Safety, Efficacy, and Biomarker Results from a Phase Ib Study of the Anti-DKK1 Antibody DKN-01 in Combination with Pembrolizumab in Advanced Esophagogastric Cancers

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ABSTRACT

Therapeutic combinations targeting innate and adaptive immunity and predictive biomarkers of response in esophagogastric cancer (EGC) are needed. We assessed safety and clinical utility of DKN-01 (a novel DKK1-neutralizing IgG4 antibody) combined with pembrolizumab and retrospectively determined DKK1 tumoral expression as a biomarker. Patients with advanced EGC received intravenous DKN-01 (150 or 300 mg) on days 1 and 15 with pembrolizumab 200 mg on day 1 in 21-day cycles. Clinical response was assessed by RECIST v1.1. Association of tumoral DKK1 mRNA expression (*H*-score: high ≥ upper-tertile, low < upper-tertile) with response was assessed with PD-L1 levels as a covariate. Sixty-three patients received DKN-01 150 mg ($n = 2$) or 300 mg ($n = 61$) plus pembrolizumab. Common adverse events were fatigue, anemia, blood alkaline phosphatase elevation, aspartate aminotransferase elevation, and hyponatremia. Among evaluable anti-PD-1/PD-L1-naïve patients receiving DKN-01 300 mg and pembrolizumab, objective response rate (ORR) was 11.4% (5/44) and 18.5% (5/27) in patients with gastroesophageal junction or gastric cancer (GEJ/GC). Among response-evaluable anti-PD-1/PD-L1-naïve patients with GEJ/GC and known tumoral DKK1 expression, ORR was 50% in DKK1-high and 0% in DKK1-low patients, median PFS was 22.1 vs. 5.9 weeks (HR, 0.24; 95% CI, 0.08–0.67), respectively, and median OS was 31.6 weeks vs. 17.4 weeks (HR, 0.41; 95% CI, 0.16–1.07), respectively. Association of DKK1 expression with PFS was independent of PD-L1 expression (adjusted HR, 0.21; 95% CI, 0.06–0.69). DKN-01 combined with pembrolizumab was well tolerated with no new safety signals. Antitumor activity was enriched in anti-PD-1/PD-L1-naïve patients with GEJ/GC whose tumors expressed high DKK1.

Introduction

Globally, esophagogastric cancers (EGC) represent a major cause of cancer-related deaths (1). The backbone of first-line (1L) systemic therapy includes a fluoropyrimidine and a platinum agent with addition of trastuzumab in HER2 overexpressing patients (2). After progression on 1L therapy, paclitaxel with or without the anti-VEGFR2 antibody ramucirumab is a global standard for second line (2L) therapy (3). Following the Keynote-059 trial (4), the anti-PD-1 antibody pembrolizumab was approved for third line (3L) in patients with PD-L1+ tumors defined by a combined positive score (CPS) of 1 or greater (CPS ≥ 1; ref. 5). Pre-specified analysis from pembrolizumab-containing trials, including Keynote-061 (2L) and Keynote-062 (1L), have identified subsets of patients more likely to benefit from immune-checkpoint inhibitors (ICI), including those with higher PD-L1 scores (CPS ≥ 10) and/or microsatellite instable (MSI-H) tumors (6–8). However, this represents the minority of patients, and intrinsic resistance to ICI remains a critical unmet need. Discriminatory biomarkers independent of PD-L1 and MSI-H represent a key area of investigation with potential to identify patients more likely to respond to ICIs.

The Wnt/β-catenin pathway has multiple roles in cancer and contributes to ICI resistance across several tumor types (9–11). Although not fully understood, mechanisms may include generation of an immunosuppressive tumor microenvironment through T-cell exclusion and decreased immune cell trafficking (9). The secreted protein Dickkopf-1 (DKK1) is best characterized as an inhibitor of the Wnt/β-catenin-dependent (canonical) pathway; however, it has been implicated in activating Wnt/β-catenin-independent (noncanonical) signaling pathway and PI3K/AKT signaling (12). Although DKK1 can have both tumor suppressing and promoting activity, elevated DKK1 expression is associated with poor prognosis in several cancers, including EGC (12). Mechanistically, DKK1 contributes to an immunosuppressive tumor microenvironment by activating the suppressive effects of myeloid-derived suppressor cells and impeding natural killer cell-mediated antitumor response (13–16). Specifically, in preclinical

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models DKN-01 treatment led to PD-L1 upregulation on MDSCs and additive activity when combined with an anti-PD-1 (17).

DKN-01 (Leap Therapeutics) is a humanized IgG4 monoclonal antibody that binds and neutralizes circulating DKK1 and has demonstrated nonclinical single-agent activity in DKK1-expressing tumor models (18). Combination work with anti-PD-1 agents suggested enhanced activity in murine models (17). We conducted a phase Ib trial exploring the safety and preliminary clinical activity of DKN-01 alone or in combination regimens in previously treated, patients with advanced EGC. We also explored the association of tumoral DKK1 mRNA expression with clinical response to DKN-01 in combination with pembrolizumab.

Patients and Methods

Study design

This open-label, nonrandomized, multicenter, dose-escalation, dose-expansion study (NCT02013154) was conducted in multiple parts, including a DKN-01 monotherapy cohort and two combination cohorts—DKN-01 + pembrolizumab and DKN-01 + paclitaxel (reported separately; refs. 19, 20). Here, we report results of the DKN-01 + pembrolizumab cohort, including its anti-PD-1/PD-L1-naïve subgroup. Methodology for DKN-01 monotherapy is provided in the Supplementary Materials and Methods (page 2).

The trial adhered to the Declaration of Helsinki and Good Clinical Practice, the trial protocol was reviewed and approved by the institutional review boards (IRB) at participating sites or a central IRB, and all patients provided written informed consent.

