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# Phase 2 trial of bintrafusp alfa as second-line therapy for patients with locally advanced/metastatic biliary tract cancers

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**Abbreviations**: AE, adverse event; BTC, biliary tract cancer; CR, complete response, DOR, duration of response; ECOG, Eastern Cooperative Oncology Group; EHCC, extrahepatic cholangiocarcinoma; 5-FU, 5-fluorouracil; FGFR, fibroblast growth factor receptor; GC, gallbladder cancer; IDH, isocitrate dehydrogenase; IHCC, intrahepatic cholangiocarcinoma; NE, not evaluable; ORR, objective response rate; OS, overall survival; PD, progressive disease; PD-L1, programmed death ligand 1; PFS, progression-free survival; PR, partial response; RECIST, Response Evaluation Criteria in Solid Tumors; SD, stable disease.

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## Abstract

**Background and Aims:** Biliary tract cancers are rare, heterogeneous cancers with poor prognoses. Bintrafusp alfa, a first-in-class bifunctional fusion protein composed of the extracellular domain of TGF-βRII (a TGF-β "trap") fused to a human IgG1 monoclonal antibody blocking programmed death ligand 1, was evaluated in patients with locally advanced/metastatic chemorefractory biliary tract cancers.

**Approach and Results:** This multicenter, single-arm, open-label, phase 2 study (NCT03833661) enrolled adults with locally advanced or metastatic biliary tract cancer that was intolerant to or had failed first-line systemic platinum-based chemotherapy. Patients received 1200 mg bintrafusp alfa intravenously Q2W. The primary endpoint was confirmed objective response according to Response Evaluation Criteria in Solid Tumors 1.1 assessed by IRC. Secondary endpoints included duration of response, durable response rate, safety, progression-free survival, and overall survival. Between March 2019 and January 2020, 159 patients were enrolled. Median follow-up was 16.1 (range, 0.0–19.3) months; 17 patients (10.7%; 95% CI: 6.4%–16.6%) achieved an objective response. Median duration of response was 10.0 (range, 1.9–15.7) months; 10 patients (6.3%; 95% CI: 3.1%–11.3%) had a durable response ($\geq$6 mo). Median progression-free survival was 1.8 months (95% CI: 1.7–1.8 mo); median overall survival was 7.6 months (95% CI: 5.8–9.7 mo). Overall survival rates were 57.9% (6 mo) and 38.8% (12 mo). Grade $\geq$3 adverse events occurred in 26.4% of patients, including one treatment-related death (hepatic failure). Frequent grade $\geq$3 adverse events included anemia (3.8%), pruritus (1.9%), and increased alanine aminotransferase (1.9%).

**Conclusions:** Although this study did not meet its prespecified primary endpoint, bintrafusp alfa demonstrated clinical activity as second-line treatment in this hard-to-treat cancer, with durable responses and a manageable safety profile.

## INTRODUCTION

Biliary tract cancer (BTC) comprises several rare, lethal, and heterogeneous cancers of the biliary tree, including intrahepatic cholangiocarcinoma (IHCC), perihilar and distal cholangiocarcinoma, gallbladder cancer (GC), and ampullary cancer.[1–4] Most patients have advanced disease at diagnosis, and the majority become refractory or experience relapse with available treatments (eg, gemcitabine plus cisplatin or oxaliplatin).[2,5] In 2019, there were ~199,000 new cases and 172,000 estimated deaths due to BTCs globally.[6] Gemcitabine plus cisplatin is the current first-line standard of care for locally advanced/metastatic BTC.[4] In a meta-analysis of 25 studies of second-line chemotherapy, the objective response rate (ORR) was 7.7%, whereas progression-free survival (PFS) and overall survival (OS) were 3.2 months and 7.2 months, respectively.[7] Although other therapeutic agents are currently being investigated, at the initiation of this study, there was no globally accepted standard of care for second-line treatment of patients with locally advanced/metastatic BTC.[8–15] Since that time, 2 key second-line studies using 5-fluorouracil (5-FU)/leucovorin/oxaliplatin versus active, supportive care (OS, 6.2 vs 5.3 mo and response rate of 5% vs 0%)[16] and 5-FU/leucovorin + liposomal irinotecan versus 5-FU/leucovorin (OS, 8.6 vs 5.5 mo and response rate of 14.8% vs 5.8%)[17] have provided context for the interpretation of data from novel agents studied in advanced BTCs in the second-line setting.

Although there have been recent FDA approvals for fibroblast growth factor receptors (FGFR) and isocitrate dehydrogenase 1 (IDH 1) inhibitors in advanced cholangiocarcinomas, fusions in *FGFR* or mutations in *IDH1* occur in <20% of patients with BTCs.[18–21] In addition, FDA approvals for pembrolizumab, a programmed death ligand 1 (PD-L1) inhibitor, in tumor mutational burden-high and microsatellite instability-high/deficient mismatch repair solid tumor indications may be an option for the few patients that have these biomarkers; however, anti–PD-L1 therapies have demonstrated limited efficacy in BTC.[22–24] The recent TOPAZ-1 study met its primary endpoint of OS and secondary endpoints of PFS and ORR without exacerbating toxicity, leading to the recent approval of durvalumab as a preferred first-line treatment in combination with gemcitabine + cisplatin for locally advanced or metastatic disease.[25,26] However, the median OS was only 12.8 months with durvalumab and chemotherapy compared with 11.5 months with gemcitabine/cisplatin alone.[26] Together, this demonstrates a significant unmet need for patients with locally advanced/metastatic BTC, as reflected by the robust pipeline of therapies under investigation.

Many preclinical studies have demonstrated a link between aberrant TGF-β signaling and the pathogenesis of a variety of cancers, including BTC.[27–29] TGF-β1 expression in BTC has been correlated with lymph node metastasis, distant metastasis, and tumor recurrence.[29,30] TGF-β receptor inhibitors, such as galunisertib, have demonstrated anticancer effects in cell line models of BTC.[31] Furthermore, TGF-β in the tumor microenvironment is also associated with resistance to therapies inhibiting the PD-L1 pathway, which is used by tumor cells to evade immune detection and elimination, and is implicated in BTC.

