Impaired Chromatin Remodeling Predicts Better Survival to Modified Gemcitabine and S-1 plus Nivolumab in Advanced Biliary Tract Cancer: A Phase II T1219 Study

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ABSTRACT

Purpose: Modified gemcitabine and S-1 (GS) is an active regimen for patients with advanced biliary tract cancer (ABTC) in our previous study. Herein, we report the results of a single-arm phase II of nivolumab plus modified GS (NGS) as first-line treatment in ABTC.

Patients and Methods: Patients received nivolumab 240 mg and 800 mg/m^2 gemcitabine on day 1 plus daily 80/100/120 mg of S-1 (based on body surface area) on days 1 to 10, in a 2-week cycle. The primary endpoint was the objective response rate (ORR). The correlation between therapeutic efficacy and genetic alterations with signatures identified by targeted next-generation sequencing panels was explored.

Results: Between December 2019 and December 2020, 48 eligible patients were enrolled. After a median of 17.6 months of follow-up, the ORR was 45.9% [95% confidence interval (CI), 31.4%–60.8%]. The median progression-free survival (PFS) and overall survival (OS) was 9.1 (95% CI, 5.8–9.6) and 19.2 (95% CI, 11.6–not reached) months, respectively. All grade 3/4 treatment-related adverse events (AE) were less than 10%, except fatigue (14.6%) and skin rash (10.4%). Eighteen patients (35.4%) experienced immune-related AEs without treatment-related death. High tumor mutational burden (TMB-H; top 20%; ≥7.1 mut/Mb) only predicted prolonged median PFS but not OS. Up to 28.9% of patients who harbored loss-of-function mutations in chromatin remodeling genes demonstrated significantly longer median PFS and OS than those without alterations.

Conclusions: NGS is a safe and promising regimen in ABTC. Impaired functions of chromatin remodeling genes may be a potential surrogate biomarker with predictive value in this study.

Introduction

Biliary tract cancer (BTC), including intrahepatic cholangiocarcinoma (iCCA), extrahepatic cholangiocarcinoma (eCCA), gallbladder cancer (GBC), and ampulla of Vater cancer (AVC), accounts for 3% of all gastrointestinal malignancies (1). Approximately 60% to 70% of advanced biliary tract cancer (ABTC) is diagnosed at an advanced stage with the historic 5-year survival rates of only 10% to 20% (2–4). Systemic therapy, including chemotherapy and targeted therapies based on genetic alternations, remains a standard treatment for ABTC (5).

Several prospective clinical trials have demonstrated an improvement in the median overall survival (OS) using combination chemotherapy for BTC. A phase III ABC-02 study established the role of gemcitabine and cisplatin (GC) in the standard first-line chemotherapy for patients with BTC having a median OS of 11 months (6). A phase III JCOG 1113 study showed a noninferiority of gemcitabine and S-1 (GS) to GC with a numerical better median OS (15.1 months vs. 13.4 months; ref. 7). In our study, modified GS as first-line treatment in patients with ABTC demonstrated acceptable efficacies of an objective response rate (ORR) of 21.7% and a median OS of 12.7 months (8). Most importantly, grade 3/4 treatment-related adverse events (AE) were less than 6% of patients in all individual items. Owing to its favorable therapeutic index, modified GS regimen could be applied for maintenance treatment after 6 months of disease

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Translational Relevance

Chemotherapy regimens remain a mainstay of systemic treatment for advanced biliary tract cancer (ABTC). Recent studies showed that adding immune checkpoint inhibitor to chemotherapy might herald the opportunity to provide more durable antitumor activity in many malignancies. This study applied a friendly chemotherapy regimen: modified gemcitabine and S-1, in combination with nivolumab as the first-line treatment in ABTC and showed promising therapeutic efficacies without increased toxicities associated with cytotoxic agents. Results of comprehensive genomic profiling of tumors collected before treatment showed that nearly one third of patients with ABTC harbored mutations in genes involved in chromatin remodeling, which directly correlated with a high tumor mutation burden (TMB-H). Both TMB-H and chromatin remodeling mutations predicted longer progression-free survival, but only truncating mutations of chromatin remodeling genes demonstrated significant value in predicting prolonged overall survival compared with those without mutations in ABTC. The predictive capability of biomarkers for chemoimmunotherapy in ABTC needs to be validated in a larger cohort.

control and an ideal chemotherapy backbone in combination with additional cytotoxic chemotherapies or immune checkpoint inhibitors (ICI) in ABTC.

Immunotherapy has opened a new era of treatment in various types of cancer, including BTC. In KEYNOTE-028 and 158 studies, pembrolizumab showed 5.8% to 17% of ORR in programmed cell death protein 1 (PD-L1)-positive BTC (9). To increase ORR, it is reasonable to combine immunotherapy with other treatments, such as chemotherapy. In a phase I study conducted in Japan, nivolumab plus GC demonstrated promising results with an ORR of 36.7% and a median OS of 15.4 months (10). Previously, the chemotherapy regimen of GS was an alternative in Asian patients with ABTC (7, 8), but more published data focused on the efficacy of GC in combination with immunotherapy. In this study, we applied the friendly modified GS regimen in combination with nivolumab and evaluated the safety and effectiveness of the triplet regimen in patients with ABTC.

Patients and Methods

Study approval

This trial was approved by the Institutional Review Board of all three participating institutes with reference numbers (2019–10–001C, CMUH108-REC1–133, and A-BR-108–073) and is registered with ClinicalTrials.gov (NCT04172402). All patients had signed informed consent forms before study treatment. This study was conducted in accordance with the Declaration of Helsinki and International Conference on Harmonization Good Clinical Practice guideline.

