Purpose: Dickkopf-1 (DKK1) modulates Wnt signaling, promoting tumor growth, metastasis, and immunosuppression. High DKK1 expression has been detected in various tumor types—including biliary tract cancer (BTC)—and is associated with poor prognosis. DKN-01—a humanized mAb targeting DKK1—was evaluated in a phase I multicenter study in combination with gemcitabine and cisplatin in patients with unresectable or metastatic BTC with no prior systemic therapy for advanced disease.

Patients and Methods: This study included a dose-escalation phase assessing DKN-01 at two dose levels (150 mg and 300 mg) combined with gemcitabine (1,000 mg/m²) and cisplatin (25 mg/m²) followed by dose expansion. Primary endpoints evaluated safety and tolerability; secondary endpoints evaluated efficacy, pharmacokinetics, and circulating biomarkers.

Results: Fifty-one patients with intrahepatic cholangiocarcinoma (63%), extrahepatic cholangiocarcinoma (8%), and gallbladder cancer (29%) were enrolled. No dose-limiting toxicities were seen, and the expansion phase proceeded with DKN-01 300 mg (N = 47). The most frequent grade 3/4 treatment-emergent adverse events included neutropenia (60%), thrombocytopenia (34%), and anemia (23%). The objective response rate was 21.3% and median progression-free survival was 8.7 months (95% confidence interval, 5.4–10.3 months). Better outcomes were associated with biomarkers of angiogenesis inhibition (increased sVEGFR1 and lower VEGF-C) and reduced inflammation (lower IL6 and decreased TNFα).

Conclusions: DKN-01 300 mg was well tolerated in this combination but did not appear to have additional activity beyond historically reported efficacy with gemcitabine/cisplatin alone. Exploratory pharmacokinetic and biomarker data indicate potential antiangiogenic and immunomodulatory activity of DKN-01 chemotherapy and the need for increased dose/intensity. A study with DKN-01 600 mg in combination with a PD-1 inhibitor in BTC is ongoing.

Introduction
Biliary tract cancer (BTC)—which includes intrahepatic and extrahepatic cholangiocarcinoma and gallbladder carcinoma—is a group of rare but aggressive malignancies with a poor prognosis (1). Surgery remains the mainstay of care for early-stage BTC, but the majority of patients recur (2–5). Up to 80% of patients present with unresectable/metastatic disease (5). The combination of gemcitabine and cisplatin has been the global first-line standard of care in advanced BTC since 2009 (6, 7), after showing objective response rates (ORR) of 20%–26%, median progression-free survival (PFS) of 6–8 months, and overall survival (OS) of 11–12 months (7, 8). More effective regimens are needed to improve clinical outcomes in BTC.

Wnt signaling is a key pathway regulating stem cell maintenance, cell proliferation, and migration during development and adult tissue homeostasis (9–13). This pathway is frequently dysregulated in cancer, and several agents targeting it are in preclinical and clinical development (14, 15). Dickkopf-1 (DKK1) is a secreted protein characterized by two cysteine-rich domains, which interacts with the LRP5/LRP6 coreceptor to inhibit canonical Wnt/b-catenin signaling (16). DKK1 plays physiologic roles in embryonic development and bone homeostasis via modulation of Wnt signaling (17–19). DKK1 also plays a role in cancer and has been shown to promote proliferation, invasion, and growth in nonclinical models (20). Recent publications have implicated DKK1 in fostering an immunosuppressive tumor microenvironment by activating myeloid-derived suppressor cells, which blunt T-cell responses, and also by downregulating natural killer (NK)-activating ligands that would otherwise allow for NK cell–mediated clearance (21, 22). Therefore, targeting DKK1 has been hypothesized to have anticancer activity through multiple mechanisms, including directly targeting tumor cells and modulating the tumor microenvironment (18).
Elevated DKK1 expression was detected in multiple tumor types, including BTC (23), and was associated with poor prognosis (18). Analysis of BTC tumor tissue samples from 138 patients revealed that 38% of tumors showed elevated DKK1 expression by IHC, and DKK1 expression was associated with significantly lower 5-year OS and higher disease recurrence rate (21). Furthermore, elevated DKK1 expression correlated with increased expression of matrix metalloproteinase 9 (MMP9) and VEGF-C, proteins with characterized roles in tumor cell invasion, angiogenesis, and lymph node metastasis (21). Of note, targeted knockdown of DKK1 expression resulted in decreased MMP9 and VEGF-C expression and reduced tumor invasiveness in cholangiocarcinoma models (21, 24).

DKN-01 is a humanized mAb (IgG4) optimized for neutralizing activity against DKK1 being investigated in multiple solid tumors. DKN-01 has demonstrated single-agent activity in esophagogastric, – small cell lung cancers (25, 26). The side-effect profile of DKN-01 has been generally consistent across studies, characterized primarily by fatigue and mild gastrointestinal symptoms, including nausea, vomiting, and diarrhea.

On the basis of the pleiotropic roles of DKK1, preclinical evidence of reduced tumor invasiveness in cholangiocarcinoma models upon DKK1 suppression, and the prior clinical evidence of tolerability and antitumor activity of DKN-01 in patients with cancer, we designed this first phase I and biomarker study of DKN-01 for BTC.