Patients

Ambulatory patients ages 18 years or older with histologically proven EGC progressing after ≥1 prior line of systemic therapy for metastatic or locally advanced disease were eligible, including those with anti-PD-1/PD-L1-naïve and anti-PD-1/PD-L1-refractory disease. Prior treatment with anti-PD-1/PD-L1 mAbs was permitted, provided disease was refractory to anti-PD-1/PD-L1 mAb with documented disease progression within 24 weeks of last anti-PD1/PD-L1 mAb dose. Patients were required to have Eastern Cooperative Oncology Group performance status 0 or 1; one or more measurable disease sites as defined by RECIST v1.1 (21); and adequate end organ function. Fresh biopsy or archival tissue within 3 months was required for study entry and patients could not have received prior systemic therapies within 21 days of study entry. Key disease-related exclusion criteria included active central nervous system metastases; preexisting osteoblastic bony metastasis; and autoimmune conditions requiring chronic steroid use. Complete eligibility criteria are available in the trial protocol (NCT02013154).

DKN-01 dosing

DKN-01 is an IgG4 mAb produced as a secreted protein in large-scale batch cell culture using a Chinese hamster ovary cell line that was stably transfected with an expression vector containing the coding sequences for both the heavy and light chains of DKN-01. Following cell culture, DKN-01 is purified from the supernatant by standard chromatography and filtration techniques, followed by aseptic fill-finish to produce DKN-01 drug product.

DKN-01 was administered as a 30-minute intravenous infusion on days 1 and 15 of a 21-day cycle. Pembrolizumab 200 mg was given as a 30-minute intravenous infusion on day 1. On days when both agents were given, DKN-01 was given prior to pembrolizumab. Imaging was performed prior to cycle 3 and prior to every odd cycle thereafter. Patients continued therapy until disease progression, unacceptable toxicity, withdrawal of consent, or at the investigator's discretion.

DKN-01 150 mg was the starting dose for combination with pembrolizumab 200 mg. Following dose-limiting toxicity (DLT) analyses, escalation was planned to a target dose of DKN-01 300 mg. This starting dose and accelerated dose escalation were informed by a phase I monotherapy study that established safety up to DKN-01 600 mg intravenously (NCT01457417; ref. 22) and a study confirming the safety of 150 and 300 mg in combination with cytotoxic chemotherapy (NCT02375880; ref. 23).

Laboratory assessments

Pharmacokinetic (PK) assessment including serum DKN-01 concentration was performed on cycle 1 day 1 (C1D1), C1D8, C1D15, C2D1, and D1 of subsequent cycles. DKN-01 antidrug antibodies were assessed prior to dosing in C1 and on day 1 of every other cycle (i.e., C3D1, C5D1, etc.). Pharmacodynamic analysis was conducted using total serum DKK1 concentrations collected at the PK time points listed above.

Formalin-fixed, paraffin-embedded patient tumor tissue was evaluated centrally at Advanced Cell Diagnostics (ACD) for DKK1 expression. DKK1 messenger ribonucleic acid (mRNA) expression was measured by a single-plex RNAscope chromogenic in-situ hybridization (CISH) assay on the Leica Biosystems BOND RX platform (23, 24). DKK1 mRNA was detected in tumor cells using QuPath open-source morphometric analysis program (25), and an *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 cells. $H\text{-score} = (\%\text{low}) \times 1 + (\%\text{medium}) \times 2 + (\%\text{high}) \times 3$. In a minority of anti-PD-1/PD-L1-naïve patients with gastroesophageal junction or gastric cancers (GEJ/GC; 3 of 31), it was not possible for the QuPath program to accurately determine an *H*-score, a manual *H*-score was calculated instead using the same formula. When possible, DKK1 expression was also semiquantified in stroma and immune cells. The majority of assessed biopsies were predose. If sufficient quality tissue was not available, on-treatment biopsy (C2D1 ± 7 days) was used.

The bioanalytical assay measured total DKN-01 and DKK1 concentrations (free analyte plus analyte derived from DKN-01/DKK1 complex). A target-mediated drug disposition (TMDD) model was used to estimate total DKN-01, total DKK1, and free serum DKK1 concentrations. Total DKN-01 PK exposure parameters [e.g., maximum concentration (C_{max}) and AUC] were calculated from the model output.

PD-L1 IHC analysis for DKN-01 + pembrolizumab-treated patients was conducted centrally by Covance (Meyrin). A slide section from a predose biopsy was stained using an investigational version of the PD-L1 IHC 22C3 pharmDx (Agilent). A CPS was measured using standard methods (26).

Historical tumor microsatellite and/or mismatch repair (MMR) status was recorded in the clinical database when available for DKN-01 + pembrolizumab-treated patients. For anti-PD-1/PD-L1-naïve patients with GEJ/GC without available historical data, MMR status was assessed centrally at Interpace Diagnostics by IHC on the Ventana Benchmark Ultra Staining Platform for MLH-1 (M1), MSH-2 (G219–1129), MSH-6 (SP93), and PMS2 (A16–4).

Clinical outcome assessments

The primary endpoint was safety and tolerability of DKN-01 alone and in combination with pembrolizumab. Adverse events (AE) were

classified using NCI Common Terminology Criteria for Adverse Events version 4.0 guidelines.

Objective response was evaluated by the investigator using RECIST v1.1 (21). In addition, blinded independent central review (BICR) using RECIST v1.1 was performed retrospectively by Imaging Endpoints. Secondary objectives included estimation of objective response rate [ORR, number of patients with complete or partial response (CR or PR) divided by number of patients in the response-evaluable population], disease control rate [DCR, number of patients exhibiting CR, PR, or stable disease (SD)], duration of response (DoR), progression-free survival [PFS, time from treatment initiation to objectively determined progressive disease (PD) or death from any cause], and overall survival (OS, time from treatment initiation until death from any cause). Evaluation of DKK1 expression in tumor tissue relative to clinical outcomes was an exploratory objective.

Statistical analysis

Safety and efficacy (PFS and OS) analyses included all patients receiving at least one dose of study drug according to the treatment initially received. The efficacy-evaluable population included all patients who completed at least one cycle of study treatment, including all planned doses of DKN-01 and pembrolizumab, as applicable. The response-evaluable population included a subset of the efficacy-evaluable population that received at least one posttreatment imaging study. Statistical analyses were performed using SAS, Version 9.3.