Given the role of TGF-β and PD-L1 in BTC, colocalized, simultaneous inhibition of these 2 nonredundant and complementary immunostimulatory pathways may provide a novel treatment approach. Bintrafusp alfa is a first-in-class bifunctional fusion protein composed of the extracellular domain of the human TGF-β receptor II (TGF-βRII or TGF-β "trap" sequestering all TGF-β isoforms) fused through a flexible linker to the *C*-terminus of each heavy chain of an IgG1 antibody blocking PD-L1 (anti–PD-L1). In an expansion cohort of an open-label, phase 1 trial, bintrafusp alfa showed signs of clinical activity and a manageable safety profile in 30 Asian patients with BTC who experienced disease progression after first-line chemotherapy, with an ORR per independent review of 20% and 5 of 6 responses ongoing at data cutoff.[13] In a follow-up analysis of this cohort with a median follow-up of 121.6 weeks, 3 responders had an ongoing response (18 +, 24 +, and 24 + months). We report results from a global, open-label, phase 2 study (NCT03833661) of bintrafusp alfa in patients with locally advanced or metastatic BTC for whom first-line standard chemotherapy had failed. At the time of writing, this report represents the largest study to date of immunotherapy in the second-line treatment setting for BTC.

# METHODS

## Study design and participants

This study is a multicenter, single-arm, open-label, phase 2 trial. Patient enrollment was conducted internationally at 36 sites in 10 countries. Eligible patients had histologically or cytologically confirmed, locally advanced or metastatic BTC, including IHCC, extrahepatic cholangiocarcinoma (EHCC), and GC subtypes, had a disease that was measurable according to the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1, and were intolerant to or had failed first-line systemic platinum-based chemotherapy. Patients could have received only 1 prior line of systemic treatment for locally advanced or metastatic BTC. Additional key inclusion criteria were age 18 years or older; Eastern Cooperative Oncology Group performance status 0–1; a fresh or archival tumor specimen; and adequate renal, cardiac, and hepatic function. Key exclusion criteria included ampullary cancer; prior treatment with immunotherapy or checkpoint inhibitors; any antibody or inhibitor targeting the TGF-β pathway; a history of noninfectious interstitial lung disease requiring systemic steroid treatment; or current pneumonitis.

The study was conducted in accordance with the ethical principles of the International Council for Harmonization guideline for Good Clinical Practice, the Japanese ministerial ordinance on Good Clinical Practice (study centers in Japan only), and the Declaration of Helsinki, as well as applicable local regulations. The complete study protocol is available in the Supplemental Digital Content.

## Procedures

Patients received 1200 mg of bintrafusp alfa intravenously every 2 weeks until confirmed disease progression, death, unacceptable toxicity, or study withdrawal. This study included a 28-day screening period, treatment period, 12-week safety, and long-term survival follow-up. Tumor response was assessed using RECIST 1.1 based on central interpretation of contrast-enhanced CT scan and/or MRI of the chest/abdomen/pelvis, every 8 weeks for the first 12 months of treatment and then every 12 weeks until confirmed disease progression. Immune-related best overall response and immune-related PFS were evaluated according to immune-related RECIST guidelines.[32]

Safety was evaluated in all patients who received at least one dose of bintrafusp alfa; the severity of treatment-related adverse events (AEs) was graded according to National Cancer Institute-Common Terminology Criteria for Adverse Events 5.0. Any AE that was believed to be a potential immune-related or potential TGF-β–related event was considered an AE of special interest. Immune-related AEs were identified using a preselected list of terms from the Medical Dictionary for Regulatory Activities version 23.1. Whole-blood samples preinfusion were obtained at day 1 for pharmacokinetic analyses, at screening for immunogenicity analyses, and at days 15, 29, 43, and 85, and every 6 weeks until week 25 and every 12 weeks thereafter for both pharmacokinetic and immunogenicity analysis. Postinfusion samples were also obtained at days 1 and 29 for pharmacokinetic analyses. Samples were, in addition, obtained at the end of treatment and up to 28 days after the last treatment. Immunogenicity of bintrafusp alfa was measured using an antidrug antibody assay, from screening through 12 weeks (±2 wk) after the last treatment. Samples with a reportable antidrug antibody titer were tested for neutralizing antibodies specific to TGF-β or PD-L1. Neutralizing antibody results were positive or negative in a single assay and were only derived when testing was not performed.

Tumor and liquid biopsy samples were tested using assays for genetic alterations (eg, *IDH* and *FGFR*) and tumor mutational burden in circulating tumor DNA (Guardant Health Inc.), microsatellite instability (Promega MSI assay), and PD-L1 expression (SP263 assay scored for positive tumor cells).

## Outcomes

The primary endpoint was confirmed objective response according to RECIST version 1.1 assessed by an independent review committee. Secondary endpoints included duration of response (DOR), durable response (defined as the proportion of patients who had a response lasting at least 6 mo), safety (occurrence of treatment-emergent AEs, treatment-related AEs, and AEs of special interest), PFS assessed by independent review, clinical efficacy (objective response, DOR, durable response, and PFS) assessed by investigator, OS, pharmacokinetics of bintrafusp alfa, and immunogenicity of bintrafusp alfa. Objective response, DOR, and durable response according to PD-L1 expression or microsatellite instability status were other secondary endpoints. Exploratory analyses included clinical efficacy by tumor mutational burden, tumor-based and blood-based genetic profiling, and efficacy based on immune-related best overall response and immune-related PFS assessed per independent review.