Patients and Methods

Study design

This single-arm, multicenter, phase I dose-escalation and dose-expansion study was conducted to determine the feasibility and preliminary efficacy of combining DKN-01 with gemcitabine/cisplatin as first-line therapy in patients with advanced BTC. In phase 1a, escalating doses of DKN-01 were administered intravenously, followed by gemcitabine 1,000 mg/m² i.v. and cisplatin 25 mg/m² i.v. on days 1 and 8 of 21-day cycles. Therapy was continued as long as patients were receiving clinical benefit in the investigator's judgment or until a criterion for discontinuation was met. The two DKN-01 doses assessed were 150 mg and 300 mg, in cohorts 1 and 2, respectively. This dosing was based on initial modeling indicating that DKN-01 dosing at 150–300 mg effectively blocked DKK1 for 30 days (>95%). Because modeling suggested that DKK1 suppression lasted for 30 days, the dosing frequency in 21-day cycles was deemed sufficient for sustained DKK1 suppression. DKN-01 was administered first, followed by cisplatin and gemcitabine. Gemcitabine/cisplatin and associated predmedication were administered per standard institutional practice. The primary objective was safety and establishing an MTD of DKN-01. For each cohort, up to 4 patients may have been screened simultaneously to ensure 3 patients would be treated; if all 4 patients were eligible, all 4 were permitted to enroll. In the phase Ib dose-expansion portion, up to 44 patients were planned to receive DKN-01 at the MTD, or highest dose tested if the MTD was not established, to further characterize safety and tolerability within the defined patient population. This sample size was not based on formal statistical calculations, given the exploratory nature of the phase I study. The secondary objectives were efficacy, pharmacokinetics, pharmacodynamics, and tissue and circulating biomarkers. Institutional review boards at all participating sites approved the study, and all patients provided written informed consent before the performance of any study-related procedures. The study was conducted in accordance with International Council for Harmonization Good Clinical Practice (ICH-GCP) guidelines and Declaration of Helsinki. The study was registered at clinicaltrials.gov (NCT02375880).

For dose escalation, the dose-limiting toxicities (DLT) were defined as grade 3 or greater occurring within cycle 1 and considered by the investigator to be possibly related to DKN-01 or gemcitabine/cisplatin. Exceptions were alopeecia; grade 3/4 anemia, thrombocytopenia, or neutropenia resolving to ≤grade 2 within 7 days; grade 3 leukopenia or lymphopenia resolving to ≤grade 2 within 14 days or grade 4 leukopenia or lymphopenia resolving to ≤grade 2 within 21 days; grade 3/4 asymptomatic laboratory values resolving to ≤grade 2 within 14 days; grade 3/4 nausea, vomiting, or diarrhea resolving to ≤grade 2 within 72 hours; grade 3 drug-related fever; and any grade 4 hypersensitivity reaction to DKN-01, regardless of whether premedication was administered, or grade 3 hypersensitivity reaction to DKN-01 after no premedication was administered. Toxicities were graded by the treating investigator using the NCI Common Terminology Criteria for Adverse Events, Version 4.03 (CTCAE v4.03).

Eligibility

Patients with histologically or cytologically confirmed intrahepatic or extrahepatic cholangiocarcinoma or gallbladder cancer with unresectable/metastatic disease were eligible. Patients were required to be at least 30 years of age because bone growth is estimated to be complete by this age. Patients had not previously received systemic therapy for advanced disease. Patients may have received prior adjuvant gemcitabine with or without cisplatin, provided 6 months had lapsed since last treatment. Patients required measurable disease, as defined by the RECIST (RECISTv1.1, ref. 27). Prior cryotherapy, radiofrequency ablation, radioembolization, ethanol injection, transarterial chemoembolization (TACE), or photodynamic therapy was permitted if at least 28 days had elapsed since that therapy, and lesions outside the treatment zone were present and measurable. Patients were required to have adequate hepatic function [total bilirubin ≤2.0× upper limit of normal (ULN); aspartate aminotransferase and alanine aminotransferase (AST/ALT) ≤2.5× ULN, ≤5× ULN if liver metastasis], renal function (calculated creatinine clearance ≥50 ml/minute using the Cockcroft–Gault method), and bone marrow function [white blood cells ≥1.5×10⁹/L, hemoglobin ≥9 g/dL (blood transfusion permitted within 30 days of study entry), platelets ≥100×10⁹/L, and Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1]. Patients with peripartum cancer, clinically significant comorbidities, or a history of osteonecrosis or structural bone

Anti-DKK1 Antibody with Chemotherapy in Biliary Tract Cancer

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abnormalities, osteoblastic bony metastases, or symptomatic central nervous system metastases were excluded (see Supplementary Materials).

Safety and efficacy assessments

Safety assessments included documentation of adverse events (AE) according to CTCAE v4.03, physical examinations, vital signs, electrocardiograms, and clinical and laboratory assessments. Disease response by CT and/or MRI was assessed by the Investigator using RECISTv1.1 at the start of cycle 3 and then every two cycles (6 weeks) thereafter. If response was noted, a follow-up radiographic assessment was to be performed at least 4 weeks later to confirm the response.

Blood analyses

Single- and multiple-dose serum samples were collected for the quantitation of DKN-01, total DKK1 (free DKK1 and DKK1/DKN-01 complex), and anti-DKN-01 antibodies. Samples were collected weekly during cycle 1 (days 1, 8, and 15), day 1 of each subsequent cycle, and at end of treatment. Serum DKK1 levels were measured by a validated ELISA, an assay designed to perform in the presence of DKN-01.

