KN026, a Bispecific Anti-HER2 Antibody, Combined with KN046, an Anti-CTLA-4/PD-L1 Antibody, in Patients with HER2-positive Advanced Gastrointestinal Cancer: a multicenter, open-label, nonrandomized, phase Ib study

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Abstract

Background

Anti-HER2 antibodies combined with immune checkpoint inhibitors exert synergistic effects on innate and adaptive immunity. This phase Ib study assessed the safety and efficacy of KN026 plus KN046 in patients with HER2-positive gastrointestinal cancer.

Methods

Treatment naïve or pretreated locally advanced or metastasis, gastrointestinal cancer patients with HER2 alterations were enrolled. Four dose combinations of KN026 (20 mg/kg Q2W or 30 mg/kg Q3w) and KN046 (3 mg/kg Q2w or 5 mg/kg Q2w/Q3w) were designed. The primary objective was to assess dose-limiting toxicity (DLT) and preliminary efficacy.

Results

Overall, 47 patients were enrolled. No DLTs were observed. Any-grade treatment-related adverse events (TRAEs) were observed in 42 patients (89.4%). Anemia (40.4%), infusion-related reactions (36.2%), and elevated alanine transaminase (ALT) (27.7%) were the most common TRAEs. Grade 3 or worse TRAEs occurred in 17% of the patients. Twenty-four of the 47 patients suffered immune-related adverse events (irAEs). Rash (14.9%), hypothyroidism (6.4%) and interstitial lung disease (6.4%) were most commonly observed. Forty of 47 patients were included in efficacy assessment. The objective response rates (ORRs) in the patients with HER2-positive gastric cancer and gastroesophageal junction (GC/GEJ) adenocarcinoma in the first-line and late-line settings were 71.4% and 37.5%, respectively. No disease remission was observed in the patients with low HER2 expression or HER2 mutation. Notably, half of the patients (2/4) pretreated with trastuzumab and a programmed cell death-1 (PD-1) blockade achieved partial response (PR).

Conclusions and Relevance

: KN026 combined with KN046 exhibited acceptable safety profiles and encouraging efficacy in patients with HER2-positive gastrointestinal cancer.

Trial registration:

ClinicalTrials.gov, NCT NCT04040699, Registered August 1,2019.

Background

Overexpression or amplification of HER2 has been validated as an essential therapeutic target for HER2-positive gastric cancer and gastroesophageal junction (GC/GEJ) adenocarcinoma (1, 2). Trastuzumab, fam-trastuzumab deruxtecan-nxki (DS-8201a), and disitamab vedotin (RC48-ADC) have been approved as treatments for locally advanced or metastatic HER2-positive GC/GEJ cancer (3–6). In a phase I/II study of RC48-ADC, tumor shrinkage was also observed in patients with HER2 2+/FISH- disease, suggesting that these patients might benefit from anti-HER2 therapy (7, 8). Studies of both preclinical and clinical models suggest that the combination of anti-HER2 antibodies and immune checkpoint inhibitors provides coordinated engagement of innate and adaptive immunity (9–12). Several clinical trials have explored the antitumor activities of treatments targeting HER2 and checkpoint inhibitors with or without chemotherapy (13–17). These results indicated that HER2-targeting agents combined with immunotherapy are a promising option for HER2-positive GC/GEJ cancer.

KN026 is a bispecific anti-HER2 antibody that simultaneously recognizes the two distinct HER2 epitopes, extracellular domains II and IV (ECD2 and ECD4), showing antitumor activities across a range of HER2 expression levels (18, 19). The recommended phase II dose (RP2D) of KN026 (KN026-CHN-001, NCT03619681) is either 20 mg/kg every 2 weeks (Q2w) or 30 mg/kg every 3 weeks (Q3w) (18). KN046 is designed to target the tumor microenvironment by blocking both PD-L1 and CTLA-4. In phase 1 study, dose escalation was conducted in dose levels of 1mg/kg Q2w, 3mg/kg Q2w, 5mg/kg or 300mg Q3w, 5mg/kg Q2w. The RP2D of KN046 was 5mg/kg Q2w. And anti-tumor activity was observed in dose levels of 3 or 5mg/kg Q2w, 5mg/kg Q3w and 300mg Q3w (20, 21).

Based on previous clinical findings, we conducted a phase Ib study to assess the safety, tolerability, and antitumor activity of KN026 combined with KN046 in both pretreated and treatment-naïve patients with HER2-positive gastrointestinal cancer.

Methods

Patient eligibility

Patients (aged ≥ 18 years) with pathologically confirmed locally advanced or metastatic gastrointestinal cancers and HER2 alterations (low expression, overexpression, mutation, or amplification) who were pretreated or treatment naïve were considered. Patients were eligible if they had measurable lesions (as defined by RECIST V1.1), an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, adequate organ and bone marrow function, and life expectancy ≥ 3 months were eligible for enrollment. Previous treatment with any anti-HER2 or anti-PD-L1/CTLA-4 agent was also permitted if any developed toxicities had returned to grade 1 or baseline.

Patients were excluded if they had a history of autoimmune diseases; active brain or meningeal metastases; previous allogeneic bone marrow, stem cell or solid organ transplantation; ongoing infection; and a left ventricular ejection fraction (LVEF) < 45% or a reduction of 15% after previous anti-HER2 therapy. All patients provided written informed consent before participation.

Study design and treatment

This multicenter, open-label, nonrandomized, phase Ib, dose-exploration study was conducted in centers in China from Nov. 2019 to Aug. 2021(ClinicalTrials. gov, NCT04040699). The study was approved by the ethics committee of each study center. And this study was performed in accordance with the International Conference on Harmonisation Guidelines for Good Clinical Practice and Declaration of Helsinki. All authors had access to the study data and reviewed and approved the final manuscript.