Patient eligibility

The key inclusion criteria were as follows: (i) histologically confirmed locally advanced or metastatic BTC including iCCA, eCCA, GBC, and AVC, with at least one measurable lesion according to the RECIST v1.1; (ii) patient age ≥ 20 years; (iii) Eastern Cooperative Oncology Group performance status (ECOG PS) score of 0 or 1; (iv) adequate bone marrow, hepatic, and renal functions [absolute neutrophil count ≥1,500/μL, platelets ≥100,000/μL, hemoglobin ≥9 g/dL, serum total bilirubin level ≤1.5 times the upper limit of normal (ULN) and <2 mg/dL, alanine aminotransferase (ALT) level ≤3 times the ULN (or ≤5 times the ULN if liver metastasis), and creatinine clearance (CCr) ≥50 mL/min]; (v) no prior chemotherapy or radiotherapy except those delivered as adjuvant setting that completed at least 6 months before documentation of recurrence by imaging study; and (vi) the ability to sign an informed consent document.

The exclusion criteria were as follows: (i) the presence of grade 2 or above ascites, pleural effusion, or diarrhea; (ii) previous or current brain metastasis; (iii) uncontrolled active infection; (iv) pregnancy or breast feeding; (v) history of active autoimmune disease within 3 years or long-term use of steroid more than prednisolone 10 mg/day; and (vi) other malignancy within the past 5 years, except for adequately treated basal or squamous cell skin cancer or cervical cancer *in situ*.

Study treatment and dose modification

The study regimen consisted of intravenous infusion of fixed-dose 240 mg nivolumab and 800 mg/m^2 gemcitabine on day 1, plus oral administration of S-1 80/100/120 mg daily, according to the initial body surface area (<1.25/m^2; ≥1.25/m^2 and <1.5/m^2; or ≥1.5/m^2) on days 1 to 10 in a 2-week cycle. The dosage of S-1 in the first cycle was applied for the following cycle until specific AEs occurred or dose reduction was required by investigators' judgments. The treatment was administered until disease progression, intolerable toxicities, withdrawal of consent, or any other reasons. The subsequent cycle could be started only if the following criteria were met on day 1: neutrophil count ≥1,500/mm^3, platelet count ≥75,000/mm^3, total bilirubin ≤2 times the ULN, ALT ≤5 times the ULN, and all other toxicities recovered to ≤grade 2. The treatment was only allowed to be delayed by up to 2 weeks. Dose modification for nivolumab was not permitted, whereas reduction of the other two drugs was allowed only twice, with the permitted nadir dose being 400 mg/m^2 for gemcitabine and 60 mg/day for S-1. No further dose reescalation was permitted after the nadir dose.

If grade 4 neutropenia, febrile neutropenia, grade 4 thrombocytopenia (or grade 3 with platelet transfusion), or grade 3 to 4 nonhematologic toxicities related to gemcitabine occurred, the subsequent dose of gemcitabine was reduced by 200 mg/m^2. If grade 3 to 4 diarrhea, stomatitis, rash, or nonhematologic toxicities associated with S-1 occurred, then the subsequent S-1 dose was reduced by 20 mg/day. Dose reduction of S-1 was according to renal function on scheduled day 1 of the subsequent cycle. If 40 mL/min ≤CCr < 50 mL/min was noted, S-1 was reduced by one dose level. In the presence of 30 mL/min ≤ CCr < 40 mL/min, the dose reduction was suggested by two dose levels if CCr did not show a value of lower than 50 mL/min previously; the dose was also adjusted to one dose level if 40 mL/min ≤ CCr < 50 mL/min had been reached previously. S-1 would be discontinued permanently once CCr <30 mL/min.

Assessment and follow-up

The radiographic assessment was performed every 6 weeks for four times, followed by every 8 weeks thereafter. The tumor responses were assessed according to RECIST v1.1 with confirmation of objective response using two successive imaging studies. Long-term disease control rate (DCR) was defined by objective response and stable disease ≥12 weeks. AEs were graded using the Common Terminology Criteria for AEs version 4.0, and causal association with study drugs was assessed by investigators. For safety follow-up, each subject was followed for occurrence of severe AEs until 30 days after the last dose of study medication, or additional antitumor therapy had been

introduced, whichever came first. Survival status will be followed after the end of treatment at least every 3 months until death or lost to follow-up.

Tumor mutation burden (TMB) and genetic analysis by targeted next-generation sequencing (NGS)

Tumor tissues collected before treatment were subject to NGS using the 440-gene panel ACTOnco (ACT Genomics) and sequenced on the Ion Torrent platform (Thermo Fisher Scientific). The detailed experimental procedure was as previously described or otherwise specified (11). All samples were sequenced at an average mean depth of ≥500× and uniformity of at least 75% of sequenced regions had been covered at ≥100×. TMB was calculated as somatic mutations detected in the sequenced genomic region of 1.1 Mb. Somatic mutations were identified by database approach, which matched each variant to records in public databases as the following order of Genome Aggregate (gnomAD, https://gnomad.broadinstitute.org/), the Cancer Genome Atlas (TCGA), and Catalogue of Somatic Mutations in Cancer (COSMIC). Variants matched to records in gnomAD were designated as germline mutations, whereas variants matched to records in TCGA and COSMIC were designated as somatic mutations. Subsequently, patient-derived factors, including variant allelic frequency, copy-number alterations, zygosity, and tumor purity were applied to the remaining mutations (12, 13). Driver mutations were filtered out to decrease potential bias in the calculation. A regression-based model is used to correct for synonymous mutations and panel size.

IHC staining of PD-L1

Archival paraffin-embedded tumor samples before treatment were used for PD-L1 IHC staining by Dako 22C3 pharmaDx assay (Dako North America). The results were evaluated by an independent pathologist at the central laboratory. The PD-L1 expression was defined as the percentage of the number of PD-L1 staining cells, including tumor cells, lymphocytes, and macrophages for combined positive score (CPS).