Serial plasma samples for biomarkers of interest, including circulating inflammatory and angiogenic molecules, were collected pre-treatment and weekly during cycle 1 (days 1, 8, and 15), cycle 2 day 1, and end of treatment in all patients.

For plasma biomarkers, blood was collected in EDTA vacutainers, processed and stored at each site using a standardized protocol, and shipped to Massachusetts General Hospital, Boston, MA for centralized analysis in a Clinical Laboratory Improvement Amendments – Certified Core of the Steele Laboratories. Plasma analysis was carried out in duplicate for the following biomarker panels: human angiogenesis array (VEGF, VEGF-C, VEGF-D, sFLT-1/sVEGFR1, PI GF, bFGF, sTIE-2) and proinflammatory array (IFNγ, IL1β, IL10, IL12/ p70, IL13, IL2, IL4, IL6, IL8, TNFα; both MesoScale Discovery). Human SDF1-α and hepatocyte growth factor (HGF) were measured by ELISA (R&D Systems).

DKK1 RNAscope ISH

Tumor tissue from consenting patients was evaluated for DKK1 messenger ribonucleic acid (mRNA) expression by a single-plex RNAscope ISH assay on the Leica Biosystems BOND RX platform (28). Formalin-fixed, paraffin-embedded sections were baked and deparaffinized, followed by target retrieval (15 minutes at 95°C) using Leica Epitope Retrieval Buffer 2 and protease III treatment for 15 minutes at 40°C. DKK1 probe (ACD, catalog no. 421418) was hybridized for 2 hours at 42°C, followed by signal amplification and chromogenic detection. Samples were quality controlled for RNA integrity and background staining with PPB and dapB probes, respectively (ACD, catalog no. 313908 and 312038). DKK1 mRNA tumor expression was measured using QuPath open-source morphometric analysis program (29), and a H-score (range 0–300) was calculated by determining the percentage of low (1–3 dots/cell), medium (4–9 dots/cell), and high (10+ dots/cell) expressing tumor cells. H-score = (%low) 1+(%medium) 2+(%high) 3.

Statistical considerations

Safety parameters and continuous and categorical measures of efficacy were summarized using standard descriptive statistics. Safety analyses were conducted on data from all patients receiving at least one dose of study drug according to the treatment initially received.

The population evaluable for efficacy analysis included all subjects who completed at least one cycle of study treatment, including all planned doses of DKN-01, gemcitabine, and cisplatin. The response-evaluable population included a subset of the efficacy population that received at least one posttreatment imaging study. ORR was defined as the number of patients who exhibited a complete or partial response (CR or PR) divided by the number of patients in the response-evaluable population. Disease control rate was the number of patients exhibiting CR, PR, or stable disease (SD) divided by the number of patients with an evaluable posttreatment response. PFS was defined as the time from treatment initiation to the first date of objectively determined progressive disease (PD) or death from any cause. Patients without evidence of PD or death were censored in the analysis. OS was defined as the time from treatment initiation until death of any cause. Patients still alive as of the data cut-off date were censored on the last known alive date in mortality status. PFS and OS were estimated using Kaplan–Meier methods by study part and dose group; median estimates were provided along with its 95% confidence intervals (CI). Biomarker changes over time were analyzed using the P values from Wilcoxon sign-rank test. Associations between biomarker data at the specific time points and outcomes were analyzed using the Wald test in a univariate Cox regression using rank-transformed covariates. The statistical analyses were performed using SAS, Version 9.3.

Pharmacokinetic/pharmacodynamic analysis

Using serial serum DKN-01 and DKK1 measurements, we developed a model to provide plausible pharmacokinetic profiles such that DKN-01 exposure pharmacokinetic parameters [e.g., maximum concentration (Cmax), and AUC] could be calculated via noncompartmental analysis. The bioanalytic assay used in these studies did not distinguish between free versus DKN-01-bound DKK1. To understand the effects of DKN-01, we used an indirect method to estimate the free DKK1 concentration. Based upon model fitting to serum concentrations of DKN-01 and total DKK1, a pharmacokinetic/pharmacodynamic target-mediated drug disposition (TMDD) model allowed for estimation of free serum DKK1. The TMDD model was able to describe both the pharmacokinetic (DKN-01) and pharmacodynamic (DKK1) data simultaneously, and at the same time estimate the in vivo binding affinity of DKN-01 for DKK1. Free DKK1 concentrations were estimated from the binding affinity (30).

Results

Patient characteristics

Fifty-one patients were enrolled and treated across 9 sites, 4 at planned doses of DKN-01, gemcitabine, and cisplatin. The response-evaluable population included a subset of the efficacy population that received at least one posttreatment imaging study. ORR was defined as the number of patients who exhibited a complete or partial response (CR or PR) divided by the number of patients in the response-evaluable population. Disease control rate was the number of patients exhibiting CR, PR, or stable disease (SD) divided by the number of patients with an evaluable posttreatment response. PFS was defined as the time from treatment initiation to the first date of objectively determined progressive disease (PD) or death from any cause. Patients without evidence of PD or death were censored in the analysis. OS was defined as the time from treatment initiation until death of any cause. Patients still alive as of the data cut-off date were censored on the last known alive date in mortality status. PFS and OS were estimated using Kaplan–Meier methods by study part and dose group; median estimates were provided along with its 95% confidence intervals (CI). Biomarker changes over time were analyzed using the P values from Wilcoxon sign-rank test. Associations between biomarker data at the specific time points and outcomes were analyzed using the Wald test in a univariate Cox regression using rank-transformed covariates. The statistical analyses were performed using SAS, Version 9.3.