The primary endpoint was to assess dose-limiting toxicity (DLT). Secondary endpoints included 6-month and 12-month progression-free survival (PFS) rates, objective response rates (ORRs), 6-month and 12-month overall survival (OS) rates and biomarkers (HER2 and PD-L1 statuses) of efficacy.

In this study, 3–6 eligible patients were treated with KN026 and KN046 in Dose 1. If no DLT was noted, the dosage was increased to the next level, and the number of subjects increased to approximately 20 simultaneously with the approval of the Site Monitoring Committee (SMC) (Fig. A.1). Based on safety, pharmacokinetics and other data from previous dose groups, a modified toxicity probability interval (mTPI-2) model was used to calculate the maximum tolerated dose (MTD).

Dose 1 was suggested as a combination of RP2D for KN026 and KN046 at the lower dosage. Meanwhile, the loading dose of KN026 in Dose 2 and Dose 3 was determined based on a combined analysis of human pharmacokinetics and antitumor activity, indicating that the rapid acquisition of the peak concentration was related to optimal antitumor activity. Subsequently, the loading dose was discarded in Dose 4 since the antitumor activity was comparable, and it was inconvenient for clinical administration. In summary, four dose levels of KN026 and KN046 were proposed: 20 mg/kg KN026 and 3 mg/kg KN046 every 2 weeks (dose 1), 20 mg/kg KN026 every 2 weeks with loading on days 1, 8, and 15 of cycle 1 and

5 mg/kg KN046 every 3 weeks (dose 2), 30 mg/kg KN026 with loading on days 1 and 8 of cycle 1 and 5 mg/kg KN046 every 3 weeks (dose 3), and 20 mg/kg KN026 and 5 mg/kg KN046 every 2 weeks (dose 4). The treatment was continued until the disease progressed, unacceptable toxicity developed, or consent was withdrawn.

Clinical assessment

All adverse events (AEs) were recorded and categorized based on severity (NCI-CTCAE V.4.03) and the relationship with KN026 or KN046. DLTs were assessed based on pre-established criteria listed in the Appendix for 21 days (Q3w) or 28 days (Q2w) after the administration of the first dose.

Imaging assessments were performed at screening and every 8 weeks thereafter. The response was evaluated based on Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 by the investigators. Patients with progressive disease were allowed to continue treatment if the investigator believed they were benefiting from the therapy.

HER2 and PD-L1 statuses

Immunohistochemistry (IHC, HercepTest, Dako, Denmark), fluorescence in situ hybridization (FISH; HER2 FISH pharmDx, Dako) or next-generation sequencing (NGS) were used to test the HER2 status of the tumors by certified pathologists on a local or central basis. Patient samples were eligible if they were IHC-positive (1+, 2+, or 3+), FISH-positive (HER2:CEP17 ratio ≥ 2) or presented HER2 mutations detected using NGS (including HER2 exon 20 insertion, the HER2 in-frame deletion and other nonsynonymous activating mutations reported in the COSMIC database). Patients who were HER2 1 + or 2 + on IHC and FISH-negative or had HER2 mutations were excluded from the KN046-IST-02 protocol v4.0 since no disease remission was observed after the administration of dose 1. Furthermore, wild-type RAS was required for patients with colorectal cancer. The HER2 status was reassessed in those patients who had previously received anti-HER2 therapy. In particular, central confirmation of the HER2 status was not needed only if the patient's test results were available before enrollment.

A combined positive score (CPS) of PD-L1 expression was determined as the number of PD-L1-positive cells (tumor cells, lymphocytes, and macrophages) divided by the total number of tumor cells * 100; at least 100 viable tumor cells needed to be present to evaluate a specimen.

Statistical analyses

Patients who received at least one dose of KN026 or KN046 were included in the safety analysis population. Patients who completed at least one efficacy assessment were included in the efficacy analysis population. Using the Clopper and Pearson method, the ORR and exact 95% confidence interval (95% CI) were determined. The Kaplan–Meier method was used to plot PFS and OS curves, with median and 95% CI values reported for each parameter. Data for analysis were collected up to August 10, 2021. HER2 positivity and PD-L1 CPS ≥ 1 were used as biomarker cutoff points for the efficacy evaluation. Statistical analyses were performed using SAS version 9.4 or GraphPad Prism software.

Results

Study population analysis

From November 6, 2019 to August 10, 2021, a total of 69 patients were screened. There were 47 patients (GC, n = 34; CRC, n = 11; another GI tumors, n = 2) finally enrolled in this study and distributed into 4 dose levels (dose 1, n = 25; dose 2, n = 3; dose 3, n = 14; dose 4, n = 5) (Fig.A.2). Baseline characteristics were showed in Table 1. More than half of the patients (68.1%) were male. The median age was 56 years (range 29–74 years). Thirty-eight (80.9%) patients were diagnosed with IHC 3 + or 2 + and FISH-positive disease, two (4.3%) of whom had IHC 0 + HER2 mutant tumors based on local IHC testing. In addition, we excluded patients with HER2 1 + or 2 + on IHC and FISH-negative or HER2 mutations in the KN046-IST-02 protocol v4.0, as no disease remission occurred in Dose 1 for this population. HER2 positivity (as detected using IHC, FISH, or tissue NGS) was unable to be centrally validated in 10 of 47 patients: 8 due to insufficient tissue for repeat IHC testing

and 2 due to discordance between the local and central IHC results. All patients had proficient DNA mismatch repair (pMMR) and negative results for EBER in situ hybridization (EBER-ISH), except for one patient with HER2 IHC 1 + and EBER-ISH positivity who received one dose of treatment only after multiline therapy due to thoracic vertebral fracture and disease progression.