The Kaplan–Meier method was used to estimate median PFS and OS with 95% confidence intervals (CI). Patients still alive as of the data cut-off date were censored on the last known alive date. Patients without evidence of PD or death were censored in the analysis. For DKN-01 + pembrolizumab patients, administration of palliative radiation therapy was considered clinical progression for the purposes of determining PFS. Clinical activity was summarized by descriptive analyses of response as determined by the investigators. For analysis of tumoral DKK1 mRNA expression, the distribution of DKK1 mRNA was assessed and tertiles were used to define two groups of DKK1 tumoral mRNA expression: ≥upper-tertile versus <upper-tertile (reference group). Association of DKK1 mRNA expression with clinical outcomes, namely clinical benefit/objective response (response-evaluable population), PFS, and OS (safety analysis population) was assessed using univariate and multivariable logistic regression (clinical benefit/objective response as outcome) and Cox proportional hazards models (PFS or OS outcome). PD-L1 expression was used as a covariate in multivariable models with different cut-offs [CPS < 1 (negative); CPS ≥ 1 to <10 (low-positive); and CPS ≥ 10 (high-positive)] and adjusted effect estimates were provided for DKK1 mRNA expression.

Results

Between November 9, 2017, and February 15, 2019, 63 patients enrolled in the DKN-01 + pembrolizumab cohort at 10 centers in the United States (**Fig. 1**). No DLT or serious AEs occurred in the 2 patients dosed at DKN-01 150 mg + pembrolizumab. Dose escalation to DKN-01 300 mg + pembrolizumab proceeded in 61 patients in 2 patient groups: anti-PD-1/PD-L1 naïve ($n = 52$) and anti-PD-1/PD-L1 refractory ($n = 9$). Results are reported after database lock on September 3, 2019, at which time 5 patients (all anti-PD-1/PD-L1 naïve treated with DKN-01 300 mg + pembrolizumab) remained on treatment. Results in the DKN-01 monotherapy cohort are provided in Supplementary Tables S1 to S3 and Supplementary Fig. S1.

Patient demographics and cancer characteristics for DKN-01 + pembrolizumab patients are summarized in **Table 1**. All DKN-01 300 mg patients received prior platinum and 95% received prior 5-fluoruracil; the majority (67%) received prior taxanes with or without ramucirumab (38%). Overall, PD-L1 CPS was negative (<1) in 29.5% of patients, low positive (≥1 to <10) in 36.1%, and high positive (≥10) in 21.3%; proportions were similar among anti-PD-1/PD-L1-naïve patients and anti-PD-1/PD-L1-naïve GEJ/GC patients. No DKN-01 300 mg + pembrolizumab patients had evidence for microsatellite instability or MMR deficiency.

Safety

Patients treated with DKN-01 300 mg + pembrolizumab completed a median 2.0 (range: 1–17) treatment cycles. No DLT events were observed. Four patients discontinued DKN-01 due to seven AEs: grade 2 pleural effusion ($n = 1$); grade 3 abdominal pain ($n = 1$); grade 3 pneumonia ($n = 1$); grade 3 syncope, grade 2 orthostatic hypotension and grade 2 dehydration ($n = 1$, all in same patient); and grade 2 neuropathy ($n = 3$). These AEs were considered possibly study-drug related by the investigator. One (1.6%) patient required dose modification of DKN-01 300 mg due to grade 3 hyperbilirubinemia. Most patients treated with DKN-01 300 mg + pembrolizumab (66%, 40 patients) had an AE related to DKN-01, most commonly fatigue (18%), aspartate aminotransferase (AST) increase (15%), and blood alkaline phosphatase (ALP; 15%); all other AEs were reported for <10% of patients (**Table 2**). Fifteen patients (24.6%) treated with DKN-01 300 mg + pembrolizumab experienced treatment-related grade ≥3 AEs. Four patients (6.6%) experienced grade 5 AEs (gastrointestinal hemorrhage in 1 patient and disease progression in 3 patients); however, none were reported as related to DKN-01 300 mg + pembrolizumab. Serious AEs were reported for 24 (39%) patients, most frequently metabolism and nutrition disorders (12%). Serious AEs of abdominal pain were reported in 7% of patients, and pneumonia, sepsis, pneumonia aspiration, and pulmonary embolism were each reported for 2 (3%) patients; no other preferred term was reported as a serious AE for more than 1 patient. No treatment-related infusion reactions or immune system disorders were reported for DKN-01.

Clinical activity

Among 63 patients treated with DKN-01 + pembrolizumab, 53 (84%) were evaluable for response (**Table 3**). Investigator-assessed ORR was 9.4% overall (DCR 39.6%), 11.4% for anti-PD-1/PD-L1-naïve patients (DCR 38.6%), and 18.5% for anti-PD-1/PD-L1-naïve patients with GEJ/GC (DCR 48.1%). There were no CRs. All PRs were in anti-PD-1/PD-L1-naïve patients with GEJ/GC. There were no CRs or PRs in anti-PD-1/PD-L1-refractory patients. Among the 16 DKN-01 300 mg patients with best overall response of SD, 12 were anti-PD-1/PD-L1-naïve (8 had GEJ/GC), and 4 were anti-PD-1/PD-L1-refractory. The retrospective BICR assessment of best overall response was generally consistent with the investigator assessment: 6 PRs in anti-PD-1/PD-L1-naïve patients, 4 PRs in patients with GEJ/GC. The median DoR was 23.9 weeks (95% CI, 6.7, NA) among all DKN-01 300 mg patients, including anti-PD-1/PD-L1-naïve patients and anti-PD-1/PD-L1-naïve patients with GEJ/GC (**Table 3**).

Median PFS was 6 weeks overall and for anti-PD-1/PD-L1-naïve patients, 6.9 weeks in anti-PD-1/PD-L1-naïve patients with GEJ/GC, and 6.6 weeks among anti-PD-1/PD-L1-refractory patients (**Table 4**). Median OS was 20.4 weeks overall and for anti-PD-1/PD-L1-naïve patients, 22.1 weeks among anti-PD-1/PD-L1-naïve patients with GEJ/GC, and 19.0 weeks among anti-PD-1/PD-L1-refractory patients.