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Results

Patient characteristics

Fifty-one patients were enrolled and treated across 9 sites, 4 at 150 mg and 3 at 300 mg DKN-01 in escalation phase, and 44 at 300 mg in the expansion phase between July 2015 and August 2018 (Fig. 1). The median age of patients was 62 years (42–83 years), and the majority of patients were White (82%) and female (63%; Table 1). One patient who had an ECOG PS of 2 but was enrolled at the medical monitor’s discretion.

The prevailing BTC type was intrahepatic cholangiocarcinoma (63%), and the remainder were extrahepatic cholangiocarcinomas (8%) and gallbladder cancers (29%). Most patients had metastatic disease (75%) at diagnosis. Nine patients received prior adjuvant systemic therapy, with 7 having received prior gemcitabine/cisplatin.

Exposure

The median duration on DKN-01 300 mg, gemcitabine, and cisplatin treatments were 155, 155, and 113 days (7, 7, and 6 cycles), respectively.
Figure 1.
CONSORT diagram of phase Ib trial of DKN-01 with gemcitabine/cisplatin chemotherapy in patients with advanced BTCs.

Enrolled
\( N = 51 \)

DKN-01 150 mg +Gem/Cis
\( N = 4 \)

Safety population
\( N = 4 \)

Efficacy population
\( N = 4 \)

Per protocol population
\( N = 2 \)

Responder population (Patients with PR)
\( N = 0 \)

Discontinued study drug
\( N = 51 \)

Reason for discontinuation:
PD: 3
Dosing delay ≥21 days: 1

Discontinued study
\( N = 51 \)

Reason for discontinuation:
Death: 4

Reason for discontinuation:
PD: 26
Dosing delay ≥21 days: 10
Investigator decision: 6
Adverse event: 3
Other: 3

Reason for discontinuation:
Death: 35
Withdrawn consent: 3
Lost to follow-up: 2
Sponsor request: 1
Study closure: 10

Enrolled
\( N = 51 \)

DKN-01 300 mg +Gem/Cis
\( N = 47 \)

Safety population
\( N = 47 \)

Efficacy population
\( N = 47 \)

Per protocol population
\( N = 38 \)

Responder population (Patients with PR)
\( N = 10 \)
Table 1. Demographics and baseline disease characteristics.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>150 mg DKN-01 + gemcitabine/cisplatin (N = 4)</th>
<th>300 mg DKN-01 + gemcitabine/cisplatin (N = 47)</th>
<th>Overall (N = 51)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (±STD) age at consent (years)</td>
<td>63.5 (4.8)</td>
<td>63.9 (9.2)</td>
<td>63.8 (8.9)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>1 (25.0)</td>
<td>31 (66.0)</td>
<td>32 (62.7)</td>
</tr>
<tr>
<td>White, n (%)</td>
<td>4 (100.0)</td>
<td>38 (80.9)</td>
<td>42 (82.4)</td>
</tr>
<tr>
<td>Primary diagnosis, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intrahepatic biliary system</td>
<td>2 (50.0)</td>
<td>30 (63.8)</td>
<td>32 (62.7)</td>
</tr>
<tr>
<td>Extrahepatic biliary system</td>
<td>0</td>
<td>4 (8.5)</td>
<td>4 (7.8)</td>
</tr>
<tr>
<td>Gallbladder</td>
<td>2 (50.0)</td>
<td>13 (27.7)</td>
<td>15 (29.4)</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma(^a), n (%)</td>
<td>4 (100.0)</td>
<td>47 (100.0)</td>
<td>51 (100.0)</td>
</tr>
<tr>
<td>Stage IV disease at time of diagnosis, n (%)</td>
<td>4 (100.0)</td>
<td>36 (76.6)</td>
<td>40 (78.4)</td>
</tr>
<tr>
<td>Median (Q1, Q3) time since disease diagnosis (months)</td>
<td>1.61 (1.25, 20.94)</td>
<td>1.54 (0.92, 3.45)</td>
<td>1.54 (0.99, 3.45)</td>
</tr>
<tr>
<td>Baseline disease status, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unresectable, locally advanced</td>
<td>0</td>
<td>11 (23.4)</td>
<td>11 (21.6)</td>
</tr>
<tr>
<td>Metastatic disease</td>
<td>4 (100.0)</td>
<td>34 (72.3)</td>
<td>38 (74.5)</td>
</tr>
<tr>
<td>Baseline ECOG PS 0 or 1, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>3 (75.0)</td>
<td>25 (54.5)</td>
<td>28 (54.9)</td>
</tr>
<tr>
<td>1</td>
<td>1 (25.0)</td>
<td>21 (44.7)</td>
<td>22 (43.1)</td>
</tr>
<tr>
<td>Mean (±STD) baseline CA 19–9 (unit/mL)</td>
<td>1,077.70 (1,242.289)</td>
<td>12,709.39 (78,787.428)</td>
<td>11,740.08 (75,430.882)</td>
</tr>
<tr>
<td>Prior therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>1 (25.0)</td>
<td>20 (42.6)</td>
<td>21 (41.2)</td>
</tr>
<tr>
<td>Adjuvant systemic therapy</td>
<td>1 (25.0)</td>
<td>8 (17.0)</td>
<td>9 (17.6)</td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>1 (25.0)</td>
<td>4 (8.5)</td>
<td>5 (9.8)</td>
</tr>
<tr>
<td>Radiotherapy with chemotherapy</td>
<td>1 (25.0)</td>
<td>3 (6.4)</td>
<td>4 (7.8)</td>
</tr>
<tr>
<td>Local therapy</td>
<td>1 (25.0)</td>
<td>1 (2.1)</td>
<td>2 (3.9)</td>
</tr>
<tr>
<td>Mean (±STD) number of prior adjuvant chemotherapy regimens</td>
<td>1.0 (–)</td>
<td>1.3 (0.46)</td>
<td>1.2 (0.44)</td>
</tr>
</tbody>
</table>