## Statistical analysis

With a planned recruitment of 141 patients, including a minimum of 30 patients from each BTC subtype (IHCC, EHCC, and GC), the study was designed to have 80% power to reject the null hypothesis of an ORR of ≤10%. The null hypothesis would be rejected if the lower limit of 95% CI exceeded 10%. Two data cutoffs were planned: a primary analysis 9 months after the accrual of the last patient and a secondary analysis 15 months after the accrual of the last patient. For objective response, exact 2-sided 95% CIs were calculated using the Clopper-Pearson method. DOR, PFS, and OS were analyzed using the Kaplan-Meier method. No statistical testing was performed between patient subgroups due to small population sizes.

## Role of the funding source

This was an industry-sponsored trial by the healthcare business of Merck KGaA, Darmstadt, Germany, which provided the study drug and worked with investigators on the trial design and plan, collection and analysis of data, and interpretation of results. Funding for a professional medical writer with access to the data was provided by the healthcare business of Merck KGaA, Darmstadt, Germany (CrossRef Funder ID: 10.13039/100009945) and was previously part of an alliance between the healthcare business of Merck KGaA, Darmstadt, Germany, and GlaxoSmithKline.

# RESULTS

Between March 25, 2019 and January 7, 2020, a total of 247 patients were screened for enrollment at 36 centers in 10 countries. Overall, 159 patients were enrolled and received bintrafusp alfa for a median of 8.0 weeks (range, 2.0–80.1 wk) (Supplemental Figure S1, http://links.lww.com/HEP/F5). Baseline patient and disease characteristics are shown in Table 1. A majority of patients had PD-L1–negative tumors (61.6%), defined as tumor cell PD-L1 expression <1% by immunohistochemistry.[13] Patients had a median CA 19-9 of 184.2 U/mL (range, 0.0–177400 U/mL). At the data cutoff of March 30, 2021, the median follow-up time was 16.1 months (range, 0.0–19.3 mo); 3 patients remained on treatment (1.9%). The most common reasons for the discontinuation of treatment were progressive disease (66.7%), AEs (18.2%), and death (8.8%). After the treatment with bintrafusp alfa, 67 patients (42.1%) received subsequent anticancer drug treatment.

The ORR by independent review was 10.7% (n = 17, 95% CI: 6.4%–16.6%), with 3 patients (1.9%)

**TABLE 1** Baseline patient and disease characteristics

| | Full analysis set (N = 159) |
|---|---|
| Median age, y (range) | 65 (39–83) |
| Sex, n (%) | |
| Male | 94 (59.1) |
| Female | 65 (40.9) |
| Geographic region, n (%) | |
| North America | 23 (14.5) |
| Europe | 61 (38.4) |
| Asia | 75 (47.2) |
| Race, n (%) | |
| White | 64 (40.3) |
| Asian | 77 (48.4) |
| Other | 1 (0.6) |
| Not collected | 17 (10.7) |
| ECOG performance status, n (%) | |
| 0 | 92 (57.9) |
| 1 | 67 (42.1) |
| Disease stage, n (%) | |
| Locally advanced | 9 (5.7) |
| Metastatic | 150 (94.3) |
| BTC subtype, n (%) | |
| IHCC | 95 (59.7) |
| EHCC | 32 (20.1) |
| GC | 32 (20.1) |
| No. prior anticancer regimens, n (%) | |
| 1 | 132 (83.0) |
| 2 | 22 (13.8) |
| 3 | 5 (3.1) |
| No. prior treatments for metastatic/locally advanced disease, n (%) | |
| 0 | 1 (0.6) |
| 1 | 157 (98.7) |
| 2 | 1 (0.6) |
| At least one previous anticancer radiotherapy, n (%) | 14 (8.8) |
| At least one previous anticancer surgery, n (%) | 63 (39.6) |
| History of hepatobiliary disorders, n (%)[a] | 30 (18.9) |
| PD-L1 expression, n (%) | |
| Positive (≥1% tumor cells) | 43 (27.0) |
| High (≥50% tumor cells) | 11 (6.9) |
| Negative (<1% tumor cells) | 98 (61.6) |
| *IDH1* mutation status, n/N (%) | |
| Positive | 14/145 (9.7) |
| Negative | 131/145 (90.3) |
| Missing | 8/153 (5.2) |
| *IDH2* mutation status, n/N (%) | |
| Positive | 11/145 (7.6) |
| Negative | 134/145 (92.4) |
| Missing | 8/153 (5.2) |
| *FGFR2* mutation status, n/N (%) | |
| Positive | 15/145 (10.3) |
| Negative | 130/145 (89.7) |
| Missing | 8/153 (5.2) |
| Microsatellite instability-high status, n/N (%) | |
| High | 3/153 (2.0) |
| Low or microsatellite stable | 150/153 (98.0) |
| Baseline CA 19-9 (U/mL), median (range) | 184.2 (0.0–177400.0) |

[a]Including cirrhosis (n = 6), cholelithiasis (n = 5), cholestatic jaundice (n = 5), cholangitis (n = 4), and hepatic steatosis (n = 4).
Abbreviations: BTC, biliary tract cancer; ECOG, Eastern Cooperative Oncology Group; EHCC, extrahepatic cholangiocarcinoma; GC, gallbladder cancer; IHCC, intrahepatic cholangiocarcinoma; PD-L1, programmed death ligand 1.

experiencing a complete response (CR) (Figure 1, Supplemental Figure S2, http://links.lww.com/HEP/F5, Table 2). The investigator-assessed ORR was 10.7% (n = 17, 95% CI: 6.4%–16.6%). Responses were durable: median DOR was 10.0 months (range, 1.9–15.7 mo), and 10 of 17 responders had a response of at least 6 months (a durable response rate based on an overall population of 6.3%; 95% CI: 3.1%–11.3%) (Table 2; Supplemental Figure S3, http://links.lww.com/HEP/F5). Of the 17 responders, 6 had an ongoing response at the data cutoff. Median PFS was 1.8 months (95% CI: 1.7–1.8 mo) per independent review (Table 2, Figure 2A) and was comparable across BTC subtypes (Figure 2B). The PFS rate was 11.7% at 6 months and 6.5% at 12 months (Figure 2A). Median OS was 7.6 months (95% CI: 5.8–9.7 mo), with survival rates of 57.9%, 38.8%, and 26.9% at 6 months, 12 months, and 18 months, respectively (Figure 2C, Table 2). OS was also similar across BTC subtypes (Figure 2D).

