD-L1) in baseline tumor tissues did not differ significantly between responders and non-responders (Fig. S8A). RNA sequencing is not a complete substitute for immunohistochemistry for tumor PD-L1 expression; however, our results are consistent with those of the TOPAZ-1 and REGOMUNE studies.

HRD refers to a phenotype characterized by the inability of cells to effectively repair DNA damage using the homologous recombination repair pathway and is one of the hallmarks of cancer.[30] Although there is no single definition of HRD, detection using genomic alterations in the homologous recombination repair pathway via NGS is commonly accepted, and the application of HRD scores using whole-exome sequencing has recently been attempted for more rigorous validation.[30] HRD has been considered a predictive marker for the anti-tumor effect of immunotherapy in various cancers.[31–33] However, the clinical implications of HRD have not been well explored in BTC. A study using the TCGA dataset showed a high correlation between HRD and high TMB in BTC,[34] suggesting that HRD can be used to select patients who may respond to immunotherapy. In this study, HRD detection results between tissue NGS and ctDNA NGS showed high concordance. Furthermore, consistent with previously reported findings in other cancer types, patients with HRD had a relatively higher TMB, and demonstrated favorable clinical outcomes with sitravatinib/tislelizumab combination treatment. Therefore, HRD assessed by NGS might be used as a biomarker to predict response to the immunotherapy/anti-angiogenic combination in advanced BTC, with assessment by tissue NGS recommended. ctDNA NGS might be used as a complementary tool to screen patients for whom tissue biopsy is not feasible.

This study has some limitations. First, this study has an inherent limitation in that it was designed as a single-arm trial. Therefore, this study had difficulty distinguishing between treatment effects and natural disease progression, limiting causal inferences about treatment effects. Second, while efficacy is typically evaluated as an objective response in phase II studies, this study used DCR as the primary endpoint. BTC is a highly aggressive tumor with poor prognosis, and many patients experienced difficulty undergoing subsequent palliative systemic treatment owing to poor performance status and complications such as cholangitis. Additionally, despite the development of various treatment options in recent years, effective subsequent systemic treatment options remain limited. Therefore, this study was designed as a signal-seeking study to investigate disease stabilization. Further large, prospective studies will be needed to validate the results of this study. Third, this study has a small sample size, making it difficult to obtain sufficient validity in exploratory analysis. Although statistical verification was supported by methods such as the bootstrap method, further validation through large-scale prospective, randomized studies is necessary to support these results. Fourth, in this study, follow-up duration was relatively short. At the data cut-off point, there were three patients undergoing ongoing treatment (Fig. S1B), and two of them are still receiving this treatment. Therefore, long-term follow-up data on responders would be meaningful. Lastly, given the lack of a standardized definition of HRD in BTC, we classified HRD as genetic alterations in a predefined set of 20 genes. Further external validation using whole-genome sequencing or other methods are needed in the future.

In conclusion, the combination of sitravatinib and tislelizumab as a second-line treatment in patients with advanced BTC demonstrated meaningful efficacy and an acceptable safety profile. Furthermore, efficacy was observed regardless of prior ICI exposure, and HRD detected by tissue NGS emerged as a potential biomarker for optimal patient selection.

Affiliations

[1]*Seoul National University Hospital, Cancer Research Institute, Seoul National University College of Medicine, Integrated Major in Innovative Medical Science, Seoul National University Graduate School, Seoul, South Korea;* [2]*Division of Medical Oncology, Department of Internal Medicine, Yonsei Cancer Center, Yonsei University College of Medicine, Seoul, South Korea;* [3]*Department of Internal Medicine, Seoul National University Bundang Hospital, Seoul National University College of Medicine, Seongnam, South Korea;* [4]*Medical Oncology, CHA Bundang Medical Center, CHA University School of Medicine, Seongnam, South Korea;* [5]*Division of Medical Oncology, Department of Internal Medicine, College of Medicine, Seoul St. Mary's Hospital, The Catholic University of Korea, Seoul, South Korea*

Abbreviations

AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BTC, biliary tract cancer; ctDNA, circulating tumor DNA; DCR, disease control rate; FGFR2, fibroblast growth factor receptor 2; HRD, homologous recombination deficiency; ICI, immune checkpoint inhibitor; IDH1, isocitrate dehydrogenase 1; irAE, immune-related adverse event; NGS, next-generation sequencing; ORR, objective response rate; OS, overall survival; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1; PFS, progression-free survival; TMB, tumor mutation burden; TRAE, treatment-related adverse event; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

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Conflict of interest

C-k.L. received honoraria from AstraZeneca, Servier, Dong-A ST, Boryung Pharmaceuticals, and Roche; consulting fees from Roche, and Daiichi Sankyo; and received research grants or supports from Ono Pharmaceuticals, Celltrion, Boryung Pharmaceuticals, GC Biopharma and Lunit Inc (outside the submitted work). JWK has been a consultant for AstraZeneca, BeiGene, Beyond Bio, BMS/Celgene, Eisai, GC cell, MSD, Ono Pharma, Sanofi-Aventis, Servier, and TCU-BEit, and has received research grants from Samyang biopharma, and Boryung. J-WK has been a consultant for Degiopharm, Pyramid Biosciences, MSD, Lilly, MitoImmune, Adlai Nortye, AstraZeneca, and Aslan. HJC has been a consultant for Roche, Ono Pharma, BeiGene, Eisai, BMS, Sanofi, AstraZeneca, Sevier, and MDS, and has received research grants from Roche, BeiGene, Dong-A ST, and Boryung Corporation. D-YO has been a consultant or advisor for AstraZeneca, Novartis, Genentech/Roche, Merck Serono, Bayer, Taiho, ASLAN, Halozyme, Zymeworks, BMS/Celgene, BeiGene, Basilea, Turning Point, Yuhan, Arcus Biosciences, IQVIA, MSD, LG Chem, Astellas, Abbvie, J-Pharma, Mirati Therapeutics, Eutilex, Moderna, and Idience, and has received research grants from AstraZeneca, Novartis, Array, Eli Lilly, Servier, BeiGene, MSD, and Handok. All other authors declare no competing interests.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

JY and D-YO conceptualized the study. JY, C-KL, JWK, HJC, MAL, and D-YO contributed to the data acquisition. JY, C-KL, JWK, BK, SJP, HJC, HJC, MAL, T-

YK, and D-YO contributed to the analysis and interpretation of data. JY, T-YK, and D-YO have accessed and verified all the data in the study. D-YO supervised the study. All authors contributed to the writing and review of the manuscript and provided final approval. All authors had full access to all the data in the study and analyses and accept responsibility to submit for publication.

Data availability

Deidentified individual participant data from this study can be made available upon request to the corresponding author following publication and are subject to approval by the study investigators.

Previous presentation

This study was presented in part at the 2024 ASCO (American Society of Clinical Oncology) annual meeting.

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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2025.10.032.

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Author names in bold designate shared co-first authorship

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