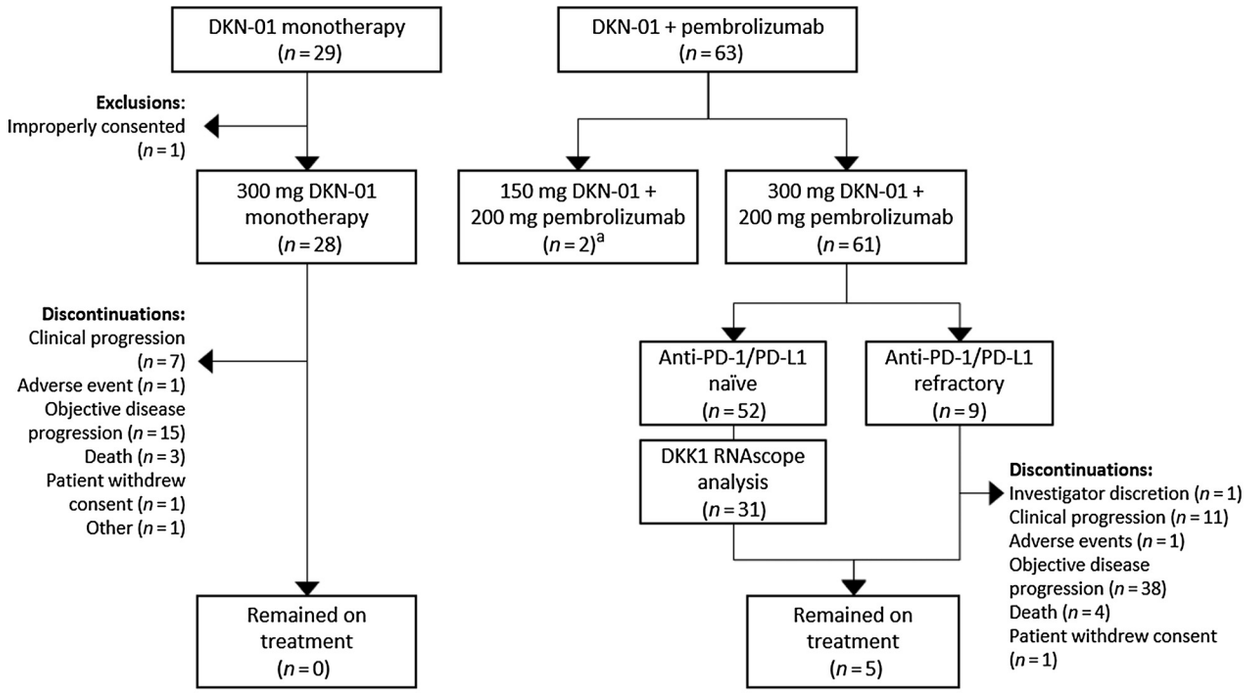

Figure 1.
Study flow diagram.

DKK1 expression and clinical outcomes

DKK1 expression by RNAscope CISH was available in 59 patients treated with DKN-01 + pembrolizumab, including 31 of 34 anti-PD-1/PD-L1-naïve patients with GEJ/GC (baseline characteristics available in Supplementary Table S4). DKK1 expression was primarily localized to tumor cells within the tumor microenvironment with little to no DKK1 staining observed in stroma or immune cells (Supplementary Table S5). Patients with tumor *H*-scores in the upper tertile (≥35) were considered high expressors (DKK1-high). Patients with *H*-scores below the upper-tertile (<35) were considered low expressors (DKK1-low).

Among the 31 anti-PD-1/PD-L1-naïve patients with GEJ/GC, 11 (35.5%) were DKK1-high. All responding anti-PD-1/PD-L1-naïve patients with GEJ/GC were DKK1-high (**Fig. 2A** and **B**), and the ORR for DKK1-high GEJ/GC patients was 50% versus 0% for DKK1-low patients (response-evaluable population). Median PFS in this subgroup was 22.1 weeks versus 5.9 weeks for DKK1-high versus DKK1-low patients, respectively (HR = 0.24; 95% CI, 0.08–0.67; **Fig. 2C**; Supplementary Fig. S2). There was also a trend toward improved OS (31.6 weeks vs. 17.4 weeks, HR = 0.41; 95% CI, 0.16–1.07) for DKK1-high versus DKK1-low patients, respectively (**Fig. 2D**; Supplementary Fig. S2). In multivariable analysis, the longer PFS and trend for longer OS in DKK1-high patients was independent of PD-L1 status (Supplementary Fig. S3). PD-L1 CPS was not a predictor for PFS or OS (Supplementary Fig. S4). DKK1 high versus low status was associated with higher odds of clinical benefit/objective response and was also independent of PD-L1 expression (Supplementary Fig. S4). Of the 31 anti-PD-1/PD-L1-naïve patients with GEJ/GC with DKK1 RNAscope data, microsatellite or MMR status was available for 26 patients, and none had evidence of microsatellite instability or deficient MMR (Supplementary Table S4).

Among anti-PD-1/PD-L1-refractory patients with GEJ/GC treated with DKN-01 300 mg + pembrolizumab, DKK1 status was available for 4 of 5 patients. Best overall response was SD for two patients (DKK1 *H*-scores 59 and 75) and PD for two patients (DKK1 *H*-scores 2 and 23). DKN-01 PK and immunogenicity analyses are reported in the Supplementary Fig S5.

Discussion

In this phase I trial, DKN-01 was well tolerated as monotherapy and in combination with pembrolizumab across a heterogenous population of advanced, previously treated patients with EGC. Consistent with the hypothesized mechanisms, encouraging antitumor activity was seen in the DKK1-high biomarker population. One subgroup in particular, anti-PD-1/PD-L1-naïve patients with GEJ/GC with DKK1-high tumoral expression, experienced an ORR of 50% and survival outcomes were longer than in the DKK1-low population. The 21-day cycle of DKN-01 300 mg on Days 1 and 15 with pembrolizumab 200 mg on Day 1 was established as the recommended phase II dose (RP2D).