Among the 159 patients, 62.3% experienced a treatment-related AE; the most common events were pruritus (12.6%), rash (9.4%), and fatigue (8.8%) (Table 3). Treatment-related AEs of grade 3 or higher occurred in 26.4% of patients; the most common grade ≥3 treatment-related AEs included anemia (3.8%), pruritus (1.9%), increased alanine aminotransferase (1.9%), and increased aspartate aminotransferase (1.9%). Grade 4 treatment-related AEs occurred in 5 patients: myocarditis, hepatitis, bacterial sepsis, aspartate aminotransferase increase, and hepatic failure (one each). One treatment-related death occurred due to hepatic failure; while the patient had a history of hepatitis *B* virus-related cirrhosis and liver lesions, hepatitis *B* virus was not detected since 2017, and the patient was on hepatitis *B* suppressive therapy (tenofovir). Therefore, it is unlikely that the hepatic failure was due to hepatitis *B* reactivation. After treatment with the first dose of bintrafusp alfa, the patient

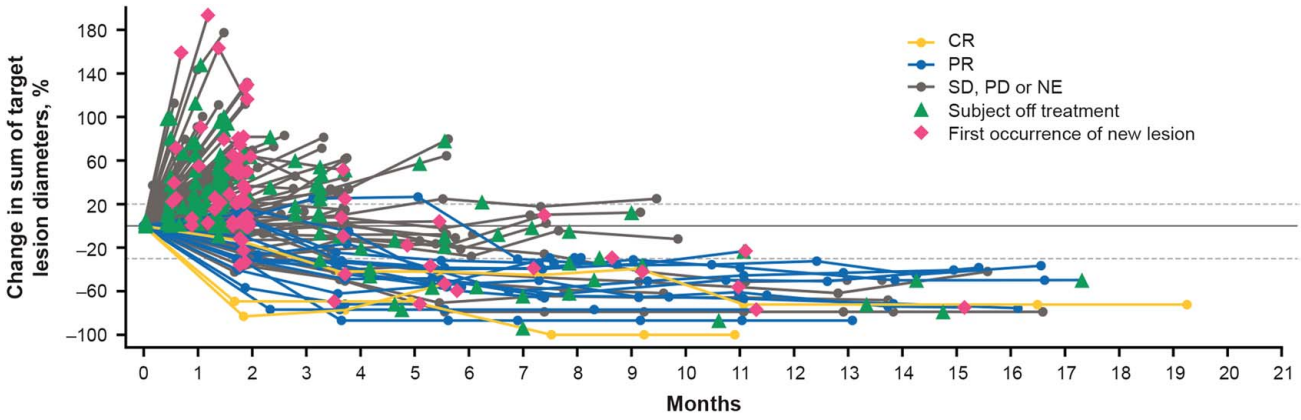


**FIGURE 1** Best percentage change in the sum of diameters according to Response Evaluation Criteria in Solid Tumors 1.1 per independent review. Abbreviations: CR, complete response, NE, not evaluable; PD, progressive disease; PR, partial response; SD, stable disease.

experienced signs of hepatic failure (asthenia and jaundice), which resulted in hospitalization 17 days after the first treatment administration and diagnosis of grade 4 hepatic failure. This resulted in treatment discontinuation, and during hospitalization, complications of cirrhosis (eg, grade 4 serious acute kidney injury and grade 3 hyperkalemia) led to fatal multiorgan failure 6 days later. Fifteen patients (9.4%) discontinued treatment due to treatment-related AEs, including 2 patients with diarrhea and 1 patient each due to hepatic failure, bacterial sepsis, alanine aminotransferase increase, aspartate aminotransferase increase, myositis, tumor hyperprogression, immune-mediated encephalitis, acute kidney injury, nephritis, renal injury, tubulointerstitial nephritis, pneumonitis, pemphigoid, rash, and toxic skin eruption.

**TABLE 2** Treatment response to bintrafusp alfa per independent review

| Variable | N = 159 |
|---|---|
| Confirmed ORR, % (95% CI) | 10.7 (6.4–16.6) |
| Confirmed best overall response, n (%) | |
| Complete response | 3 (1.9) |
| Partial response | 14 (8.8) |
| Stable disease | 19 (11.9) |
| Progressive disease | 105 (66.0) |
| Not evaluable | 18 (11.3) |
| Median DOR, months (range) | 10.0 (1.9–15.7) |
| Durable response rate,[a] % (95% CI) | 6.3 (3.1–11.3) |
| Median PFS, months (95% CI) | 1.8 (1.7–1.8) |
| Median OS, months (95% CI) | 7.6 (5.8–9.7) |
| ORR by subtype, % (n/N) | |
| IHCC | 12.6 (12/95) |
| EHCC | 9.4 (3/32) |
| GC | 6.3 (2/32) |
| ORR by tumor cell PD-L1 expression, % (n/N) | |
| Positive | 7.0 (3/43) |
| Negative | 11.2 (11/98) |

[a]Durable response of at least 6 months according to Response Evaluation Criteria in Solid Tumors 1.1, assessed per independent review.
Abbreviations: DOR, duration of response; EHCC, extrahepatic cholangiocarcinoma; GC, gallbladder cancer; IHCC, intrahepatic cholangiocarcinoma; OS, overall survival; ORR, objective response rate; PD-L1, programmed death ligand 1; PFS, progression-free survival.