Table 1
Baseline Demographic and Clinical Characteristics of Patients Receiving KN026 Plus KN046

Characteristic	Dose 1	Dose 2	Dose 3	Dose 4	All
Number	25	3	14	5	47
Median age, years (range)	55 (38–74)	56 (37–67)	56 (29–74)	48 (32–58)	56 (29–74)
Age < 65 years	20 (80.0)	2 (66.7)	12 (85.7)	5 (100.0)	39 (83.0)
Sex					
Male	16 (64.0)	3 (100.0)	9 (64.3)	4 (80.0)	32 (68.1)
Female	9 (36.0)	0	5 (35.7)	1 (20.0)	15 (31.9)
ECOG					
0	3 (12.0)	1 (33.3)	1 (7.1)	1 (20.0)	6 (12.8)
1	22 (88.0)	2 (66.7)	13 (92.9)	4 (80.0)	41 (87.2)
Primary tumor location					
GC					
Proximal	8 (32.0)	1 (33.3)	4 (28.6)	1 (20.0)	14 (29.8)
Distal	10 (40.0)	0 (0.0)	6 (42.9)	2 (40.0)	18 (38.3)
Unknown	0 (0.0)	0 (0.0)	2 (14.3)	0 (0.0)	2 (4.3)
CRC					
Right	0 (0.0)	0 (0.0)	1 (7.1)	0 (0.0)	1 (2.1)
Left	5 (20.0)	2 (66.7)	1 (7.1)	2 (40.0)	10 (21.3)
Other					
Duodenum	1 (4.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.1)
Abdominal cavity	1 (4.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.1)
Liver metastasis					
Yes	14 (56.0)	2 (66.7)	11 (78.6)	3 (60.0)	30 (63.8)
No	11 (44.0)	1 (33.3)	3 (21.4)	2 (40.0)	17 (36.2)
Peritoneal metastasis					
Yes	6 (24.0)	0 (0.0)	0 (0.0)	0 (0.0)	6 (12.8)
No	19 (76.0)	3 (100.0)	14 (100.0)	5 (100.0)	41 (87.2)
Lauren type of GC					

NOTE: Values are presented as numbers and percentages, unless otherwise indicated.

Abbreviations: CPS, combined positive score; FISH, fluorescence in situ hybridization; ECOG, Eastern Cooperative Oncology Group; PD-L1, programmed death ligand-1; PD-1, programmed cell death-1.

[a]Based on local IHC testing.

[b]Two patients (1 patient receiving Dose 1 and 1 patient receiving Dose 3) had high HER2 expression based on local IHC testing but low HER2 expression based on central testing.

Characteristic	Dose 1	Dose 2	Dose 3	Dose 4	All
Intestinal	12 (48.0)	1 (33.3)	6 (42.9)	2 (40.0)	21 (44.7)
Diffuse	2 (8.0)	0 (0.0)	1 (7.1)	0 (0.0)	3 (6.4)
Mixed	1 (4.0)	0 (0.0)	2 (14.3)	1 (20.0)	4 (8.5)
Unknown	3 (12.0)	0 (0.0)	3 (21.4)	0 (0.0)	6 (12.8)
HER2 status[a]					
3+	18 (72.0)[b]	1 (33.3)	8 (57.1)	1 (20.0)	28 (59.6)
2+, FISH+	1 (4.0)	1 (33.3)	4 (28.6)[b]	4 (80.0)	10 (21.3)
2+, FISH-	3 (12.0)	0 (0.0)	1 (7.1)	0 (0.0)	4 (8.5)
1+	1 (4.0)	1 (33.3)	1 (7.1)	0 (0.0)	3 (6.4)
Mutation					
G776V	1 (4.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.1)
L755S	1 (4.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.1)
PD-L1 status					
Negative (CPS < 1)	14 (56.0)	1 (33.3)	5 (35.7)	2 (40.0)	22 (46.8)
Positive (CPS ≥ 1)	6 (24.0)	1 (33.3)	5 (35.7)	2 (40.0)	14 (29.8)
Unknown	5 (20.0)	1 (33.3)	4 (28.6)	1 (20.0)	11 (23.4)
Prior regimens					
0	6 (24.0)	0 (0.0)	1 (7.1)	0 (0.0)	7 (14.9)
1	10 (40.0)	0 (0.0)	1 (7.1)	1 (20.0)	12 (25.5)
≥ 2	9 (36.0)	3 (100.0)	12 (86.7)	4 (80.0)	28 (59.6)
Prior therapies					
Anti-HER2	11 (44.0)	2 (66.7)	7 (50.0)	2 (40.0)	22 (46.8)
Anti-PD-1	3 (12.0)	0 (0.0)	5 (35.7)	3 (60.0)	11 (23.4)
Both	3 (12.0)	0 (0.0)	2 (14.3)	1 (20.0)	6 (12.8)

NOTE: Values are presented as numbers and percentages, unless otherwise indicated.

Abbreviations: CPS, combined positive score; FISH, fluorescence in situ hybridization; ECOG, Eastern Cooperative Oncology Group; PD-L1, programmed death ligand-1; PD-1, programmed cell death-1.

[a]Based on local IHC testing.

[b]Two patients (1 patient receiving Dose 1 and 1 patient receiving Dose 3) had high HER2 expression based on local IHC testing but low HER2 expression based on central testing.