The reported AEs were consistent with those reported from trials in similar populations and reflect the highly symptomatic nature of advanced EGC (27, 28). The most common drug-related AEs were fatigue, asymptomatic liver function abnormalities, decreased appetite, and anemia. Importantly, there was no evidence of synergistic immune toxicity or infusion reactions.

Table 1. Baseline demographic, disease, and tumor characteristics in patients receiving DKN-01 + pembrolizumab by anti-PD-1/PD-L1 status.

		300 mg DKN-01 + Pembro			
	150 mg DKN-01 + Pembro (*N* = 2)	**Anti-PD-1/ PD-L1-Naïve (*N* = 52)**	**Anti-PD-1/PD-L1-Naïve, GEJ/GC (*N* = 34)**	**Anti-PD-1/PD-L1-Refractory (*N* = 9)**	**All 300 mg DKN-01 + Pembro (*N* = 61)**
Age (years)[a]					
Mean (SD)	68.0 (1.41)	62.8 (12.48)	61.6 (12.36)	61.3 (13.28)	62.6 (12.50)
Min, Max	67, 69	28, 81	28, 80	40, 74	28, 81
Gender					
Male	1 (50.0)	49 (94.2)	31 (91.2)	6 (66.7)	55 (90.2)
Female	1 (50.0)	3 (5.8)	3 (8.8)	3 (33.3)	6 (9.8)
Race					
White	2 (100.0)	48 (92.3)	30 (88.2)	9 (100.0)	57 (93.4)
Asian	0	2 (3.8)	2 (5.9)	0	2 (3.3)
Other	0	2 (3.8)	2 (5.9)	0	2 (3.3)
Type of cancer/histology					
Esophageal	1 (50.0)	18 (34.6)	0	4 (44.4)	22 (36.1)
Squamous cell carcinoma	0	4 (7.7)	0	1 (11.1)	5 (8.2)
Adenocarcinoma	1 (50.0)	14 (26.9)	0	3 (33.3)	17 (27.9)
Gastroesophageal junction adenocarcinoma	1 (50.0)	27 (51.9)	27 (79.4)	5 (55.6)	32 (52.5)
Gastric adenocarcinoma	0	7 (13.5)	7 (20.6)	0	7 (11.5)
Months since diagnosis[b]					
Mean (SD)	51.9 (40.42)	15.8 (12.12)	16.2 (10.72)	27.2 (8.36)	17.5 (12.29)
Min, Max	23, 80	3, 68	3, 52	18, 42	3, 68
Disease stage at diagnosis					
Stage I	0	2 (3.8)	0	0	2 (3.3)
Stage II	1 (50.0)	6 (11.5)	2 (5.9)	0	6 (9.8)
Stage III	0	5 (9.6)	3 (8.8)	2 (22.2)	7 (11.5)
Stage IV	1 (50.0)	39 (75.0)	29 (85.3)	7 (77.8)	46 (75.4)
No. of prior systemic regimens					
Mean (SD)	3.0 (0.00)	1.9 (0.92)	2.0 (1.00)	3.6 (0.88)	2.1 (1.09)
Median	3.0	2.0	2.0	4.0	2.0
Min, Max	3, 3	1, 5	1, 5	2, 5	1, 5
Type of prior systemic therapy					
Chemotherapy	2 (100.0)	52 (100.0)	34 (100.0)	9 (100.0)	61 (100.0)
5-Fluoruracil	2 (100.0)	49 (94.2)	34 (100.0)	9 (100.0)	58 (95.1)
Platinum	2 (100.0)	52 (100.0)	34 (100.0)	9 (100.0)	61 (100.0)
Taxane	1 (50.0)	32 (61.5)	19 (55.9)	9 (100.0)	41 (67.2)
Trastuzumab	0	13 (25.0)	10 (29.4)	2 (22.2)	15 (24.6)
PD-1/PD L1 inhibitor	1 (50.0)	0	0	9 (100.0)	9 (14.8)
Ramucirumab	1 (50.0)	16 (30.8)	12 (35.3)	7 (77.8)	23 (37.7)
Tumor PD-L1: CPS, *n* (%)					
CPS < 1 (negative)	1 (50.0)	15 (28.8)	7 (20.6)	3 (33.3)	18 (29.5)
CPS ≥ 1 to <10 (positive, low)	0	18 (34.6)	13 (38.2)	4 (44.4)	22 (36.1)
CPS ≥ 10 (positive, high)	1 (50.0)	12 (23.1)	7 (20.6)	1 (11.1)	13 (21.3)
Missing	0	7 (13.5)	7 (20.6)	1 (11.1)	8 (13.1)
Microsatellite status (MSS), *n* (%)					
MSS/pMMR	1 (50.0)	43 (82.7)	28 (82.4)	3 (33.3)	46 (75.4)
MSI-H/dMMR	1 (50.0)	0	0	0	0
Unknown	0	9 (17.3)	6 (17.6)	6 (66.7)	15 (24.6)
DKK1 RNAScope *H*-score	*n* = 2	*n* = 49	*n* = 31	*n* = 8	*N* = 57
Mean (SD)	147.0 (35.36)	45.4 (56.33)	46.7 (58.47)	49.8 (81.07)	46.0 (59.53)
Min, Max	122, 172	0, 210	0, 210	0, 237	0, 237

Abbreviations: dMMR, deficient mismatch repair; pMMR, proficient mismatch repair.
[a]Age at time of informed consent.
[b]Time since disease diagnosis is the number of months between the date of initial diagnosis and date of first study treatment.

Although clinical activity of DKN-01 300 mg + pembrolizumab in this anti-PD-1/PD-L1-naïve, unselected, heterogenous population is modest at 11.4% ORR, this study did not include patients treated at the RP2D and predicted to have higher ICI response (MSI-H/deficient MMR). PD-L1 status has limitations as a predictive biomarker (spatial and temporal heterogeneity; ref. 29) in EGC, and rare responses (ORR ∼5%–6%) occur in PD-L1-negative patients. Preliminary data from our study suggest tumoral DKK1 expression is a predictive response biomarker for DKN-01-based therapy independent of known ICI response biomarkers. DKK1 expression is known to carry a poor

Table 2. Treatment-emergent adverse events occurring in 5% or more patients receiving DKN-01 + pembrolizumab by anti-PD-1/PD-L1 status.