Immune-related AEs were reported in 28.9% of patients (Supplemental Table S1, http://links.lww.com/HEP/F5); 15 patients (9.4%) permanently discontinued treatment due to immune-related AEs. Grade ≥3 immune-related AEs were reported in 20 patients (12.6%), including one grade 4 myocarditis and one grade 4 hepatitis. Of the 46 patients with immune-related AEs, 31 patients (67.4%) required steroids. Skin AEs, potentially mediated by TGF-β inhibition (eg, keratoacanthomas, squamous cell carcinoma of the skin), were reported in 8.2% of patients (Supplemental Table S1, http://links.lww.com/HEP/F5). Only one patient (0.6%) reported a TGF-β inhibition-mediated skin AE (eg, keratoacanthomas, squamous cell carcinoma of the skin, hyperkeratosis) of grade 3 or higher. Keratoacanthoma was the most common TGF-β inhibition-mediated skin AE and was reported in 9 patients (5.7%). TGF-β inhibition-mediated skin AEs were managed through observation and surgical excision (n = 4) and did not lead to permanent treatment discontinuation (Supplemental Table S1, http://links.lww.com/HEP/F5). Treatment-related bleeding events were reported in 5.7% of patients. Infusion-related reactions that were assessed by the investigator as being related to bintrafusp alfa occurred in 6.3% of patients.

No clinically meaningful differences were observed among subtypes for efficacy endpoints (Supplemental Table S2 in the http://links.lww.com/HEP/F5). The ORR by BTC subtype was 12.6%, 9.4%, and 6.3% for IHCC, EHCC, and GC, respectively. Survival was comparable across BTC subtypes. The response rate per immune-related RECIST by independent review was 12.6% (95% CI: 7.9%–18.8%) (Supplemental Table S3, http://links.lww.com/HEP/F5), with a PFS per immune-related

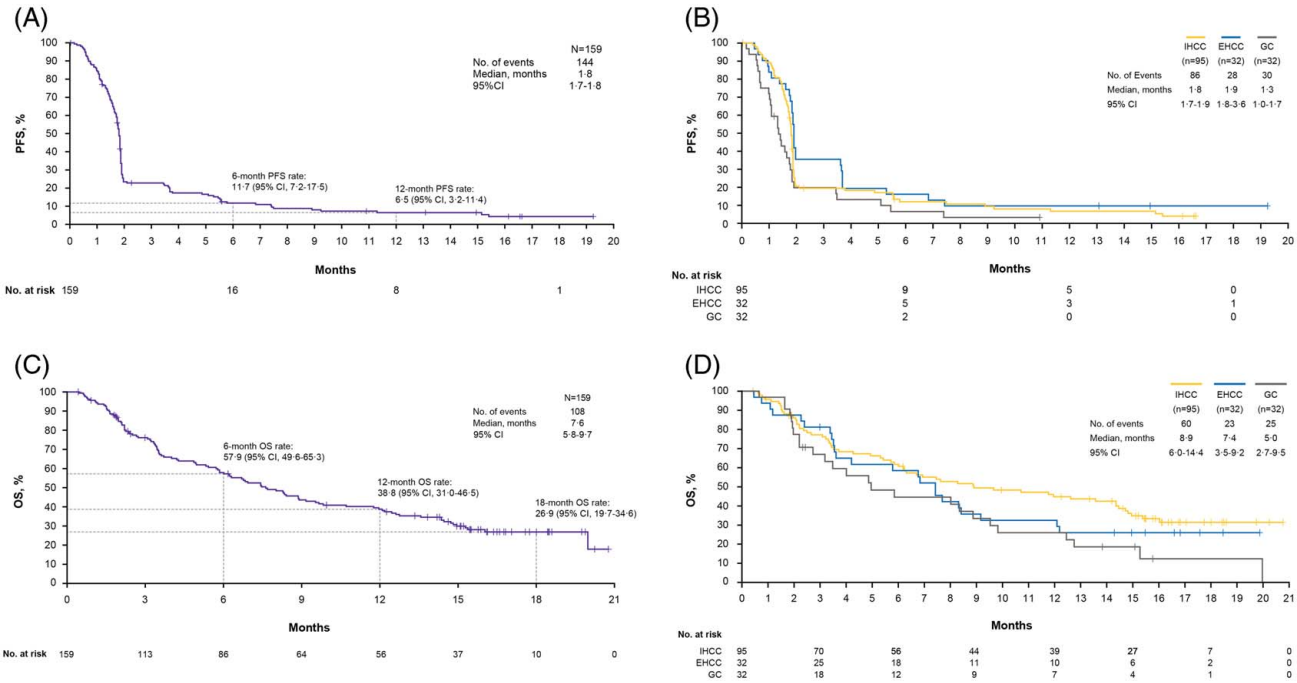


**FIGURE 2** Kaplan-Meier analysis of (A) PFS as adjudicated by the independent review, (B) PFS by BTC subtype, (C) OS for all patients, and (D) OS by BTC subtype. Abbreviations: BTC, biliary tract cancer; EHCC, extrahepatic cholangiocarcinoma; GC, gallbladder cancer; IHCC, intrahepatic cholangiocarcinoma; OS, overall survival; PFS, progression-free survival.

RECIST of 1.8 months (Supplemental Figure S4 and Table S3 in the http://links.lww.com/HEP/F5).