Statistical and data analysis

Simon's optimal two-stage design was applied for the sample size with the primary endpoint of ORR (14). The secondary endpoints included long-term DCR, PFS, OS, and safety profiles. Considering the lowest ORR of patients treated with GC was 15% in a phase III study (KHBO1401-MITSUBA) and 21.6% under modified GS in our previous TG1308 study, the p0 and p1 were set as 15% and 35%, respectively (8, 15). With a significance level of 0.05 and a power of 90%, 19 evaluable patients would be accrued in the first stage. If more than 4 patients achieved objective response, additional 25 evaluable patients were enrolled in the second stage. The null hypothesis was rejected if ≥10 responders existed from 44 evaluable patients. Assuming 10% of drop-out rate, a total of 48 patients should be enrolled. All efficacy analyses were applied to the intention-to-treat population, and safety profiles were evaluated in subjects who had received at least one dose. PFS was calculated from the time of the first dose to the first documented disease progression/ to death/ to be censored, whichever occurred first. Kaplan–Meier method was used to determine median PFS and OS with 95% confidence intervals (CI) and the log-rank test was used to compare PFS between different subgroups. The correlation between PD-L1 and TMB was calculated by the Chi-square test. Univariate Cox regression model based on selected clinicopathological factors and genetic alterations was applied to estimate the HR with 95% CI of survival. All analyses were performed using GraphPad Prism (v.9.0; GraphPad Inc.) and SAS version 9.4 (SAS Institute). A two-sided $P < 0.05$ was considered statistically significant.

Data availability statement

The data generated in this study are available upon request from the corresponding authors. The NGS analysis data can be obtained in the Sequence Read Archive (https://www.ncbi.nlm.nih.gov/Traces/study/?acc=PRJNA856807&o=acc_s%3Aa) with accession no. SRP385653.

Results

Patient characteristics

Between December 27, 2019 and December 3, 2020, 48 eligible patients were enrolled. The data cutoff was December 31, 2021. The baseline demographics of all patients are summarized in **Table 1**. The median age was 66 years (range, 30–80), 26 were female (54%), 38 (79.2%) had an ECOG PS of 0, 41 (85.4%) had metastatic disease, and 16 had undergone surgery previously. Most patients had iCCA (29 patients, 60%). Other types included eCCA (12 patients, 25%), GBC (5 patients, 10%), and AVC (2 patients, 2%). The most common metastasis site is the liver (75%). Fourteen patients (29%) had chronic hepatitis infection.

Treatment-related toxicities

All 48 patients receiving at least one treatment were evaluated for treatment-related AEs. The most frequent AEs were anemia (27%), thrombocytopenia (25%), fatigue (25%), and stomatitis (21%), as listed in **Table 2**. The most common grade 3 AEs were fatigue (15%), skin rash (10%), and thrombocytopenia (8%). No grade 4 AE was reported. Twenty-seven episodes of immune-related AEs (irAE) occurred in 18 patients, with the most common one being skin rash, followed by adrenal insufficiency and hypothyroidism. Three patients stopped nivolumab permanently due to grade 3 adverse effects of pneumonitis, arthritis, and skin toxicity. By the time of data cutoff, no treatment-related deaths were recorded. Eight patients were withdrawn from the study due to delayed recovery from AEs (hyperbilirubinemia: 4, biliary tract infection with sepsis: 3, stomatitis: 1).

Tumour response and survival

In the first stage, confirmed partial response (PR) was identified in 6 patients of 19 evaluable subjects, which met the criteria for progressing to the second stage. Of the 48 patients enrolled, 47 were evaluable and one was nonevaluable owing to obstructive jaundice and early withdrawal by the patient's choice. Twenty-two patients had objective response (45.9%) with 95% CI of 31.4% to 60.8%, including one in pathologic CR (2.1%), 21 in PR (43.8%), stable disease (SD) in 20 (41.7%), and progressive disease (PD) in 5 patients (10.4%), as shown in the **Table 3**. Furthermore, 16 patients had SD ≥12 weeks (33.3%) with long-term DCR of 79.2% with 95% CI of 65% to 89.5% (**Fig. 1A**). Two patients with PD showed a <20% increase in target lesions, but the appearance of new lesions or progression of nontarget lesions was noted. The duration of response (DoR) to data cutoff was 9.6 months (95% CI, 6.7–not reached), as shown in **Fig. 1B** and **C**. Two patients with significant tumor shrinkage during treatment were subject to salvage operations, and one patient had pathologic complete response without microscopic tumor cells over the primary tumor and lymph nodes. Six patients were still on PR or SD status at the time of data cutoff (**Fig. 1C**). A total of 41 patients (85.4%) had either disease progression or death. The median number of treatment cycles was 14 (range, 1–38). Gemcitabine dose was reduced in 7 patients (14.6%),

Table 1. Baseline demographics and clinical characteristics.

	Patients ($N = 48$)
Age (years)	
Median (range)	66 (30–80)
<65	18 (37.5)
≥65	30 (62.5)
Gender	
Male	22 (45.8)
Female	26 (54.2)
ECOG performance status	
0	38 (79.2)
1	10 (20.8)
Primary site	
Intrahepatic	29 (60.4)
Extrahepatic	12 (25)
Gallbladder	5 (10.4)
Ampulla of Vater	2 (4.2)
Disease status at entry	
Locally advanced	7 (14.6)
Distant metastasis	41 (85.4)
Previous surgery	
Yes	16 (33.3)
No	32 (66.7)
Metastatic sites	
Liver	36 (75)
Lung	10 (20.8)
Distant lymph node	22 (45.8)
Bone	6 (12.5)
Peritoneum	7 (14.6)
Chronic hepatitis	
HBV	12 (25)
HCV	2 (4)
PD-L1 expression	
CPS ≥1	20 (42.6)
CPS ≥10	14 (29.2)
Not assessable	1 (2.1)

Note: Data are *n* (%), unless otherwise specified.
Abbreviations: CPS, combined positive score; ECOG, Eastern Cooperative Oncology Group; HBV, hepatitis B virus; HCV, hepatitis C virus.

and S-1 dosage was reduced in 29 patients (60.4%). The dose intensity (DI) of gemcitabine and S-1 during the 12 cycles from the start of chemotherapy was 94% and 85% of planned DI, respectively.