Abbreviations: CA, cancer antigen; ECOG, Eastern Cooperative Oncology Group; PS, performance status; Q, quartile; STD, standard deviation.

\(^a\)One case out of the 47 in the expansion cohort (2.1%) was a glandular carcinoma.

Safety

DKN-01 combined with gemcitabine/cisplatin was well tolerated, and consistent with the toxicity profile of gemcitabine/cisplatin for this population (Table 2). The MTD of DKN-01 with gemcitabine/cisplatin was not reached, as no patients experienced a DLT in dose escalation. The highest dose of DKN-01 tested (300 mg i.v.) was selected for dose expansion.

Across both DKN-01 doses, hematologic abnormalities, including thrombocytopenia (73%), neutropenia (71%), anemia (61%), and leukopenia (57%), were the most common treatment emergent AEs (TEAE), including with grade 3/4 neutropenia (33%), leukopenia (26%), and anemia (22%). Other common (incidence ≥25%) DKN-01–related nonhematologic AEs included fatigue (49%), nausea (37%), AST increased (35%), ALT increased (28%), and alkaline phosphatase increased (26%).

A total of 25/51 (49%) patients experienced serious AEs (SAE) during the treatment period: 1 patient in the DKN-01 150 mg group (in cycle 10 and unrelated to study treatment) and 24 patients in the DKN-01 300 mg group. The most frequently reported SAEs were infections [28%, most commonly sepsis (6%) and bacteremia (4%)], blood and lymphoid system disorders [12%, most commonly neutropenia and thrombocytopenia (each 8%)], and hepatobiliary disorders [10%, most commonly cholangitis (6%)]. Other SAEs reported for >1 patient each included acute kidney injury, pyrexia, and upper gastrointestinal hemorrhage. Six patients experienced SAEs considered by the investigator to be related to the DKN-01 addition to chemotherapy: 1 patient had sepsis, peritonitis, thrombocytopenia, and neutropenia; another had thrombocytopenia and neutropenia; another had neutropenia and leukopenia; and 1 patient each had thrombocytopenia, sepsis, and failure to thrive.

Overall, 4 patients experienced an AE leading to death, with 1 patient each having nephrotic syndrome, acute kidney injury, septic shock, and sepsis. Neither the septic shock nor the sepsis occurred in the setting of neutropenia. None of the patient deaths were attributed by the Investigator to DKN-01 or gemcitabine/cisplatin. No patient experienced DKN-01–related hypersensitivity or immune-mediated reactions.

Efficacy

Among 47 subjects receiving at least one cycle with DKN-01 at a dose of 300 mg, the ORR was 21.3%, with 10 patients having a best response of confirmed PR. The median and maximum times to first PR were 2.9 months (~3 cycles) and 10.8 months (~12 cycles; Fig. 2) and a median duration of PR of 6.8 months. Among the 43 response-evaluable patients (i.e., those with at least one postbaseline tumor assessment), the ORR was 23.3%. Thirty-one subjects (66.0%) had SD.

Median PFS was 8.7 months (95% CI, 5.4–10.3 months) and median OS was 12.4 months (95% CI, 9.0–16.1 months). Median PFS and OS are displayed by tumor type in Fig. 3.

Drug pharmacokinetic evaluation

DKN-01 was administered intravenously on days 1 and 8 of a repeating 21-day cycle. Both C\(_{\text{max}}\) and AUC increased with increasing dose. After the first dose in cycle 1, the mean C\(_{\text{max}}\) (±STD) was 42,000 ng/mL at 150 mg (N = 2) and 105,000 ± 29,500 ng/mL at 300 mg (N = 39). The first dose interval AUC (AUC\(_{\text{1–24}}\), mean ± STD)
Table 2. Common (overall incidence >20%) TEAEs.