Safety

All enrolled patients (n = 47) who received at least one dose of KN026 or KN046 were included in the safety assessment. No DLTs or grade 4 AEs were observed. Treatment-related adverse events (TRAEs) of any grade occurred in 42 (89.4%) of the 47 patients, commonly including anemia (n = 19, 40.4%), infusion-related reactions (n = 17, 36.2%), elevated ALT levels (n = 13,

27.7%), diarrhea (n = 12, 25.5%) and elevated AST levels (n = 11, 23.4%) (Table 2). Eight patients (17.0%) had grade 3 or worse TRAEs, including anemia (n = 2), thrombocytopenia (n = 1), neutropenia (n = 1), elevated GGT levels (n = 1), endocrine system dysfunction (n = 1), encephalitis (n = 1) and infusion-related reactions (n = 1). In particular, KN046 was more prone to causing severe infusion-related reactions (dose 1, n = 1), although these TRAEs were also commonly observed in patients receiving KN026 treatment (23.4% vs. 19.1%).

Table 2
Common (≥ 5%) TRAEs in Patients Receiving KN026 Plus KN046

	Dose 1 N = 25 (%)		Dose 2 N = 3 (%)		Dose 3 N = 14 (%)		Dose 4 N = 5 (%)		Total N = 47 (%)	
	Grade ≥ 3	All Grades	Grade ≥ 3	All Grades	Grade ≥ 3	All Grades	Grade ≥ 3	All Grades	Grade ≥ 3	All Grades
All	6 (24.0)	25 (100.0)	1 (33.3)	3 (100)	1 (7.1)	11 (78.6)	0 (0.0)	3 (60.0)	8 (17.0)	42 (89.4)
Anemia	1 (4.0)	13 (52.0)	1 (33.3)	2 (66.7)	0 (0.0)	4 (28.6)	0 (0.0)	0 (0.0)	2 (4.3)	19 (40.4)
Infusion-related reaction	1 (4.0)	9 (36.0)	0 (0.0)	1 (33.3)	0 (0.0)	7 (50.0)	0 (0.0)	0 (0.0)	1 (2.1)	17 (36.2)
Elevated ALT level	0 (0.0)	7 (28.0)	0 (0.0)	1 (33.3)	0 (0.0)	5 (35.7)	0 (0.0)	1 (20.0)	0 (0.0)	13 (27.7)
Diarrhea	0 (0.0)	6 (24.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (35.7)	0 (0.0)	1 (20.0)	0 (0.0)	12 (25.5)
Elevated AST level	0 (0.0)	7 (28.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (28.6)	0 (0.0)	0 (0.0)	0 (0.0)	11 (23.4)
Elevated bilirubin level	0 (0.0)	6 (24.0)	0 (0.0)	1 (33.3)	0 (0.0)	3 (21.4)	0 (0.0)	0 (0.0)	0 (0.0)	10 (21.3)
Rash	0 (0.0)	8 (32.0)	0 (0.0)	1 (33.3)	0 (0.0)	1 (7.1)	0 (0.0)	0 (0.0)	0 (0.0)	10 (21.3)
Leukopenia	0 (0.0)	6 (24.0)	0 (0.0)	1 (33.3)	0 (0.0)	1 (7.1)	0 (0.0)	0 (0.0)	0 (0.0)	8 (17.0)
Neutropenia	1 (4.0)	5 (20.0)	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.1)	6 (12.8)
Thrombocytopenia	1 (4.0)	6 (24.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.1)	6 (12.8)
Proteinuria	0 (0.0)	4 (16.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (7.1)	0 (0.0)	0 (0.0)	0 (0.0)	5 (10.6)
Hypothyroidism	0 (0.0)	4 (16.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	24 (8.5)
Elevated TSH level	0 (0.0)	3 (12.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (6.4)
Hypokalemia	0 (0.0)	2 (8.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (7.1)	0 (0.0)	0 (0.0)	0 (0.0)	3 (6.4)
Interstitial lung disease	0 (0.0)	2 (8.0)	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (6.4)
Pyrexia	0 (0.0)	1 (4.0)	0 (0.0)	1 (33.3)	0 (0.0)	1 (7.1)	0 (0.0)	0 (0.0)	0 (0.0)	3 (6.4)
Thyroid dysfunction	0 (0.0)	2 (8.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (20.0)	0 (0.0)	3 (6.4)

Note: Grade 5 TRAEs occurred in 1 patient receiving Dose 1 with pulmonary arterial hypertension. No grade 4 TRAEs were observed in the study.

Abbreviations: ALT, alanine transaminase; AST, aspartate aminotransferase; TSH, thyroid-stimulating hormone.

Treatment-related serious adverse events (SAEs) occurred in 7 patients (5 receiving dose 1 and 1 each receiving dose 2 and dose 3) within a median of 1.26 months (range 0.5–7.6 months) after the start of treatment. One treatment-related death was observed among patients receiving Dose 1 at 1.4 months due to pulmonary arterial hypertension. Seven treatment interruptions and 3 treatment discontinuations were associated with KN026; similarly, 12 treatment interruptions and 5 treatment discontinuations were associated with KN046. Three patients discontinued both KN026 and KN046 because of TRAEs.

More than half of the patients (n = 24, 51.1%) developed immune-related adverse events (irAEs). The most common irAEs were rash (n = 7, 14.9%), hypothyroidism (n = 3, 6.4%) and interstitial lung disease (n = 3, 6.4%) (Table A.1, online only). Two patients receiving Dose 1 developed grade 3 irAEs: endocrine damage (n = 1) and encephalitis (n = 1).