After a median follow-up of 17.6 months (95% CI, 15.5–21.8 months), the median PFS was 9.1 months (95% CI, 5.8–9.6 months; **Fig. 1D**). The median OS was 19.2 months (95% CI, 11.6 months–not reached) with 12- and 18-month OS rates of 60.4% (95% CI, 45.2%–72.6%) and 51.2% (95% CI, 36.1%–64.5%), respectively (**Fig. 1E**). Thirty-five of 41 (85.4%) of the withdrawn patients had post-study treatments. Fourteen received gemcitabine plus platinum-based regimens, 3 received 5-FU–based regimens plus oxaliplatin and irinotecan, 3 received salvage operation, and the remaining received anti-angiogenesis agents of lenvatinib or bevacizumab, radiotherapy, clinical trial, and continuous modified GS.

The association of PD-L1 expression and treatment outcomes

PD-L1 expression could be evaluated in 47 pretreatment samples. Using the cutoff CPS of ≥1 or ≥10, 43% and 29% of patients had positive PD-L1 expression, respectively (**Table 1**). There was no statistically significant increase in ORR (60% vs. 37%, $P = 0.15$) nor long-term DCR (85% vs. 74.1%, $P = 0.48$) for patients with PD-L1 expression than those without PD-L1 expression (CPS of <1; **Table 3**).

Table 2. Treatment-related adverse events ($n = 48$).

	Grade 1–2		Grade 3	
	n	%	*n*	%
Hematologic toxicities				
Leukopenia	2	4.2	2	2.0
Neutropenia	5	10.4	3	6.3
Febrile neutropenia	0	0	0	0
Thrombocytopenia	8	16.7	4	8.3
Anemia	10	20.9	3	6.3
Nonhematologic toxicities				
Anorexia	7	14.6	0	0
Fatigue	5	10.4	7	14.6
Nausea	3	6.3	0	0
Vomiting	5	10.4	0	0
Diarrhea	7	14.6	2	4.2
Stomatitis	7	14.6	3	6.3
Elevated AST	1	2.1	1	2.1
Elevated ALT	1	2.1	0	0
Elevated lipase	0	0	1	2.1
Skin rash	3	6.3	5	10.4
Allergic reaction	1	2.1	1	2.1
Hypothyroidism	4	8.4	0	0
Adrenal insufficiency	5	10.4	0	0
Arthritis	0	0	1	2.1
Interstitial pneumonitis	1	2.1	2	4.2
Elevated lipase	0	0	1	2.1
Colitis	0	0	1	2.1
Arthritis	0	0	1	2.1

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase.
No treatment-related grade 4 adverse events were reported.

Median PFS or OS was not significantly different between PD-L1–positive and -negative patients, defined by CPS ≥1 and ≥10 as the cutoff of positive expression (Supplementary Fig. S1).

Association of genetic alterations with TMB and clinical response

All genomic alterations detected in 39 tumor samples with adequate DNA amount were collected for analysis. As shown in **Fig. 2**, the most frequently mutated genes in the entire cohort were *TP53* (53.8%), *KMT2C* (23.1%), *KRAS* (15.4%), *USH2A* (12.8%), *MUC16* (12.8%),

Table 3. Efficacy results.

	All patients ($n = 48$)	PD-L1 CPS ≥1 ($n = 20$)	PD-L1 CPS <1 ($n = 27$)
Best overall response			
Complete response	1 (2.1)	1 (5)	0 (0)
Partial response	21 (43.8)	11 (55)	10 (37)
Stable disease	20 (41.7)	6 (30)	13 (48.1)
Progressive disease	5 (10.4)	2 (10)	3 (11.1)
Not evaluated	1 (2.1)	0 (0)	1 (3.7)
ORR	22 (45.9)	12 (60)	10 (37)
95% CI	31.4%–60.8%	36.1%–80.9%	19.4%–57.6%
		$P = 0.15$	
Long-term DCR	38 (79.2)	17 (85)	20 (74.1)
95% CI	65%–89.5%	62.1%–96.8%	53.7%–88.9%
		$P = 0.48$	

Note: Values are presented as *n* (%). ORR, complete response + partial response. Long-term DCR, complete response + partial response + stable disease ≥12 weeks.