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>All grades, n (%)</th>
<th>Related to DKN-01, n (%)</th>
<th>&gt;Grade 3, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombocytopenia</td>
<td>37 (72.5)</td>
<td>25 (49.0)</td>
<td>17 (33.3)</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>56 (70.6)</td>
<td>27 (52.9)</td>
<td>30 (58.8)</td>
</tr>
<tr>
<td>Anemia</td>
<td>31 (60.8)</td>
<td>17 (33.3)</td>
<td>11 (21.6)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>29 (56.9)</td>
<td>25 (49.0)</td>
<td>5 (9.8)</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>29 (56.9)</td>
<td>19 (37.3)</td>
<td>13 (25.5)</td>
</tr>
<tr>
<td>Aspartate aminotransferase</td>
<td>28 (54.9)</td>
<td>18 (35.3)</td>
<td>4 (7.8)</td>
</tr>
<tr>
<td>Blood alkaline phosphatase</td>
<td>28 (54.9)</td>
<td>13 (25.5)</td>
<td>5 (9.8)</td>
</tr>
<tr>
<td>Nausea</td>
<td>28 (54.9)</td>
<td>19 (37.3)</td>
<td>0</td>
</tr>
<tr>
<td>Alkaline phosphatase increased</td>
<td>21 (41.2)</td>
<td>14 (27.5)</td>
<td>3 (5.9)</td>
</tr>
<tr>
<td>Constipation</td>
<td>21 (41.2)</td>
<td>5 (9.8)</td>
<td>0</td>
</tr>
<tr>
<td>Hypoalbuminemia</td>
<td>18 (35.3)</td>
<td>2 (9.8)</td>
<td>0</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>17 (33.3)</td>
<td>7 (13.7)</td>
<td>2 (3.9)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>17 (33.3)</td>
<td>2 (9.8)</td>
<td>8 (15.7)</td>
</tr>
<tr>
<td>Hypomania</td>
<td>17 (33.3)</td>
<td>7 (13.7)</td>
<td>5 (9.8)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>16 (31.4)</td>
<td>1 (2.0)</td>
<td>2 (3.9)</td>
</tr>
<tr>
<td>Decreased appetite</td>
<td>14 (27.5)</td>
<td>7 (13.7)</td>
<td>0</td>
</tr>
<tr>
<td>Hyperbilirubinemia</td>
<td>14 (27.5)</td>
<td>5 (9.8)</td>
<td>8 (15.7)</td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>14 (27.5)</td>
<td>1 (2.0)</td>
<td>1 (2.0)</td>
</tr>
<tr>
<td>Edema peripheral</td>
<td>14 (27.5)</td>
<td>5 (9.8)</td>
<td>0</td>
</tr>
<tr>
<td>Gamma-glutamyltransferase</td>
<td>12 (23.5)</td>
<td>2 (3.9)</td>
<td>6 (11.8)</td>
</tr>
<tr>
<td>Pyrexia</td>
<td>12 (23.5)</td>
<td>2 (3.9)</td>
<td>0</td>
</tr>
<tr>
<td>Vomiting</td>
<td>12 (23.5)</td>
<td>8 (15.7)</td>
<td>1 (2.0)</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>11 (21.6)</td>
<td>2 (3.9)</td>
<td>0</td>
</tr>
<tr>
<td>Weight decreased</td>
<td>11 (21.6)</td>
<td>5 (9.8)</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 2.
Spider plot of time to best response among patients receiving DKN-01, 150 mg or 300 mg, plus gemcitabine/cisplatin.

was 192,000 ng/day/mL (N = 2) and 462,000 ± 143,000 ng/day/mL (N = 33) for 150 mg and 300 mg, respectively. The Cmax for both doses was achieved between 2 and 3 hours on day 1, postdose. When Cmax was evaluated across both of the doses administered in cycle 1, the mean Cmax was 51,200 ± 12,900 ng/mL at 150 mg (N = 4) and 141,000 ± 39,800 ng/mL at 300 mg (N = 37). In this case, where both doses were included in the analysis, Cmax was reached after the second dose on day 8 and the increase in Cmax relative to the first dose interval reflects moderate drug accumulation. The corresponding mean AUC from day 1 to day 21 (AUC1–21) was 647,000 (N = 2) and 1,630,000 ± 526,000 ng/day/mL (N = 30), respectively. On the basis of the mean trough profiles, steady state was achieved by approximately the start of the fifth or sixth cycles. No patient was positive for anti-DKK1 antibodies at any time after DKN-01 administration.

Drug pharmacodynamics

DKN1 serum concentrations were assessed in all 51 patients. After a single administration of DKN-01, total (bound and free) DKK1 concentrations increased from 6.4 ng/mL to a range of 97–125 ng/mL at the 150 mg dose level and to a range of 133–140 ng/mL at the 300 mg dose level.

In patients with PR, the median free DKK1 Cmax (at the last dose administered) was 0.50 ng/mL, and the highest individual free DKK1 Cmax was 0.77 ng/mL. In nonresponders, the median Cmax was 0.61 ng/mL and the highest individual free Cmax observed was 10.8 ng/mL. However, the difference between the two groups was not statistically significant (P = 0.5).

Tumor tissue biomarker analysis

Thirteen patients had sufficient pretreatment tumor tissue for analysis for DKK1 mRNA expression by RNAscope ISH assay. DKK1 mRNA expression in the tumor cells was generally low (H-score range, 0–105). An analysis of clinical response demonstrated no clear trend for association between DKK1 expression and patient outcome (Fig. 4A).