Efficacy

By August 10, 2021, the median follow-up was 9.5 months (95% CI: 7.7–11.3). Nine patients (19.1%) had died, 33 patients (70.2%) had discontinued treatment mainly due to disease progression (38.3%), and 14 patients (29.8%) remained in the study. The median treatment duration for KN026 was 4.5 months (range, 0.8 to 15.7 months) and that for KN046 was 4.4 months (range, 0.8 to 15.5 months).

A total of 47 patients were enrolled and treated with at least one dosage of KN026 and KN046. In seven patients, efficacy was not assessed due to disease progression (n = 4) and treatment-emergent adverse events (n = 3). Shrinkage of target lesions compared with the baseline value was observed in 26 patients (65.0%), including 14 patients with confirmed PRs and 1 patient with an unconfirmed PR (Fig. 1–3). In the first-line group, the ORR was 71.4% (95% CI: 29.0-96.3), and the disease control rate (DCR) was 85.7% (95% CI: 42.1–99.6). The median progression-free survival (mPFS) was 10.9 months (95% CI: 0.9-not estimable (NE)), while the median overall survival (mOS) was not reached.

In the late-line group, the ORR was 30.3% (95% CI: 15.6–48.7), and the DCR was 75.8% (95% CI: 57.7–88.9), with a median duration of response of 6.6 months (95% CI 4.1-NE). The mPFS was 4.4 months (95% CI: 3.6–6.9), and the 6-month PFS rate was 37.5% (95% CI 20.6–54.4), while the 12-month OS rate was 68.5% (95% CI: 47.0-82.7). Among pretreated patients with GC/GEJ cancer, the ORR was 27.3% (95% CI: 10.7–50.2), the DCR was 63.6% (95% CI: 40.7–82.8), and the mPFS was 3.6 months (95% CI: 1.7–8.3) (Table 3).

Table 3
Clinical Activity Outcomes in the Evaluable Population Stratified by Tumor Type, HER2 Status and PD-L1 Status

	First line	Late line			
	GC/GEJ	GC/GEJ	CRC	Non-GC/CRC	Total
	N = 7 (%)	N = 22 (%)	N = 9 (%)	N = 2 (%)	N = 33 (%)
Best overall response					
CR	0	0	0	0	0
PR	5 (71.4)	6 (27.3)	1 (11.1)	2 (100.0)	9 (27.3)
uPR	0	0	1 (11.1)	0	1 (3.0)
SD	1 (14.3)	8 (36.4)	7 (77.8)	0	15 (45.5)
PD	1 (14.3)	8 (36.4)	0	0	8 (24.2)
ORR% (95% CI)	71.4 (29.0, 96.3)	27.3 (10.7, 50.2)	22.2 (2.8, 60.0)	100 (15.8, 100.0)	30.3 (15.6, 48.7)
DCR% (95% CI)	85.7 (42.1, 99.6)	63.6 (40.7, 82.8)	100 (66.4, 100.0)	100 (15.8, 100.0)	75.8 (57.7, 88.9)
DOR, months					
Median (95% CI)	9.9 (NE, NE)	8.7 (4.2, NE)	NE (NE, NE)	4.1 (4.1, NE)	6.6 (4.1, NE)
HER2 status[a]	7	16	7	2	25
Positive					
ORR% (95% CI)	71.4 (29.0, 96.3)	37.5 (15.2, 64.6)	28.6 (3.7,71.0)	100 (15.8, 100.0)	40.0 (21.1, 61.3)
DCR% (95% CI)	85.7 (42.1, 99.6)	75.0 (47.6, 92.7)	100 (59.0, 100.0)	100 (15.8, 100.0)	84.0 (63.9, 95.5)
DOR, months (95% CI)	9.9 (NE, NE)	8.7 (4.2, NE)	NE (NE, NE)	4.1 (4.1, NE)	6.6 (4.1, NE)
Low expression	0	6	0	0	6
ORR% (95% CI)	-	0 (0.0, 45.9)	-	-	0 (0.0, 45.9)
DCR% (95% CI)	-	33.3 (4.3, 77.7)	-	-	33.3 (4.3, 77.7)
DOR, months (95% CI)	-	-	-	-	-
Mutation	0	0	2	0	2
ORR% (95% CI)	-	-	0 (0.0, 84.2)	-	0 (0.0, 84.2)

[a]HER2 positivity was defined as IHC 3 + or IHC 2 + and ISH-positive disease; low HER2 expression was defined as IHC 1 + or IHC 2 + and ISH-negative disease; HER2 mutation types included G776V and L755S.

[b]The PD-L1 CPS was not available for 11 patients due to insufficient tissue, including 7 evaluable patients.

Abbreviations: CPS, combined positive score; CR, complete response; DCR, disease control rate; DOR, duration of response; NE, not estimable; ORR, objective response rate; PD, progressive disease; PR, partial response; SD, stable disease; uPR, unconfirmed partial response.