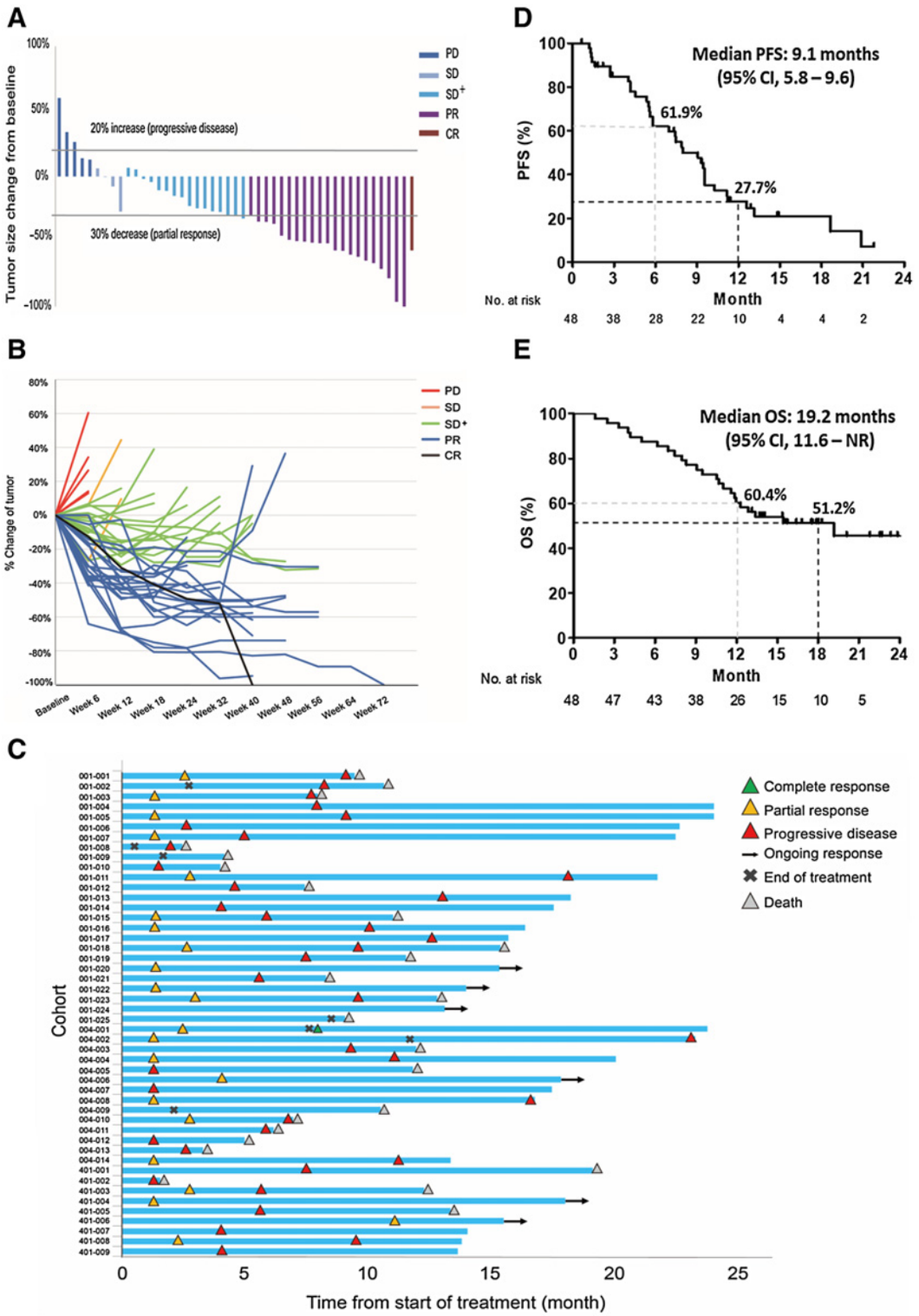
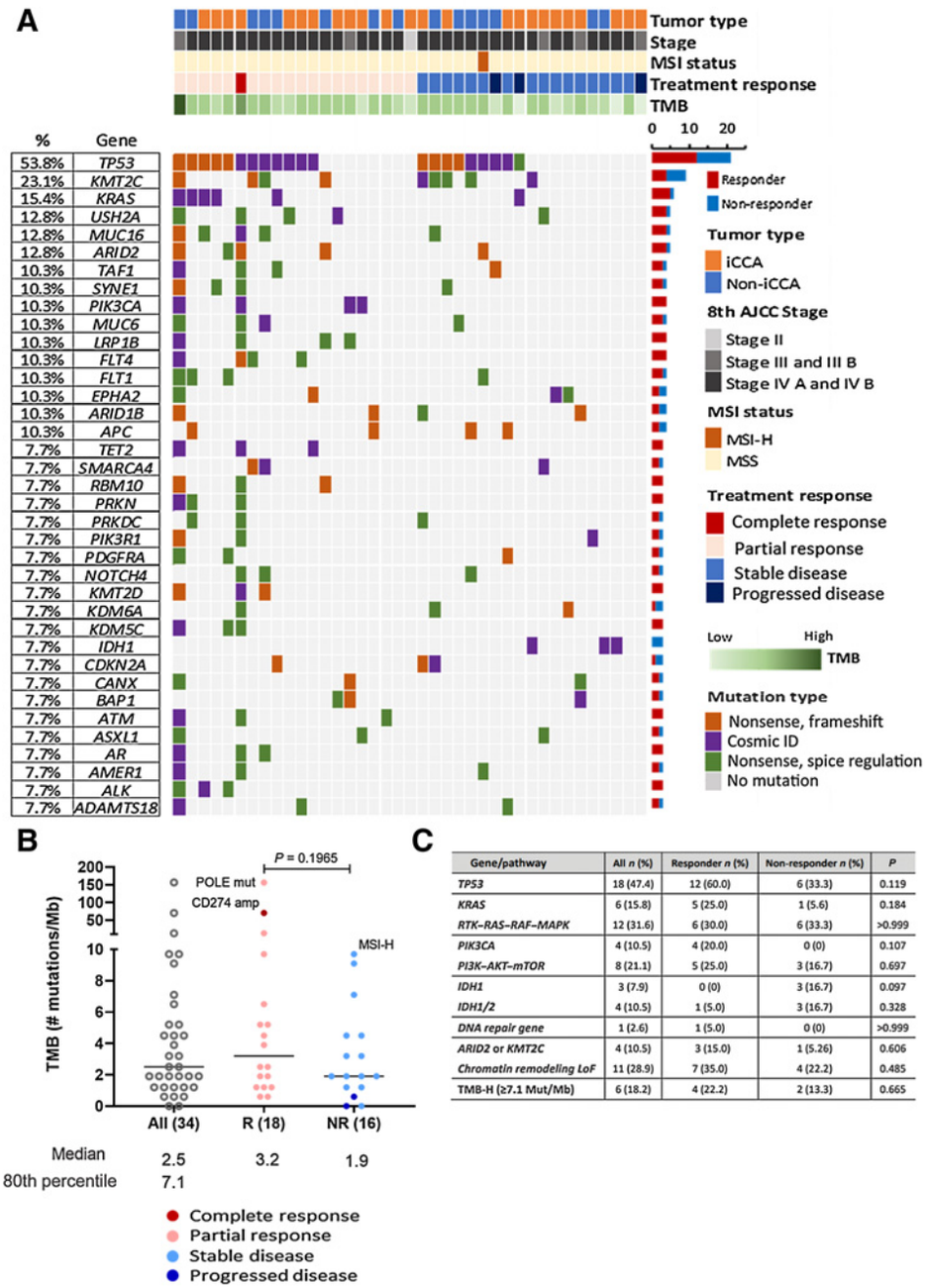


Figure 1.