Responses were observed regardless of PD-L1 expression, with a response rate of 11.2% and 7.0% in patients with tumor cell PD-L1 expression <1% and ≥1%, respectively (Table 2). These responses were durable (durable response rate of 8.2% and 2.3% of patients with tumor cell PD-L1 expression <1% and ≥1%, respectively), as shown in Supplemental Table S2 (http://links.lww.com/HEP/F5). No responses were seen in patients with microsatellite instability-high status, but the sample size was small (n = 3). The ORR was 15.5% (95% CI: 7.3–27.4) and 3.7% (95% CI: 0.5–12.7) for low (≤10.53 mutations/Mb; n = 58) and high (>10.53 mutations/Mb; n = 54) blood-based tumor mutational burden, respectively. In patients with (n = 14) and without (n = 131) *IDH1* mutations at baseline, the ORR was 21.4% (95% CI: 4.7–50.8) and 8.4% (95% CI: 4.3–14.5), respectively. No responses were seen in patients with *IDH2* mutations (n = 11). The ORR was 6.7% (95% CI: 0.2–31.9) and 10.0% (95% CI: 5.4–16.5) for patients with (n = 15) and without (n = 130) *FGFR2* mutations, respectively. The ORR among Asian patients (n = 77) was 9.1% (95% CI: 3.7–17.8) and among White patients (n = 64) was 12.5% (95% CI: 5.6–23.2). Of the 17 patients whose race data were not collected, 2 had a response. OS was comparable across patient subgroups defined based on PD-L1 status, tumor mutational burden, and *IDH1* and *FGFR2* mutation status (Supplemental Figure S5, http://links.lww.com/HEP/F5). Among the 10 patients with responses ≥6 months, there were no clear predictors of response or survival based on baseline characteristics or biomarkers (Supplemental Table S4, http://links.lww.com/HEP/F5).

Overall, 45 patients (28.7%) were antidrug antibody positive at least once (antidrug antibody ever positive): 8 patients had pre-existing antidrug antibodies and 37 had treatment-emergent antidrug antibody responses, with the majority persistent positive. The incidence of treatment-emergent neutralizing antibodies in either assay was 28/147 (19.0%) [ie, 16 and 12 participants who had treatment-emergent neutralizing antibodies in 1 assay (PD-L1 or TGF-β) and both assays (PD-L1 and TGF-β), respectively]. There were no notable differences in efficacy in patients who were either antidrug antibody positive or neutralizing antibody positive compared with those who were not. The pharmacokinetic profile for bintrafusp alfa was similar in antidrug antibody status, neutralizing antibody status, race, and ethnicity. Beyond day 43, a geometric mean $C_{trough}$ of >100 μg/mL was achieved after dosing 1200 mg every 2 weeks, and minimal accumulation occurred (<2-fold).

## DISCUSSION

Although the ORR seen here (10.7%) was above the prespecified limit of 10%; the primary endpoint was not met as the lower bound of the 95% CI was below 10%. Notably, the responses were durable in nature, with a

**TABLE 3** Treatment-related AEs occurring at any grade in ≥10% of patients or at grade ≥3 severity, and any AEs of special interest

| | N = 159, n (%) | | | |
|---|---|---|---|---|
| **Preferred term** | **Any grade** | **Grade 3** | **Grade 4** | **Grade 5[a]** |
| Any treatment-related AEs | 99 (62.3) | 42 (26.4) | 5 (3.1) | 1 (0.6) |
| Treatment-related AEs | | | | |
| Pruritus | 20 (12.6) | 3 (1.9) | 0 | 0 |
| Rash | 15 (9.4) | 2 (1.3) | 0 | 0 |
| Fatigue | 14 (8.8) | 1 (0.6) | 0 | 0 |
| Alanine aminotransferase increased | 11 (6.9) | 3 (1.9) | 0 | 0 |
| Aspartate aminotransferase increased | 10 (6.3) | 2 (1.3) | 1 (0.6) | 0 |
| Nausea | 10 (6.3) | 0 | 0 | 0 |
| Anemia | 9 (5.7) | 6 (3.8) | 0 | 0 |
| Diarrhea | 6 (3.8) | 3 (1.9) | 0 | 0 |
| Hyperthyroidism | 6 (3.8) | 1 (0.6) | 0 | 0 |
| Pyrexia | 6 (3.8) | 1 (0.6) | 0 | 0 |
| Rash maculo-papular | 6 (3.8) | 1 (0.6) | 0 | 0 |
| Dermatitis acneiform | 5 (3.1) | 2 (1.3) | 0 | 0 |
| Lipase increased | 4 (2.5) | 3 (1.9) | 0 | 0 |
| Blood alkaline phosphatase increased | 4 (2.5) | 2 (1.3) | 0 | 0 |
| Erythema | 4 (2.5) | 1 (0.6) | 0 | 0 |
| Malaise | 4 (2.5) | 1 (0.6) | 0 | 0 |
| Hyperkeratosis | 3 (1.9) | 1 (0.6) | 0 | 0 |
| Pneumonitis | 3 (1.9) | 1 (0.6) | 0 | 0 |
| Colitis | 3 (1.9) | 2 (1.3) | 0 | 0 |
| Acute kidney injury | 2 (1.3) | 2 (1.3) | 0 | 0 |
| Hepatitis | 2 (1.3) | 0 | 1 (0.6) | 0 |
| Pneumonia | 2 (1.3) | 1 (0.6) | 0 | 0 |
| Dyspnea | 2 (1.3) | 1 (0.6) | 0 | 0 |
| Hepatic failure | 1 (0.6) | 0 | 0 | 1 (0.6) |
| Bacterial sepsis | 1 (0.6) | 0 | 1 (0.6) | 0 |
| Myocarditis | 1 (0.6) | 0 | 1 (0.6) | 0 |
| Ascites | 1 (0.6) | 1 (0.6) | 0 | 0 |
| Hemolytic anemia | 1 (0.6) | 1 (0.6) | 0 | 0 |
| Hemorrhoidal hemorrhage | 1 (0.6) | 1 (0.6) | 0 | 0 |
| Hypercalcemia | 1 (0.6) | 1 (0.6) | 0 | 0 |
| Hypertension | 1 (0.6) | 1 (0.6) | 0 | 0 |
| Immune-mediated encephalitis | 1 (0.6) | 1 (0.6) | 0 | 0 |
| Latent autoimmune diabetes in adults | 1 (0.6) | 1 (0.6) | 0 | 0 |
| Myositis | 1 (0.6) | 1 (0.6) | 0 | 0 |
| Nephritis | 1 (0.6) | 1 (0.6) | 0 | 0 |
| Neutropenia | 1 (0.6) | 1 (0.6) | 0 | 0 |
| Pemphigoid | 1 (0.6) | 1 (0.6) | 0 | 0 |
| Renal injury | 1 (0.6) | 1 (0.6) | 0 | 0 |
| Toxic skin eruption | 1 (0.6) | 1 (0.6) | 0 | 0 |
| Tubulointerstitial nephritis | 1 (0.6) | 1 (0.6) | 0 | 0 |
| Tumor hyperprogression | 1 (0.6) | 1 (0.6) | 0 | 0 |
| Any AE of special interest | | | | |
| TGF-β inhibition-mediated skin AEs[b] | 13 (8.2) | 1 (0.6) | 0 | 0 |
| Any immune-related AEs | 46 (28.9) | 10 (12.6) | 2 (1.3) | 0 |
| Immune-related rash | 27 (17.0) | 9 (5.7) | 0 | 0 |
| Immune-related endocrinopathies | 13 (8.2) | 1 (0.6) | 0 | 0 |