	First line	Late line			
	GC/GEJ	GC/GEJ	CRC	Non-GC/CRC	Total
	N = 7 (%)	N = 22 (%)	N = 9 (%)	N = 2 (%)	N = 33 (%)
DCR% (95% CI)	-	-	100 (15.8, 100.0)	-	100 (15.8, 100.0)
DOR, months (95% CI)	-	-	-	-	-
PD-L1 status[b]					
Positive (CPS ≥ 1)	4	6	0	2	8
ORR% (95% CI)	100 (39.8, 100.0)	33.3 (4.3, 77.7)	-	100 (15.8, 100.0)	50.0 (15.7, 84.3)
DCR% (95% CI)	100 (39.8, 100.0)	50.0 (11.8, 88.2)	-	100 (15.8, 100.0)	62.5 (24.5, 91.5)
DOR, months (95% CI)	9.9 (NE, NE)	NE (NE, NE)	-	4.1 (4.1, NE)	4.1 (4.1, NE)
Negative (CPS < 1)	2	12	6	0	18
ORR% (95% CI)	50.0 (1.3, 98.7)	25.0 (5.5, 57.2)	16.7 (0.4, 64.1)	-	22.2 (6.4, 47.6)
DCR% (95% CI)	50.0 (1.3, 98.7)	66.7 (34.9, 90.1)	100 (54.1, 100.0)	-	77.8 (52.4, 93.6)
DOR, months (95% CI)	NE (NE, NE)	10.8 (4.2, NE)	NE (NE, NE)	-	10.8 (4.2, NE)
[a]HER2 positivity was defined as IHC 3 + or IHC 2 + and ISH-positive disease; low HER2 expression was defined as IHC 1 + or IHC 2 + and ISH-negative disease; HER2 mutation types included G776V and L755S.					
[b]The PD-L1 CPS was not available for 11 patients due to insufficient tissue, including 7 evaluable patients.					
Abbreviations: CPS, combined positive score; CR, complete response; DCR, disease control rate; DOR, duration of response; NE, not estimable; ORR, objective response rate; PD, progressive disease; PR, partial response; SD, stable disease; uPR, unconfirmed partial response.					

Forty patients (85.1%) received multiline treatments, with 22 receiving Herceptin, 11 receiving a programmed cell death-1 (PD-1) blocker, and 6 receiving both regimens. Among the 18 patients evaluated, the ORR was 44.4% (95% CI: 21.5–69.2), and the DCR was 88.9% (95% CI: 65.3–98.6) following Herceptin treatment. Notably, four assessable patients had previously received both Herceptin and anti-PD-1 antibodies, and two of these patients achieved a PR with an ORR of 50.0% (95% CI: 6.8–93.2) and a mPFS of 4.4 months (95% CI: 1.6-NE).

Regarding the different dose groups, the ORR values were 35.3% (95% CI: 14.2–61.7), 100% (95% CI: 2.5–100), 16.7% (95% CI: 2.1–48.4), and 33.3% (95% CI: 0.8–90.6) for dose 1, dose 2, dose 3, and dose 4, respectively, in accordance with RECIST v1.1 (Table A.2).

HER2 status of tumors

A central reassessment of HER2 positivity was conducted in 39 of the 47 enrolled patients, and the results were consistent with the local results obtained for those patients pretreated with anti-HER2 therapy. Two patients (1 patient who received dose 1 and 1 patient who received dose 3) presented high HER2 expression upon local IHC testing but low HER2 expression based on the central results. Antitumor activity was analyzed based on the local HER2 status.

No disease remission was observed in the patients with low HER2 expression or the patients with HER2 mutations, who had DCRs of 33.3% (95% CI: 4.3–77.7) and 100% (95% CI: 15.8–100.0), respectively. Nevertheless, the ORRs were 37.5% (95% CI: 15.2–64.6) and 71.4% (95% CI: 29.0-96.3) for HER2-positive patients treated in the late- and first-line settings, respectively. Moreover, HER2-positive pretreated patients with GC/GEJ cancer experienced a longer PFS than patients with low HER2 expression (median, 4.4 vs. 1.7 months, $p < 0.05$, Fig. A.3), with ORRs of 37.5% (95% CI: 15.2–64.6) and 0% (95% CI: 0.0-45.9), respectively. Among the patients with HER2-positive colorectal cancer, the ORR was 28.6% (95% CI: 3.7–71.0), with a mPFS of 4.8 months (95% CI: 3.4-NE) (Table 3).

PD-L1 expression in tumors

In addition to the analysis of the HER2 status, the PD-L1 CPS was also calculated to determine its relevance to efficacy. PD-L1-positive (CPS ≥ 1) samples (29.8%) and PD-L1-negative (CPS < 1) samples (46.8%) were identified by performing IHC staining with the 22C3 antibody. The PD-L1 CPS was not available for 11 patients because of insufficient tissue. The ORR was 50.0% (95% CI: 15.7–84.3) in PD-L1-positive patients compared to 22.2% (95% CI: 6.4–47.6) in PD-L1-negative pretreated patients, with mPFS values of 5.8 months (95% CI: 0.3-NE) and 3.9 months (95% CI: 3.4–6.2), respectively, but the difference in mPFS was not statistically significant ($p = 0.679$) (Table 3).

The patients were divided into four groups based on the PD-L1 CPS and HER2 status: HER2 + PD-L1+, HER2 + PD-L1-, HER2- PD-L1-, and HER2-PD-L1+ (11, 14, 6 and 1 evaluable patients, respectively). The results of the efficacy analyses of ORR and PFS after stratification by HER2 and PD-L1 status are shown in Tables A.3 and A.4.

Discussion

HER2 is overexpressed in various types of cancer, such as breast, gastroesophageal, colorectal, urothelial, and biliary cancer (22). The introduction of HER2-targeted therapy for patients with breast and gastric cancer presenting HER2 amplification or overexpression has led to remarkable improvements in clinical outcomes (23, 24). Nevertheless, trastuzumab has been shown to increase both PD-1 and PD-L1 expression levels in clinical and preclinical models, suggesting that anti-HER2 therapies combined with immune checkpoint inhibitors might enhance the antitumor activity (25, 26). This study explored the safety and preliminary efficacy of the HER2-targeted bispecific antibody KN026 and the anti-PD-L1/CTLA4 bispecific antibody KN046 in patients with HER2-positive advanced gastrointestinal cancers.