Clinical response and treatment duration. **A,** Best tumor response with the highest percentage change of the size of targe lesions from baseline during whole course of treatment. The upper and lower horizontal lines indicate a 30% decrease with the presentation of a partial response and 20% increase with the presentation of a progressive disease, according to RECIST criteria. (*Continued on the following page.*)



Gene/pathway	All *n* (%)	Responder *n* (%)	Non-responder *n* (%)	*P*
TP53	18 (47.4)	12 (60.0)	6 (33.3)	0.119
KRAS	6 (15.8)	5 (25.0)	1 (5.6)	0.184
RTK–RAS–RAF–MAPK	12 (31.6)	6 (30.0)	6 (33.3)	>0.999
PIK3CA	4 (10.5)	4 (20.0)	0 (0)	0.107
PI3K–AKT–mTOR	8 (21.1)	5 (25.0)	3 (16.7)	0.697
IDH1	3 (7.9)	0 (0)	3 (16.7)	0.097
IDH1/2	4 (10.5)	1 (5.0)	3 (16.7)	0.328
DNA repair gene	1 (2.6)	1 (5.0)	0 (0)	>0.999
ARID2 or KMT2C	4 (10.5)	3 (15.0)	1 (5.26)	0.606
Chromatin remodeling LoF	11 (28.9)	7 (35.0)	4 (22.2)	0.485
TMB-H (≥7.1 Mut/Mb)	6 (18.2)	4 (22.2)	2 (13.3)	0.665

Figure 2.

Genomic landscape and TMB distribution in 39 patients with BTC. **A,** Genomic landscape of the somatic mutations. Genetic alternations were sorted by the driver pathways in BTC, including chromatin remodeling, IDH1/2, PI3K–AKT–mTOR, and RTK–RAS–RAF–MEK pathway. **B,** Patients were ranked by TMB distribution and best response. The TMB distribution in all patients (n = 34, only samples with tumor purity ≥30%), responders, and nonresponders. Among them, 3 patients with each MSI-H, *POLE* mutation, and *CD274* amplification were labeled. **C,** The percentage of genetic alterations in MSS population (n = 38) and TMB population (n = 33, only MSS samples with tumor purity ≥30%) with the comparison between the responders and nonresponders. P value was calculated by the Fisher exact test. $P < 0.05$ was considered as significant difference. iCCA, intrahepatic cholangiocarcinoma; LoF, loss of function; MSI-H, microsatellite instability-high; MSS, microsatellite stable; NR, nonresponders (stable disease and progressive disease); R, responders (complete response and partial response); TMB-H, tumor mutation burden-high.

and *ARID2* (12.8%). TMB and microsatellite instability (MSI) status were evaluated in 34 samples containing ≥30% of tumor purity. The median TMB in all BTC was 2.5, with no significant difference between responders and nonresponders (3.2 vs. 1.9 mut/Mb, P = 0.1965; **Fig. 2B**). Using the top 20th percentile for the threshold setting, the cutoff of high TMB (TMB-H) for all BTC in this study was 7.1 mut/Mb (**Fig. 2B**). Within the subgroup of responders, a subject whose tumor demonstrated an extremely high TMB of 156.4 mut/Mb was found to harbor a deleterious *POLE* exonuclease mutations p.Ser297Phe and a patient with pathologic CR had *CD274*

(*Continued.*) Two patients with PD had less than 20% increase of target lesions but progression in nontarget lesions and newly formed lesions. One patient showed pathologic complete response with a necrotic liver tumor before salvage operation. One patient with 100% shrinkage of target lesions had only PR status due to preserved nontarget lesions. **B,** The percentage change of target lesions over time from baseline in patients with at least one posttreatment image examination. One unevaluable patient was not included in the waterfall and spider plot because of early withdrawal. **C,** Time to response and duration of response with patient survival and ongoing status. **D,** Kaplan–Meier curves of progression-free survival and (**E**) OS with presentation of median time and 95% CI. CR, complete response; NR, not reached; PD, progressive disease; PR, partial response; SD, stable disease; SD+, SD ≥ 12 weeks.

(*PD-L1*) gene amplification of 20 copies with TMB of 70.4 mut/Mb (**Fig. 2B**). One MSI-high (MSI-H) patient harboring TMB of 9.7 mut/Mb only achieved transient SD with PFS of 2.7 months (**Fig. 2B**).

We then integrated all oncogenic/likely oncogenic genetic alterations into pathway analyses. Although 31.6% (12/38) and 21.1% (8/38) of patients with microsatellite stable (MSS) tumors had aberrant RTK–RAS–RAF–MAPK and PIK3–AKT–mTOR pathways, respectively, and 28.9% (11/38) of patients harbored at least one inactivating mutation in genes of chromatin remodeling complex (**Fig. 2C**). Despite not being statistically significant, a discrepant frequency of *TP53* (60% vs. 33%), *KRAS* (25% vs. 6%), *PIK3CA* (20% vs. 0%), and *IDH1* mutations (0% vs. 17%) was identified between responders and nonresponders (**Fig. 2C**). Interestingly, we found that chromatin remodeling gene mutations were significantly associated with higher TMB (mutant vs. wild-type: 5.2 vs. 1.2 mut/Mb, $P = 0.0004$; Supplementary Fig. S3A) in 33 patients with MSS tumors. The correlation between TMB and RTK–RAS–RAF–MAPK or PIK3–AKT–mTOR pathway was shown in Supplementary Figs. S3B and S3C.

The association of genetic alterations and PFS and OS

The associations of the TMB ($n = 33$) and oncogenic pathways ($n = 38$) with survival were further evaluated in patients with MSS tumors. The MSI-H patient only had a PFS of 2.7 months, likely due to acquired loss of the second allele of the *B2M* gene (16), and was excluded from the analysis. HR of PFS and OS for other genetic variables was shown in Supplementary Table S1. As shown in **Fig. 3A**, patients with TMB-H (≥7.1 mut/Mb) tumor had significantly longer median PFS than those without (20.9 months vs. 9.1 months, HR = 0.2040, $P = 0.0093$), but no difference in median OS (**Fig. 3A**). Given its positive association with TMB-H, the impact of chromatin remodeling gene mutations was only evident in PFS but not OS (**Fig. 3B**). Strikingly, further analysis showed that patients with truncating mutations in chromatin remodeling genes demonstrated a significantly longer PFS (20.9 months vs. 7.4 months; HR = 0.287; $P = 0.0040$; **Fig. 3C**, left) and OS (undefined vs.15.4 months; HR = 0.253; $P = 0.0475$; **Fig. 3C**, right).