Plasma biomarker analysis

DKN-01 with gemcitabine/cisplatin was associated with significant changes in plasma biomarkers of angiogenesis at days 8 and 15, such as increased PIGF, VEGF-D, sTIE2, and sVEGFR1 but decreases in VEGF, VEGF-C, and bFGF. The decrease in VEGF and increase in sVEGFR1 indicates an inhibition of angiogenesis; however, plasma VEGF, VEGF-C, and bFGF levels were significantly increased at day 22, after the 1-week drug break in the first cycle of treatment, consistent with the 1-week half-life of DKN-01 (Fig. 4B–I; Supplementary Table S1). The combination regimen was associated with transient increases in circulating inflammatory cytokines, including plasma IFNα, IL6, IL8, and IL10, and with a decrease in TNFα levels (Supplementary Table S1). High pretreatment VEGF-C, and decreased sVEGFR1 at day 8 and change in sTIE2 at day 15 were associated with better survival outcomes (Supplementary Table S2). A high plasma IL6

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and an increase (or no decrease) in TNFα were associated with worse outcomes (Supplementary Tables S2 and S3). Finally, while plasma HGF level was not changed by treatment, patients with high plasma HGF at any of the time points had significantly worse outcomes after DKN-01 with gemcitabine/cisplatin (Supplementary Table S2 and S3).

Discussion

Multiple prior attempts of combining targeted therapies with a gemcitabine/platinum backbone have been made in patients with advanced BTC. However, trials of inhibitors of the VEGF (e.g., cediranib, ramucirumab), EGFR (e.g., cetuximab, panitumumab), and MET (e.g., merestinib) pathways have demonstrated limited efficacy when combined with gemcitabine/platinum (31–34). DKK1 blockade is of particular therapeutic interest in BTC because of its potential impact on Wnt signaling as well as on tumor inflammation. This is the first study combining a Wnt pathway modulator (DKN-01, a humanized IgG4 neutralizing mAb against DKK1) with gemcitabine/cisplatin as first line in patients with advanced BTC.

In this phase I multicenter study, safety/tolerability was the primary endpoint. While this study did confirm the safety and tolerability of combining DKN-01 with gemcitabine/cisplatin, the combination showed an ORR of 21% and PFS of 8.7 months, suggesting that the addition of DKN-01 does not add significant efficacy to standard of care. Of note, some patients had a PFS of greater than 1 year, while others had a much shorter duration of benefit, both within and across the anatomic subtypes of BTC. Whether this heterogeneity represents a differential activity for DKN-01 in different genomic subsets of BTC or subsets with high DKK1 expression could not be determined due to the limited amount of viable biopsy samples. Of note, high DKK1

Figure 3.
PFS and OS among patients receiving DKN-01 300 mg plus gemcitabine/cisplatin. Kaplan-Meier estimates by anatomic site of PFS (A) and OS (B).
expression was associated with better response to DKN-01 in a study of DKN-01 combined with pembrolizumab in anti–PD-1/PD-L1 inhibitor–naïve advanced gastroesophageal junction and gastric adenocarcinoma (35).

Despite the limited efficacy of adding DKN-01 to gemcitabine/cisplatin in BTC, the safety, pharmacokinetic, pharmacodynamic, and biomarker analyses from this study provided important insights for the development of DKN-01 in this disease. First, safety analyses in 51 patients with advanced BTC demonstrated that the AE profile was consistent with gemcitabine/cisplatin alone. Side effects such as nausea, constipation, diarrhea, peripheral edema, anorexia, and peripheral neuropathy are similar to those seen with gemcitabine/cisplatin alone, and hematologic toxicity was the most common grade 3/4 toxicity seen in the ABC-02 study as it was in this study. Overall, the safety and

Figure 4.
Potential biomarkers of DKN-01/gemcitabine/cisplatin treatment in tumor tissue and plasma from patients with BTC. A, DKK1 mRNA expression by response to therapy in tumor biopsies as assessed by RNAscope ISH assay. PR, partial response; SD, stable disease; PD, progressive disease; NE, nonevaluable. B–I, Changes in key plasma biomarkers during treatment at days 8, 15, and 21, shown as percent change from pretreatment values (N = 45). *P < 0.05 from Wilcoxon sign-rank test. Error bars indicate interquartile ranges for pretreatment values and for fold changes during treatment (see Supplementary Table S1).
tolerability profile of DKN-01 in this study was reasonable for consideration as a partner in other combination studies of systemic therapy.

Second, the circulating angiogenesis and inflammation biomarker and pharmacokinetic analyses provided insights on dosing and intensity of DKN-01 for future studies. Plasma for biomarker analysis was drawn weekly during cycle 1 and at the start of cycle 2. DKN-01 was given on day 1 and 8 of a 21-day cycle, and thus, there was a 2-week break before the start of cycle 2. Notably, treated patients experienced significant decreases in plasma concentrations of the proangiogenic factors VEGF, VEGF-C, and bFGF at days 8 and 15, but these levels rebounded by the start of cycle 2 (day 22). These data indicate that DKN-01 combined with gemcitabine/cisplatin significantly decreases these angiogenic biomarkers, and that evaluating their change is of value, as they appear to be related to the half-life and individual PK of DKN-01.

Finally, the circulating biomarker analysis showed immunomodulatory effects of DKN-01 with chemotherapy. The combined regimen was associated with transient increases in key immune cytokines—IFNγ, IL6, and IL8—all seen at day 15 and not at day 22. Of note, elevated pretreatment levels of plasma IL6, an immunosuppressive cytokine that may mediate antitumor immunity and response to anti-PD-1/PD-L1 immunotherapy, were associated with worse outcomes (36, 37).

While we cannot exclude that these changes are mainly due to the chemotherapy, it is important to note that the changes in VEGF, PIGF, VEGF-C, bFGF, and sVEGFR1 are not consistent with those seen in the biomarker study conducted in the randomized ABC-03 study (38). These data suggest that DKN-01 combined with gemcitabine/cisplatin significantly decreases these angiogenic biomarkers, and that evaluating their change is of value, as they appear to be related to the half-life and individual pharmacokinetics of DKN-01.