KN026 combined with KN046 showed an acceptable safety profile, and no DLTs were observed. In general, adverse reactions were similar across dose cohorts. Anemia (40.4%), infusion-related reactions (36.2%), elevated alanine transaminase (ALT)/aspartate aminotransferase (AST) levels (27.7%/23.4%) and diarrhea (25.5%) were the most common TRAEs. Grade 3 TRAEs mainly included hematological toxicities, such as anemia, thrombocytopenia, and neutropenia, with an incidence of less than 5%. The most common irAEs were rash (14.9%), hypothyroidism (6.4%) and interstitial lung disease (6.4%), which were all manageable.

The AE spectrum and incidence were similar to those patients who received KN026 or KN046 monotherapy, respectively. Notably, infusion-related reactions were more common in paients treated with the combined regimen (36.2% compared with 12.7% for KN026 monotherapy and 13.8% for KN046 monotherapy) (18, 21), which led to premedication administration. One patient in the Dose 1 cohort died from treatment-related pulmonary arterial hypertension 1.4 months after the first treatment. Further studies are needed to investigate the relationship between pulmonary arterial hypertension and medications. Certain risks associated with immunotherapy, such as myocarditis (2.1%) and encephalitis (2.1%), should be explored. Dose 3 was selected for further investigation in the dose expansion phase of the ongoing study considering the convenience of administration to ensure an optimal balance between activity and safety.

KN026 combined with KN046 also displayed promising efficacy, with an ORR of 30.3% (95% CI: 15.6,48.7) and DCR of 75.8% (95% CI: 57.7–88.9) for pretreated patients. Among patients with GC/GEJ cancer, the ORR was 37.5% (95% CI: 15.2–64.6), and the mPFS was 4.4 months (95% CI: 1.7–12.6) for HER2-positive patients with GC/GEJ treated in the late-line setting

compared with 18.48% (95% CI: 11.15–27.93) for patients receiving pembrolizumab and margetuximab therapy (13), indicating an additional synergistic effect. Of the 7 patients who received first-line treatment in our study, 5 patients (71.4%) achieved an objective response with a mPFS of 10.9 months (95% CI: 0.9-NE), and OS was not reached. Based on the ToGA study, the standard first-line treatment for patients with HER2-overexpressing GC/GEJ is chemotherapy combined with trastuzumab, which resulted in an ORR of 47% and a mOS of 13.8 months (3). The ORR increased to 74.4% when pembrolizumab was added in combination according to the results of the KEYNOTE-811 study (27). These encouraging results support the standard first-line regimen in the development of chemo-free therapy, but biomarkers that clarify the population remain to be discovered. Importantly, antitumor activity was also observed in patients previously treated with either trastuzumab, an anti-PD-1 antibody, or both. Following Herceptin treatment, the ORR was 44.4% (95% CI: 21.5–69.2), and two of four patients who had previously received both trastuzumab and a PD-1 blocker achieved PR, suggesting the advantage of this regimen as a late-line treatment.

Furthermore, HER2 status was a strong biomarker of the efficacy of bispecific antibody therapy, as no disease remission was observed in the population with low HER2 expression or HER2 mutation, suggesting that only HER2 protein overexpression predicts the efficacy of KN026. In the present study, the PD-L1-positive patients did not show any survival benefit compared with their PD-L1-negative counterparts possibly because a relatively small number of these patients was included.

This study is limited by the lack of more specific efficacy biomarkers and the identification of resistance mechanisms. Efforts should be made to identify the population who will benefit most from this therapy.

In conclusion, the regimen combining KN026 and KN046 produced impressive response rates in patients with HER2-positive GC/GEJ receiving both first-line and later-line therapy. These promising results warrant further investigation in patients with HER2-positive gastroesophageal cancers.

Abbreviations

PD-L1, programmed death ligand-1; PD-1, programmed cell death-1; ALT, alanine transaminase; AST, aspartate aminotransferase; TSH, thyroid-stimulating hormone; CPS, combined positive score; CR, complete response; DCR, disease control rate; DOR, duration of response; NE, not estimable; ORR, objective response rate; PD, progressive disease; PR, partial response; SD, stable disease; uPR, unconfirmed partial response; FISH, fluorescence in situ hybridization; ECOG, Eastern Cooperative Oncology Group.

Declarations

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Author contributions

Conceptualization Ideas: All authors

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Formal analysis: All authors

Funding acquisition: Lin Shen

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Data sharing statement

The protocol of study is available in the appendix. All data generated or analyzed during this study will be considered for sharing after the product and indication has been approved by major health authorities. Data may be request 24 months after study completion. Qualified researchers should submit a proposal to the corresponding author outlining the reasons for requiring the data. The sponsor will provide the data if the proposal is approved, provided that the requestor signs a data–access agreement. Use of data must comply with the requirements of Human Genetics Resources Administration of China and other country or region–specific regulations.

Ethics approval and consent to participate

The study was performed in accordance with the Declaration of Helsinki and the Good Clinical Practice guidelines. The protocol and all amendments were approved by the ethics committee at each site, and all patients provided written informed consent.

Consent for publication

Not applicable.

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Tai W, Mahato R, Cheng K. The role of HER2 in cancer therapy and targeted drug delivery. J Control Release 2010;146:264–75.
2. Yan M, Parker BA, Schwab R, Kurzrock R. HER2 aberrations in cancer: implications for therapy. Cancer Treat Rev 2014;40:770–80.
3. Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, *et al.* Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. Lancet 2010;376:687–97.