Discussion

The efficacy and safety of nivolumab plus modified GS in patients with ABTC were assessed in this single-arm phase II research. The current regimen had promising efficacies with an ORR of 45.9%, a median PFS of 9.1 months, and a median OS of 19.2 months. Notably, the AE profiles were excellent. The incidence of grade 3 or higher hematologic toxicities was less than 10%, which is consistent with our prior modified GS study and far lower than current GC therapy data (6, 7, 8). Because of cumulative neuropathy and probable renal toxicity, both GC were stopped (6) or only gemcitabine was kept after eight cycles with dose reduction of gemcitabine in 50% of patients during treatment (10). In contrast, the well-tolerated regimen of nivolumab plus modified GS with only 15% of patients for gemcitabine reduction promoted long-term treatment until disease progression. The continuous application of modified GS with high-dose intensity when in combination with ICIs may lead to synergic and sustained treatment outcomes. In our study, one patient exhibited a confirmed PR after treatment for more than 10 months (**Fig. 1C**).

Gemcitabine-based regimens such as GC or GS are the standard first-line chemotherapies for patients with ABTC, with an ORR of 26% to 32%, median PFS of 5.8 to 6.8 months, and median OS of 11 to 15 months (6, 7). Nivolumab plus GC had a higher 6-month PFS rate than double ICIs with nivolumab and ipilimumab (70% vs. 19%; ref. 17), indicating chemotherapy should be the backbone as the first-line treatment. Recently, a phase III randomized trial (TOPAZ-1) of GC with or without durvalumab as first-line setting significantly improved PFS (7.2 months vs. 5.7 months; HR = 0.75; $P = 0.001$) and OS (12.8 months vs. 11.5 months; HR = 0.8; $P = 0.021$) compared with chemotherapy alone (18), showing a synergistic effect of chemotherapy with ICIs in ABTC. Gemcitabine exerts variable immune-modulatory effects in the tumor microenvironment by suppressing T-regulatory cells and reducing the numbers of myeloid suppressor cells (19, 20). Interestingly, either gemcitabine or 5-FU could induce the expression of PD-L1 in cholangiocarcinoma cells, partially explaining the potent and durable treatment outcome of ICIs plus GS regimen (21). The promising results of this study with longer DoR than that of the TOPAZ-1 study (9.6 months vs. 6.4 months) indicate ICIs plus modified GS warrants further development (18).

PD-L1 expression has been applied as predictive markers for ICI response in various cancer types (22–25), but not in BTC (10, 26). Whether PD-L1 could serve as a predictive biomarker for ICI treatment remained controversial by the results of two studies of nivolumab plus GC in ABTC (10, 26). In our study, patients with PD-L1 CPS ≥1 had numerically better ORR than those with CPS <1 (**Table 3**), but did not show a significant difference in PFS or OS (Supplementary Fig. S1). In TOPAZ-1 study, two subgroups with PD-L1 expression of tumor area positivity score (combined tumor cells and immune cells) ≥5 and tumor cells ≥1% had significant OS benefits compared with opposite subgroups (18). The definite criteria and cutpoint of PD-L1 positivity for predicting treatment outcomes from first-line chemoimmunotherapy in ABTC should be further validated.

TMB-H and MSI-H are two well-recognized complex genomic biomarkers to independently predict better response to ICIs in various cancer types, based on the theory of high neoantigen load (27). This is the first prospective cohort study to demonstrate the utility of TMB-H in predicting better outcomes of chemoimmunotherapy in patients with ABTC. The panel TMB (pTMB) applied in the study was calculated from the 440-gene cancer panel (ACTOnco), which demonstrated a good correlation with WES-TMB in the *in silico* and empirical studies of the TMB Harmonization Project (28, 29). The robustness of pTMB for predicting ICI treatment response across solid cancers has led to the tissue agnostic approval of pembrolizumab for TMB-H (≥10 mut/Mb) tumors as measured by the F1CDx assay (30). However, a universal TMB-H cutoff of 10 may not be practical for all solid tumors (31, 32), especially those with low TMB, such as BTC (30, 33, 34). A recent pan-cancer study showed that a cancer type–specific TMB cutoff determined from decile within histology could successfully stratify patients who may benefit from ICIs across solid tumors (35). In this study, although TMB-H patients exhibited significantly longer PFS, they did not achieve significant OS benefit after a median follow-up of 17.6 months. The lack of significant OS difference between TMB-H and TMB-L populations may be explained by the small sample size and other factors associated with acquired resistance to immune checkpoint blockade, such as T-cell exhaustion or altered composition of T-cell subpopulation in the tumor microenvironment (36).

The accuracy of TMB measurement could be influenced by many factors, including tumor cellularity, sequencing breadth, and bioinformatic algorithm (37). TMB may be underestimated in real-world samples due to limited biopsy tissues and poor tumor cellularity, especially in pancreatic cancer and BTC. In this cohort, up to 12.8% (5/39) of tumors had undetermined TMB due to extremely low tumor purity and their probability of responding to ICI was unpredictable.