**TABLE 3.** (*continued*)

| Preferred term | N = 159, n (%) | | | |
|---|---|---|---|---|
| | Any grade | Grade 3 | Grade 4 | Grade 5[a] |
| Immune-related colitis | 2 (1.3) | 3 (1.9) | 0 | 0 |
| Immune-related hepatitis | 3 (1.9) | 2 (1.3) | 1 (0.6) | 0 |
| Immune-related nephritis and renal dysfunction | 3 (1.9) | 3 (1.9) | 0 | 0 |
| Immune-related pneumonitis | 3 (1.9) | 1 (0.6) | 0 | 0 |
| Other immune-related AEs | 3 (1.9) | 3 (1.9) | 1 (0.6) | 0 |

[a]There were 23 treatment-emergent adverse events leading to death; 14/23 were due to disease progression.
[b]Includes actinic keratosis, basal cell carcinoma, Bowen disease, hyperkeratosis, keratoacanthoma, lip squamous cell carcinoma, and squamous cell carcinoma of the skin MedDRA v23.1 preferred terms.
Abbreviation: AE, adverse event.

median DOR of 10.0 months and clinically meaningful landmark survival (57.9% at 6 mo, 38.8% at 12 mo, and 26.9% at 18 mo). Given that the PFS was only 1.8 months, this would indicate that only a subset of patients derived benefits, but those who did have durable benefits. As such, future efforts should involve studies that aim to identify biomarkers that are predictive or able to enrich patients most likely to yield responses.

Despite not meeting its primary endpoint, the clinical activity demonstrated by bintrafusp alfa in this phase 2 study has some clinical relevance in the context of results from other trials in this setting. A meta-analysis of 25 studies of second-line chemotherapy showed an ORR of 7.7% (95% CI: 4.6%–10.9%).[7] Two recent trials have helped establish two combinations as the benchmark for second-line chemotherapy.[16,17] The NIFTY trial showed the addition of liposomal irinotecan in combination with 5-FU and leucovorin improved PFS and OS versus 5-FU and leucovorin alone (median PFS of 7.1 vs 1.4 mo; median OS of 8.6 vs 5.5 mo).[17] The ABC-06 trial demonstrated clinical activity with 5-FU/leucovorin/oxaliplatin (ORR of 5%) and improved survival (6.2 vs 5.3 mo with active symptom control).[16] Although these outcomes are positive, the high rate of grade $\geq$3 AEs in both trials demonstrates a need for more tolerable treatments for patients with locally advanced or metastatic BTC.[16,17] Moreover, both 5-FU/leucovorin/oxaliplatin and 5-FU/nanoliposomal irinotecan comprise multiagent cytotoxic chemotherapy regimens. These regimens may not be feasible in all patients who have clinically significant myelosuppressive toxicities during/persistent from their first-line cytotoxic exposure. As such, immunotherapy agents, such as bintrafusp alfa, might provide some potential benefit in this setting, especially when responses are achieved that are durable in nature.

The largest study of anti–PD-1 therapies in refractory BTC, the KEYNOTE-158 trial of pembrolizumab, reported an ORR of 5.8% (95% CI: 2.1%–12.1%).[12] A phase 2 study has, in addition, shown that treatment with a combination of ipilimumab and nivolumab had an ORR of 23% exclusively in patients as a second-line therapy.[14] Second-line combination therapy with pembrolizumab and granulocyte-macrophage colony-stimulating factor had an ORR of 12% (95% CI: 4%–26%) in patients with advanced BTC.[15] Moreover, the TOPAZ-1 trial demonstrated that the PD-L1 inhibitor durvalumab, alongside first-line gemcitabine and cisplatin, had a higher median OS (12.8 mo) when compared with gemcitabine and cisplatin alone (11.5 mo), changing the first-line treatment landscape.[26] Although this supports the potential for patients with immunotherapy, the fact that this benefit is in the first-line setting underscores the need for second-line treatment options, which typically have worse outcomes.[33]

Furthermore, therapies targeting actionable mutations have shown efficacy, leading to accelerated FDA approval of the FGFR inhibitors pemigatinib and infigratinib and the IDH1 inhibitor ivosidenib in patients with cholangiocarcinoma subtypes.[19,20,34–36] Although treatment outcomes with FGFR inhibitors are promising, only 10%–20% of patients are eligible, highlighting the need for additional treatment options.[37,38] Second-line ivosidenib, an inhibitor of mutant *IDH1* found in ~15% of IHCC, demonstrated better PFS and OS than placebo, despite a high rate of crossover and 2% ORR per independent review.[16,34]