		300 mg DKN-01+ Pembro		
Preferred term	**150 mg DKN-01 + Pembro (*N* = 2)**	**Anti-PD-1/PD-L1-Naïve (*N* = 52)**	**Anti-PD-1/PD-L1-Refractory (*N* = 9)**	**All 300 mg DKN-01 + Pembro (*N* = 61)**
Any treatment-emergent AE with overall incidence >5%	2 (100.0)	51 (98.1)	7 (77.8)	58 (95.1)
Fatigue	1 (50.0)	30 (57.7)	2 (22.2)	32 (52.5)
Anemia	1 (50.0)	16 (30.8)	5 (55.6)	21 (34.4)
Blood alkaline phosphatase increased	1 (50.0)	17 (32.7)	4 (44.4)	21 (34.4)
Hyponatraemia	0	16 (30.8)	3 (33.3)	19 (31.1)
Aspartate aminotransferase increased	0	16 (30.8)	2 (22.2)	18 (29.5)
Decreased appetite	1 (50.0)	15 (28.8)	2 (22.2)	17 (27.9)
Hypoalbuminemia	1 (50.0)	15 (28.8)	1 (11.1)	16 (26.2)
Abdominal pain	1 (50.0)	11 (21.2)	1 (11.1)	12 (19.7)
Alanine aminotransferase increased	0	11 (21.2)	2 (22.2)	13 (21.3)
Constipation	1 (50.0)	8 (15.4)	4 (44.4)	12 (19.7)
Diarrhea	1 (50.0)	10 (19.2)	2 (22.2)	12 (19.7)
Dyspnea	0	10 (19.2)	1 (11.1)	11 (18.0)
Nausea	0	10 (19.2)	1 (11.1)	11 (18.0)
Hypertension	0	8 (15.4)	2 (22.2)	10 (16.4)
Back pain	0	9 (17.3)	0	9 (14.8)
Oedema peripheral	0	8 (15.4)	1 (11.1)	9 (14.8)
Pyrexia	0	7 (13.5)	2 (22.2)	9 (14.8)
Abdominal distension	1 (50.0)	5 (9.6)	2 (22.2)	7 (11.5)
Dehydration	0	8 (15.4)	0	8 (13.1)
Dizziness	0	7 (13.5)	1 (11.1)	8 (13.1)
Vomiting	0	6 (11.5)	2 (22.2)	8 (13.1)
Arthralgia	0	6 (11.5)	1 (11.1)	7 (11.5)
Dysphagia	0	3 (5.8)	4 (44.4)	7 (11.5)
Hyperbilirubinemia	1 (50.0)	6 (11.5)	0	6 (9.8)
Hypokalemia	0	6 (11.5)	1 (11.1)	7 (11.5)
Hypophosphatemia	0	7 (13.5)	0	7 (11.5)
Hypocalcemia	0	5 (9.6)	1 (11.1)	6 (9.8)
Musculoskeletal pain	0	4 (7.7)	2 (22.2)	6 (9.8)
Myalgia	1 (50.0)	5 (9.6)	0	5 (8.2)
Proteinuria	0	5 (9.6)	1 (11.1)	6 (9.8)
Weight decreased	0	5 (9.6)	1 (11.1)	6 (9.8)
Cough	0	4 (7.7)	1 (11.1)	5 (8.2)
Dyspnea exertional	0	4 (7.7)	1 (11.1)	5 (8.2)
Hyperglycemia	0	5 (9.6)	0	5 (8.2)
Insomnia	0	4 (7.7)	1 (11.1)	5 (8.2)
Muscular weakness	0	4 (7.7)	1 (11.1)	5 (8.2)
Thrombocytopenia	0	4 (7.7)	1 (11.1)	5 (8.2)
Ascites	1 (50.0)	3 (5.8)	0	3 (4.9)
Asthenia	0	4 (7.7)	0	4 6.6)
Blood creatinine increased	0	4 (7.7)	0	4 (6.6)
Dry skin	0	3 (5.8)	1 (11.1)	4 (6.6)
Fall	0	3 (5.8)	1 (11.1)	4 (6.6)
Gastroesophageal reflux disease	0	4 (7.7)	0	4 (6.6)
Hypotension	0	4 (7.7)	0	4 (6.6)
Influenza like illness	0	4 (7.7)	0	4 (6.6)
Noncardiac chest pain	0	4 (7.7)	0	4 (6.6)
Oral candidiasis	0	3 (5.8)	1 (11.1)	4 (6.6)

prognosis (12), and the lack of interaction with PD-L1 suggests it is highly unlikely DKK1 expression is simply marking a more ICI-sensitive population. Although all responders had DKK1-high tumors, two DKK1-high patients did not respond to therapy. Importantly, unlike MSI-high or EBV positivity, which are uncommon in GEJ/GC, approximately one-third of anti-PD-1/PL-L1-naïve patients with GEJ/GC in our study were considered DKK1-high (i.e., *H*-score >35; refs. 30, 31).

RNAscope is a highly sensitive and specific expression technique that overcomes antibody reagent limitations common with IHC. RNAscope was recently utilized to identify patients with elevated FGFR mRNA expression in a phase I dose-escalation trial for an orally available inhibitor of FGFR1–4 kinase activity, and the DKK1 RNAscope assay has been validated as a laboratory developed test for the prospective screening of patient tumoral tissue (32, 33). Our findings suggest that DKK1 expression assessed by RNAscope CISH

Table 3. Clinical response in patients receiving DKN-01 + pembrolizumab.