On the basis of the above data and rationale, a phase II trial of DKN-01 combined with nivolumab has been initiated in patients with advanced refractory BTC (NCT04057365). The study is testing a higher starting dose of DKN-01 600 mg i.v. and includes extensive correlative studies to further validate the effects of DKN-01 with PD-1 blockade on biomarkers of angiogenesis and inflammation in tissue as well as in blood samples. The higher DKN-01 dose of 600 mg was selected on the basis of PK modeling that implies higher DKN-01 exposure is associated with lower free DKK1, potentially leading to enhanced efficacy. The planned translational studies stand to inform the clinical development of DKN-01 as well as other Wnt signaling modulators in oncology.

Disclosure of Potential Conflicts of Interest

L. Goyal reports personal fees from Agios Pharmaceuticals (scientific advisory board), Alentis Therapeutics (scientific advisory board), QED Therapeutics (scientific advisory board and consulting), Taiho Pharmaceuticals (scientific advisory board and consulting), Debiopharm (scientific advisory board and consulting), Incyte Corporation (scientific advisory board and consulting), SIRTEX (scientific advisory board and consulting), Perris Pharmaceuticals (scientific advisory board), KLUS Pharmaceuticals (scientific advisory board), and AstaZzeneca (IDMC member) outside the submitted work. C. Sirard reports other from Leap Therapeutics (CMO of Leap therapeutics salaried employee) during the conduct of the study; other from Leap Therapeutics (stockholder) outside the submitted work; and is listed as a coinventor on 3 patents regarding the use of certain biomarkers for treatment with DKK1 inhibitor therapy which will be owned by Leap Therapeutics. M. Schrag reports personal fees from Leap Therapeutics during the conduct of the study. M.H. Kagey reports personal fees from Leap Therapeutics (full-time employee) during the conduct of the study and outside the submitted work. J.R. Eads reports other from Leap Therapeutics (funding for study conduct) during the conduct of the study, and personal fees from Novartis, Pfizer, Lexicon, Advanced Accelerator Applications, Ipsen, and Bristol-Myers Squibb (husband is an employee) as well as from other (research support) outside the submitted work. S. Stein is a member of the advisory boards of Eisai, Exelixis, QED, and Genentech. A.B. El-Khoueiry reports personal fees from Bayer, Bristol-Myers Squibb, Merck, Eisai, Roche/Genentech, Exelixis, Astrazeneca, Zymeworks, Agenus, Cytoxton, EMD Serono, Gilead, and Piersis, as well as grants from Astex and Astrazeneca outside the submitted work. G.A. Manji reports grants from Merck, BioInelixis, and Regeneron; and grants and other from Roche/Genentech (advisory board); and other from Ipsen (advisory board) outside the submitted work. T.A. Abrams reports personal fees from Bristol-Myers Squibb, Agios, and Merck outside the submitted work. A.A. Khorana reports grants from Leap [to institution (Cleveland Clinic)] during the conduct of the study; nonfinancial support from Janssen, Bayer, Sanofi, Halozyneme, Bristol-Myers Squibb, Pfizer, Seattle Genetics, Pharmaciescics, Pharmacyce, and Mentscape; as well as grants from Merck (to institution), Array (to institution), and Bristol-Myers Squibb (nonfinancial support) outside the submitted work. R. Miksad reports other from Leap Therapeutics during the conduct of the study, as well as other from Flatiron Health [employment, equity ownership in Flatiron Health Inc. (initiated before acquisition by Roche in April 2018) and Roche (stock)] outside the submitted work. D. Mahalingam reports personal fees from Amgen, Exelixis, Eisai, EMD Serono, Bayer, Genentech; grants and personal fees from Merck; and grants from OncoMetrix outside the submitted work. A.X. Zhu reports other from Bayer, Lilly, Eisai, Roche-Genentech, Sanofi Aventis, Merck, Exelixis, and Gilead (consulting/advisory) outside the submitted work. D.G. Duda reports grants and personal fees from Bayer and Bristol-Myers Squibb, grants from Exelixis; and personal fees from Sincere outside the submitted work. No other potential conflicts of interest were disclosed.

Authors’ Contributions

L. Goyal: Conceptualization, resources, data curation, formal analysis, supervision, funding acquisition, investigation, writing-original draft, project administration, writing-review and editing. C. Sirard: Resources, data curation, supervision, writing-original draft, project administration, writing-review and editing. M. Schrag: Data curation, formal analysis, writing-review and editing. M.H. Kagey: Conceptualization, data curation, formal analysis, writing-review and editing. T.A. Abrams: Resources, investigation, writing-review and editing. S. Stein: Resources, data curation, investigation, writing-review and editing. A.B. El-Khoueiry: Conceptualization, resources, data curation, investigation, writing-review and editing. A.A. Khorana: Resources, data curation, investigation, writing-review and editing. R. Miksad: Resources, data curation, investigation, writing-review and editing. D. Mahalingam: Resources, data curation, investigation, writing-review and editing. A.X. Zhu: Conceptualization, resources, data curation, investigation, writing-review and editing. D.G. Duda: Conceptualization, resources, data curation, formal analysis, supervision, funding acquisition, investigation, methodology, writing-original draft, project administration, writing-review and editing.

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