Clinical activity was observed across BTC subtypes, although a numerically higher response rate was seen among patients with IHCC (12.6%) than extrahepatic (9.4%) or GC (6.3%) (Table 2 ,http://links.lww.com/HEP/F5). Responses were also observed irrespective of PD-L1 expression on tumor cells, *FGFR2*, and *IDH1* mutation status, particularly in patients with *IDH1* mutations compared with those without (ORR, 21.4% vs 8.4%, respectively) (Supplemental Table S2, http://links.lww.com/HEP/F5). In studies of anti–PD-(L)1 therapy in BTC, the ORR tends to be higher in the PD-L1-selected population compared with the PD-L1-unselected population. The efficacy of bintrafusp alfa in both populations may be related to simultaneous, dual inhibition of the TGF-$\beta$ and PD-L1, nonredundant but complementary

pathways. Only 3 patients in this study had microsatellite instability-high tumors, and while none of these patients were responders, the low numbers limit the interpretation of this result. Responses to bintrafusp alfa have been observed in patients with microsatellite instability high tumors in BTC and gastric cancer.[13,39] While there were more responders among patients with low tumor mutational burden versus high tumor mutational burden (ORR of 15.5% vs 3.7%, respectively), the CIs were wide, preventing any conclusions regarding response and tumor mutational burden. In addition, responses were observed irrespective of race or ethnicity. While, to our knowledge, these subgroup analyses have not been presented for immunotherapies in the second-line setting, durvalumab added to gemcitabine + cisplatin in the first-line setting from the TOPAZ-1 study demonstrated improvements versus gemcitabine + cisplatin for PFS and OS in Asian patients but not non-Asian patients.

Pharmacokinetic data showed that the target exposures were reached with 1200 mg every 2 weeks in this patient population and that antidrug antibodies did not seem to impact the benefit or risk of treatment with bintrafusp alfa. The safety profile of bintrafusp alfa was consistent with TGF-β and PD-L1 inhibition and with previous trials of bintrafusp alfa in the biliary tract and other cancers. The AEs associated with bintrafusp alfa were manageable by temporary treatment discontinuation, infusion rate reduction, use of appropriate medication, or operation. In particular, the incidence of TGF-β inhibition-mediated skin AEs was low, with only one (0.6%) grade 3 event and no grade 4/5 events. Other TGF-β pathway targeting agents (eg, fresolimumab, an antibody that can bind TGF-β1, TGF-β2, and TGF-β3) have also exhibited actinic keratosis, hyperkeratosis, keratoacanthoma, basal cell carcinoma, and squamous cell carcinoma as dermatological AEs, indicating that these events are associated with inhibition of the TGF-β pathway.[40]

The work we have presented carries a number of limitations. Given that the trial was a nonrandomized, single-arm trial without a control arm (eg, active, supportive care in ABC-06 or 5-FU/leucovorin in NIFTY), definitive conclusions are not feasible. With the FDA approval of durvalumab in the first-line setting pursuant to the TOPAZ-1 trial, the application of bintrafusp in unselected patients becomes inherently challenging. The discontinuation of an ongoing phase 2/3 study of bintrafusp in the first-line setting for BTCs that did not demonstrate PFS improvement at an interim analysis highlights the evolving nature of the biliary cancer therapy landscape. Evaluation of biomarkers such as activity in *IDH1*-mutant patients could provide context for future evaluation of bifunctional agents, such as bintrafusp alfa, should such investigations be pursued.

## CONCLUSIONS

This study did not meet its prespecified primary endpoint. However, taken together, the clinically relevant ORR (10.7%) and durability of response (6.3% of patients had a response $\geq$6 mo) support the potential for clinical benefit with bifunctional immunotherapies across BTC subtypes regardless of PD-L1 expression. The tolerability profile of bintrafusp alfa was consistent with the previous phase 1 trial. Efficacy was observed across BTC subtypes and PD-L1 expression. Ongoing efforts for the development of bintrafusp alfa should be directed toward identifying biomarkers of response and combinatorial therapies, including novel immune-oncology agents.

### AUTHOR CONTRIBUTIONS

Changhoon Yoo, Milind M. Javle, Helena Verdaguer Mata, Filippo de Braud, Jörg Trojan, Jean-Luc Raoul, Jin Won Kim, Makoto Ueno, Choong-kun Lee, Susumu Hijioka, Antonio Cubillo, Junji Furuse, Nilofer Azad, John Bridgewater, Do-Youn Oh, and Mitesh J. Borad: conduct of the study and recruited patients. Masashi Sato, Yulia Vugmeyster, Andreas Machl, Marcis Bajars, and Mitesh J. Borad: conceptualization and design of the study, formal analysis, verifying underlying data, data curation, methodology, and visualization of data. All authors had access to all the data, and were responsible for drafting, reviewing, and editing the manuscript.

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## CONFLICTS OF INTEREST

Changhoon Yoo consults, advises, and received grants from Servier, Bayer, AstraZeneca, Celgene, Ipsen, and Boryung Pharmaceuticals. He consults and advises Merck and Co., Kenilworth, NJ, Eisai, Bristol Myers Squibb, Debiopharm, Kyowa Kirin, Novartis, the healthcare business of Merck KGaA, Darmstadt, Germany, Mundipharma, Roche, and Janssen. He received grants from Ono Pharmaceuticals, Ildong Pharmaceuticals, CKD Pharmaceuticals, and HK inno.N. Milind M. Javle advises and received grants from Incyte, Merck and Co., Kenilworth, NJ, QED, and the healthcare business of Merck, KGaA, Darmstadt, Germany. He advises Mundipharma, and OncoSil. He received grants from Bayer, Beigene, Novartis, Pieris, Rafael and Seattle Genetics. Helena Verdaguer Mata advises the healthcare business of Merck, KGaA, Darmstadt, Germany. She is on the speakers' bureau for AstraZeneca. Filippo de Braud advises, is on the speakers' board, received grants and compensation from Bristol Myers Squibb and Roche. 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