		300 mg DKN-01 + Pembro			
	150 mg DKN-01 + Pembro (*N* = 2)	**Anti-PD-1/PD-L1-Naïve (*N* = 50)**	**Anti-PD-1/PD-L1-Naïve, GEJ/GC (*N* = 32)**	**Anti-PD-1/PD-L1-Refractory (*N* = 9)**	**All 300 mg DKN-01 + Pembro (*N* = 59)**
Best overall response, *n* (%)					
Complete response (CR)	0	0	0	0	0
Confirmed CR	0	0	0	0	0
Partial response (PR)	0	5 (10.0)	5 (15.6)	0	5 (8.5)
Confirmed PR	0	4 (8.0)	4 (12.5)	0	4 (6.8)
Stable disease (SD)	1 (50.0)	12 (24.0)	8 (25.0)	4 (44.4)	16 (27.1)
Progressive disease (PD)	1 (50.0)	27 (54.0)	14 (43.8)	5 (55.6)	32 (54.2)
Not evaluable (NE)	0	0	0	0	0
Not done/missing	0	6 (12.0)	5 (15.6)	0	6 (10.2)
Objective disease response[a]	0	5 (11.4)	5 (18.5)	0	5 (9.4)
95% CI[b]	(0.0–84.2)	(3.8–24.6)	(6.3–38.1)	(0.0–33.6)	(3.1–20.7)
Confirmed objective disease response[a,c]	0	4 (9.1)	4 (14.8)	0	4 (7.5)
95% CI[b]	(0.0–84.2)	(2.5–21.7)	(4.2–33.7)	(0.0–33.6)	(2.1–18.2)
Objective disease control[d]	1 (50.0)	17 (38.6)	13 (48.1)	4 (44.4)	21 (39.6)
95% CI[b]	(1.3–98.7)	(24.4–54.5)	(28.7–68.1)	(13.7–78.8)	(26.5–54.0)
Confirmed objective disease control[c,d]	1 (50.0)	15 (34.1)	11 (40.7)	3 (33.3)	18 (34.0)
95% CI[b]	(1.3–98.7)	(20.5–49.9)	(22.4–61.2)	(7.5–70.1)	(21.5–48.3)
Median duration of response, weeks	NA	23.9	23.9	NA	23.9
95% CI[b]	NA	(6.7, NA)	(6.7, NA)	NA	(6.7, NA)

Abbreviation: NA, not applicable.
[a]Objective disease response is defined as the number of patients with a BOR of CR or PR divided by the number of patients with an evaluable posttreatment response.
[b]95% CI is calculated on the basis of the exact Clopper–Pearson formula for binomial proportions.
[c]Requires a confirmed response. Confirmed CR is defined as an overall response finding of CR followed by a subsequent overall response finding of CR at least 4 weeks later. Confirmed PR is an overall response finding of either PR followed by a subsequent overall response finding of PR or CR at least 4 weeks later or CR followed by a subsequent overall response finding of PR at least 4 weeks later. For BOR of SD, confirmation is defined as SD duration of at least 6 weeks.
[d]Objective disease control rate is defined as the number of patients with a best overall response of CR, PR, or SD divided by the number of patients with an evaluable posttreatment response.

Table 4. PFS and OS in patients receiving DKN-01 + pembrolizumab.

		300 mg DKN-01 + Pembro			
	150 mg DKN-01 + Pembro (*N* = 2)	**Anti-PD-1/PD-L1-Naïve (*N* = 52)**	**Anti-PD-1/PD-L1 Naïve, GEJ/GC (*N* = 34)**	**Anti-PD-1/PD-L1-Refractory (*N* = 9)**	**All 300 mg DKN-01 + Pembro (*N* = 61)**
PFS (weeks)[a], *n*	2	52	34	9	61
Median (95% CI)	15.7	6.0	6.9	6.6	6.0
95% CI for median	3.1–28.3	5.7–8.7	5.7–12.0	3.0–12.1	5.9–10.0
Q1, Q3	3.1, 28.3	5.3, 18.3	5.4, 22.1	6.0, 12.1	5.3, 13.4
Min, Max	3.1, 28.3	0.1, 54.1	0.1, 54.1	3.0, 13.4	0.1, 54.1
Number of events	2 (100.0)	45 (86.5)	27 (79.4)	8 (88.9)	53 (86.9)
Number of censored events	0	7 (13.5)	7 (20.6)	1 (11.1)	8 (13.1)
Probability of survival (SE) by time[b]					
6 months	0.50 (0.354)	0.19 (0.058)	0.25 (0.080)	0.00	0.17 (0.052)
12 months	0.00	0.04 (0.036)	0.10 (0.063)	0.00	0.04 (0.032)
OS (weeks)[a], *n*	2	52	34	9	61
Median (95% CI)	NA	20.4	22.1	19.0	20.4
95% CI for median	7.1–NA	14.4–31.6	14.4–42.4	10.7–37.4	16.0–28.6
Q1, Q3	7.1, NA	11.0, 43.7	11.1, 43.7	13.4, 37.4	11.1, 43.7
Min, Max	7.1, 84.9	2.0, 63.0	2.0, 63.0	10.7, 53.9	2.0, 63.0
Number of events	1 (50.0)	37 (71.2)	25 (73.5)	8 (88.9)	45 (73.8)
Number of censored events	1 (50.0)	15 (28.8)	9 (26.5)	1 (11.1)	16 (26.2)
Probability of survival (SE) by time[b]					
6 months	0.50 (0.354)	0.39 (0.070)	0.41 (0.087)	0.44 (0.166)	0.40 (0.065)
12 months	0.50 (0.354)	0.25 (0.068)	0.24 (0.084)	0.15 (0.133)	0.23 (0.061)

Abbreviation: NA, not applicable.
[a]Estimates based on Kaplan–Meier methodology.
[b]SEs computed using Greenwood formula.