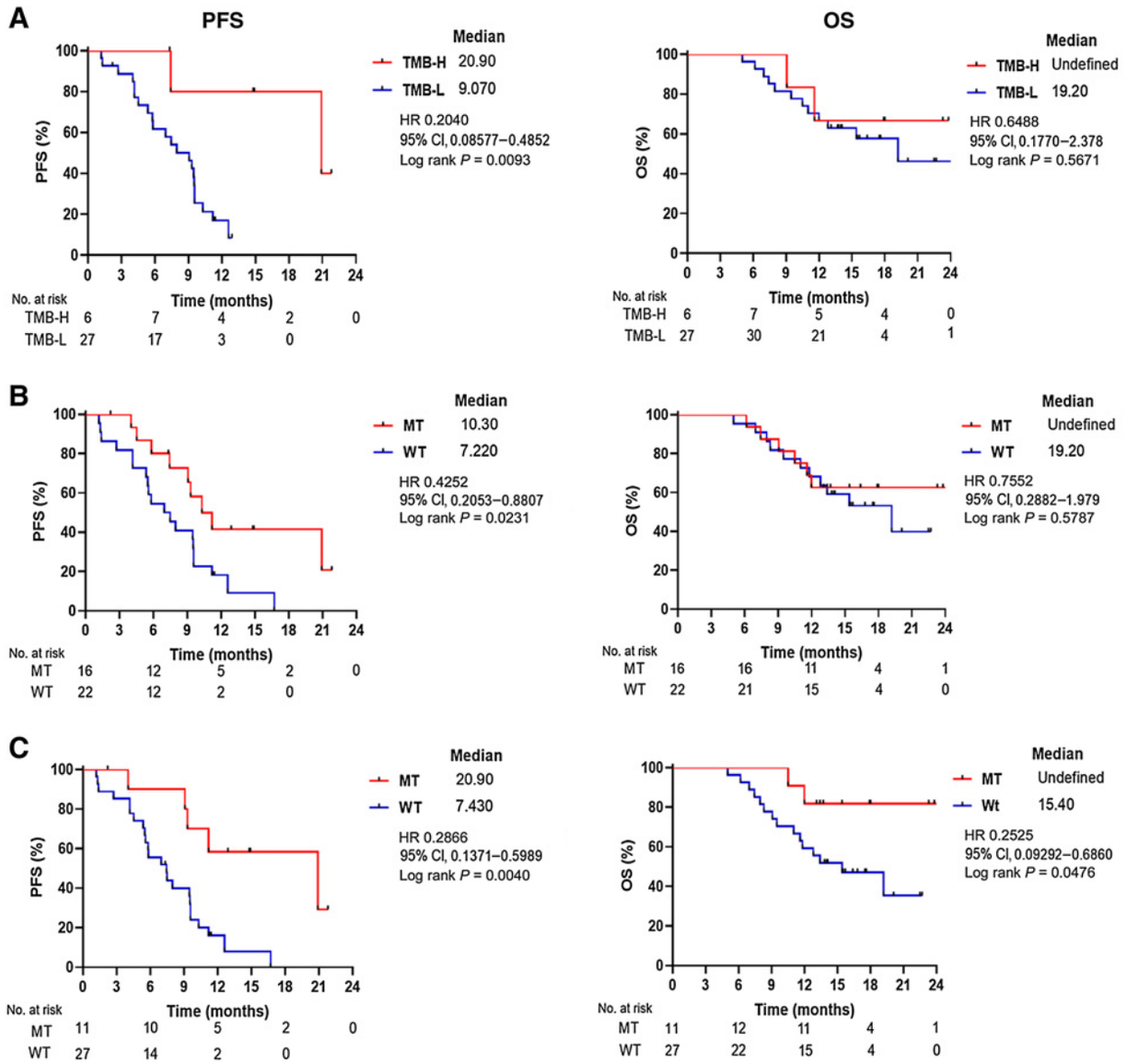


Figure 3.

Progression-free survival and OS in patients with BTC with MSS tumor by the classification of different biomarkers. The comparison of PFS and OS between subpopulations with (**A**) TMB-H and TMB-L, (**B**) all mutations of chromatin remodeling genes and wild-type, and (**C**) LoF mutations of chromatin remodeling genes and wild-type. One MSI-H patient was excluded from the analysis. LoF, loss of function; MSI-H, microsatellite instability-high; MT, mutation; TMB-H, tumor mutation burden-high; TMB-L, tumor mutation burden-low; WT, wild type. The *P* value was analyzed by log-rank test.

A high frequency of chromatin remodeling gene mutations in ABTC suggested that it could be a more robust and precise surrogate genomic biomarker to predict better survival outcomes than TMB-H did. In addition to TMB-H and MSI-H, other oncogenic genetic alterations identified in ABTC had been reported to possess predictive value for the response of chemotherapy and ICIs (38, 39). In our study, up to 28.9% (11/38) patients with ABTC harbored at least one oncogenic truncating mutation of chromatin remodeling genes and demonstrated significantly better PFS and OS than those without mutation. The association between chromatin remodeling gene truncating mutations and improved OS was also evident in patients with esophagogastric cancer receiving ICI treatment in the MSKCC cohort (Supplementary Fig. S4; ref. 35). These findings suggested that the DNA remodeling program could be implicated in regulating the prolonged effect of immune-mediated tumor inhibition. In line with our notion, previous studies reported that the epigenetic program regulates T-cell differentiation and exhaustion in the immune response (40, 41). To inhibit chromatin remodeling genes, such as *DNMT3A* (42), *EZH2* (43), and *TET2* (44) could exert a synergistic effect with PD-1 blockade. Furthermore, improved ICI treatment effectiveness was seen in patients with non–small cell lung cancer with mutations of chromatin remodelers (*ARID1A/B* or *ARID2*), as explained by increased neoantigen load and altered immune microenvironments (45). The significant treatment effectiveness of ICI-containing regimens in our study and the improved

OS for durvalumab plus chemotherapy in Asian than non-Asian patients with ABTC of TOPAZ-1 study [HR with 95% CI, 0.73 (0.57–0.94) vs. 0.89 (0.66–1.19); ref. 46] could be attributed to the genetic difference between racial and ethnic groups (47).

This study was associated with several limitations. Because of insufficient biopsy tissue for RNA extraction and fusion panel analysis, the information of *FGFR2* fusion and its impact on treatment response was unavailable. Given a small sample size, dominant genetic alterations could not be further analyzed in subtypes of BTC. Finally, a single-arm study can never confirm superior outcomes than other regimens or prognostic and predictive capability of biomarkers.

In conclusion, nivolumab plus modified GS is a well-tolerated regimen with promising outcomes and excellent safety profiles. The predictive capability of TMB-H and chromatin remodeling gene LoF mutations for chemoimmunotherapy in ABTC needs to be validated and explored in a larger cohort.

Consent to Publish

All authors had already reviewed and agreed on the concept of this manuscript. N.-J. Chiang, K.T. Tan, L.-T. Chen, and M.-H. Chen have full access to all data and final responsibility for submission and publication of this work.

Authors' Disclosures

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Authors' Contributions

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