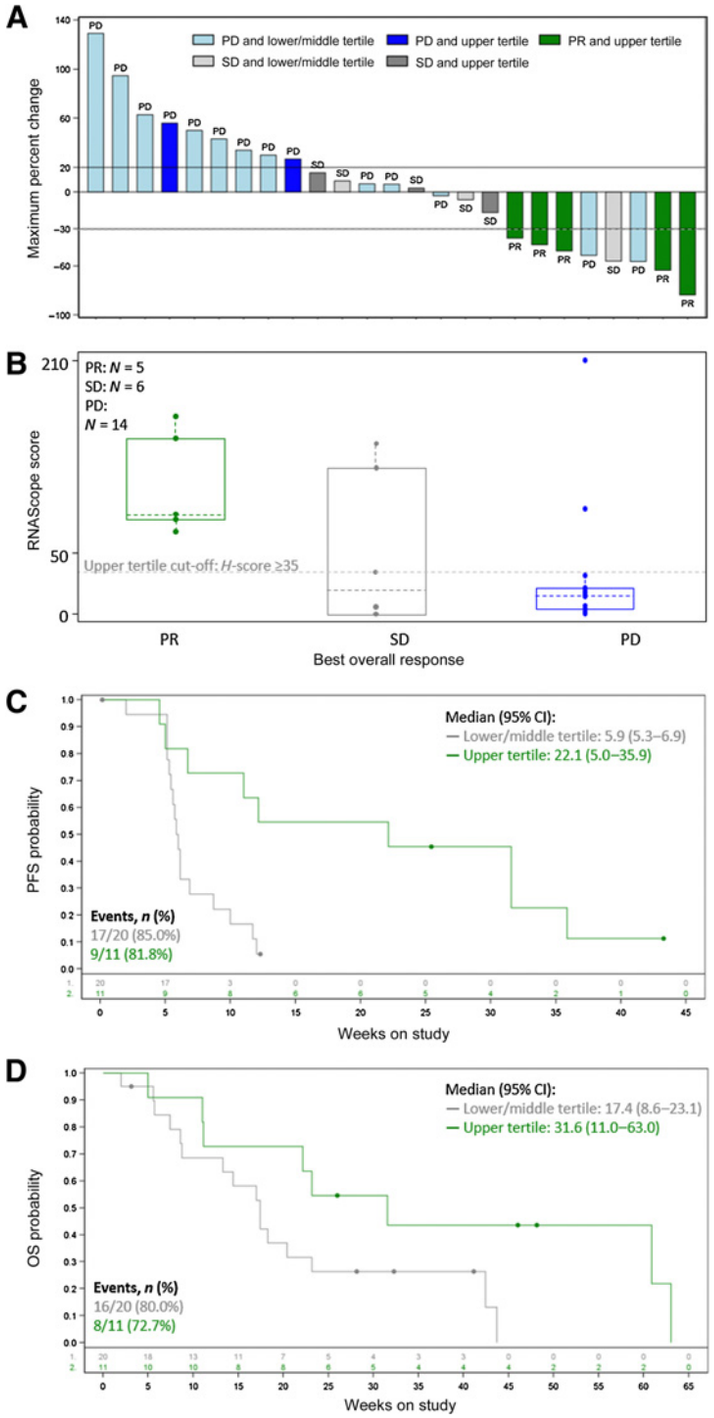

Figure 2.

Outcomes for DKN-01 + pembrolizumab in patients with GEJ/GC who are anti-PD-1/PD-L1-naïve by DKK1 RNAscope *H*-score among patients who had tumoral DKK1 mRNA expression available. Best overall response in the response-evaluable population ($n = 25$) (**A**, **B**). Kaplan–Meier estimates of PFS (**C**) and OS (**D**) in the safety analysis population ($N = 31$). Upper tertile defined as ≥35 DKK1 *H*-score derived from the safety analysis population ($N = 31$).

potentially has substantial discriminatory ability in identifying patients more likely to benefit from DKN-01-based therapies.

This small, phase I, nonrandomized study was not powered to examine clinical efficacy. Furthermore, the exploratory analysis of DKK1 expression and clinical outcomes was conducted retrospectively and lacked a validation set, and it was not possible to distinguish between prognostic and predictive biomarkers. Currently, a phase II second-line trial of DKN-01 in combination with the anti-PD-1 antibody tislelizumab ± chemotherapy (DisTinGuish) is ongoing to validate antitumor activity in the context of elevated DKK1 expression (NCT04363801). Evaluating DKN-01 in this setting will be important, given the significantly improved OS when the immune checkpoint inhibitor, nivolumab, was given with chemotherapy in the CheckMate 649 trial (34, 35). Importantly, analyses from CheckMate-649 suggest a

lesser magnitude of benefit in PD-L1 CPS <5 ($n = 606$; HR = 0.94 for OS) and this may be an area where additional agents like DKN-01 could improve benefit.

In summary, the novel DKK1-neutralizing IgG4 antibody, DKN-01, was safe and tolerable in combination with pembrolizumab in patients with advanced EGC. Common AEs were manageable, with no evidence of enhanced immune-related toxicity. Durable antitumor activity was correlated with elevated tumoral DKK1 expression in patients with GEJ/GC naïve to anti-PD-1/PD-L1 therapy. The ORR and PFS in this subgroup warrant further investigation of DKN-01 in combination with anti-PD-1 agents in biomarker-enriched EGC populations.

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Data Availability Statement

Disclosures provided by the authors are available with this article at DOI 10.1158/1535-7163.MCT-21-0273. Data underlying the findings described in this article may be obtained in accordance with the Leap Therapeutics data sharing policy described at https://www.leaptx.com.

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S.J. Klempner: Data curation, validation, investigation, visualization, writing–original draft, writing–review and editing. **J.C. Bendell:** Investigation, visualization, writing–review and editing. **V.M. Villaflor:** Investigation, visualization, writing–review and editing. **L.L. Tenner:** Investigation, visualization, writing–review and editing. **S.M. Stein:** Validation, investigation, writing–review and editing. **J.B. Rottman:** Formal analysis, investigation, visualization, writing–review and editing. **G.S. Naik:** Conceptualization, resources, data curation, software, formal analysis, validation, investigation, visualization, methodology, writing–review and editing. **C.A. Sirard:** Conceptualization, resources, data curation, formal analysis, supervision, funding acquisition, validation, investigation, visualization, writing–original draft, project administration, writing–review and editing. **M.H. Kagey:** Conceptualization, resources, data curation, formal analysis, validation, investigation, visualization, methodology, writing–original draft, writing–review and editing. **M.F. Chaney:** Investigation, visualization, writing–review and editing. **J.H. Strickler:** Data curation, validation, investigation, visualization, writing–original draft, writing–review and editing.

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