4. FDA. FDA approves fam-trastuzumab deruxtecan-nxki for HER2-positive gastric adenocarcinomas. Available at https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-fam-trastuzumab-deruxtecan-nxki-her2-positive-gastric-adenocarcinomas. Accessed January 15, 2021.
5. Shitara K, Bang YJ, Iwasa S, Sugimoto N, Ryu MH, Sakai D, *et al.* Trastuzumab deruxtecan in previously treated HER2-positive gastric cancer. N Engl J Med 2020;382:2419–30.
6. Deeks ED. Disitamab vedotin: first approval. Drugs 2021;81:1929–35.
7. Xu Y, Wang Y, Gong J, Zhang X, Peng Z, Sheng X, *et al.* Phase I study of the recombinant humanized anti-HER2 monoclonal antibody-MMAE conjugate RC48-ADC in patients with HER2-positive advanced solid tumors. Gastric Cancer 2021;24:913–25.
8. Peng Z, Liu T, Wei J, Wang A, He Y, Yang L, *et al.* Efficacy and safety of a novel anti-HER2 therapeutic antibody RC48 in patients with HER2-overexpressing, locally advanced or metastatic gastric or gastroesophageal junction cancer: a single-arm phase II study. Cancer Commun (Lond) 2021;41:1173–82.
9. Bianchini G, Gianni L. The immune system and response to HER2-targeted treatment in breast cancer. Lancet Oncol 2014;15:e58–68.
10. Park S, Jiang Z, Mortenson ED, Deng L, Radkevich-Brown O, Yang X, *et al.* The therapeutic effect of anti-HER2/neu antibody depends on both innate and adaptive immunity. Cancer Cell 2010;18:160–70.
11. Müller P, Kreuzaler M, Khan T, Thommen DS, Martin K, Glatz K, *et al.* Trastuzumab emtansine (T-DM1) renders HER2 + breast cancer highly susceptible to CTLA-4/PD-1 blockade. Sci Transl Med 2015;7:315ra188.
12. Chaganty BKR, Qiu S, Gest A, Lu Y, Ivan C, Calin GA, *et al.* Trastuzumab upregulates PD-L1 as a potential mechanism of trastuzumab resistance through engagement of immune effector cells and stimulation of IFNγ secretion. Cancer Lett 2018;430:47–56.
13. Catenacci DVT, Kang YK, Park H, Uronis HE, Lee KW, Ng MCH, *et al.* Margetuximab plus pembrolizumab in patients with previously treated, HER2-positive gastro-oesophageal adenocarcinoma (CP-MGAH22-05): a single-arm, phase 1b-2 trial. Lancet Oncol 2020;21:1066–76.
14. Janjigian YY, Maron SB, Chatila WK, Millang B, Chavan SS, Alterman C, *et al.* First-line pembrolizumab and trastuzumab in HER2-positive oesophageal, gastric, or gastro-oesophageal junction cancer: an open-label, single-arm, phase 2 trial. Lancet Oncol 2020;21:821–31.
15. Stein A, Paschold L, Tintelnot J, Goekkurt E, Thuss-Patience PC, Lorenzen S, *et al.* LBA54 Ipilimumab or FOLFOX in combination with nivolumab and trastuzumab in previously untreated HER2 positive locally advanced or metastatic esophagogastric adenocarcinoma (EGA): results of the randomized phase II INTEGA trial (AIO STO 0217). Ann Oncol 2021;32:S1331.
16. Rha SY, Lee C-K, Kim HS, Kang B, Jung M, Kwon WS, *et al.* A multi-institutional phase Ib/II trial of first-line triplet regimen (Pembrolizumab, Trastuzumab, Chemotherapy) for HER2-positive advanced gastric and gastroesophageal junction cancer (PANTHERA Trial): Molecular profiling and clinical update. J Clin Oncol 2021;39:218–18.
17. Lee MS, Chao J, Mulcahy MF, Kasi PM, Alistar AT, Mukherjee S, *et al.* Abstract CT174: phase II study of avelumab and trastuzumab with FOLFOX chemotherapy in previously untreated HER2-amplified metastatic gastroesophageal adenocarcinoma. Cancer Res 2021;81:CT174.
18. Zhang J, Ji D, Cai L, Yao H, Yan M, Wang X, *et al.* First-in-human HER2-targeted bispecific antibody KN026 for the treatment of patients with HER2-positive metastatic breast cancer: results from a phase I study. Clin Cancer Res 2022;28:618–28.
19. Xu J, Zhang Y, Wu J, Xu N, Ying J, Xiang X, *et al.* The preliminary efficacy of KN026 (Anti-HER2 BsAb) in advanced gastric and gastroesophageal junction cancer patients with HER2 expression. J Clin Oncol 2021;39:e16005–05.
20. Coward J, Ganju V, Behzadigohar R, Kwong K, Xu J, Van H, *et al.* Preliminary safety, efficacy, and pharmacokinetics (PK) results of KN046 (bispecific anti-PD-L1/CTLA4) from a first-in-human study in subjects with advanced solid tumors. J Clin Oncol 2019;37:2554–54.

21. Zhao H, Ma Y, Zhang Y, Hong S, Yang Y, Fang W, *et al.* The preliminary efficacy and safety data of KN046 in patients failed on prior immune checkpoint inhibitors therapy. J Clin Oncol 2020;38:3020–20.
22. AACR Project GENIE Consortium. AACR project GENIE: powering precision medicine through an international consortium. Cancer Discov 2017;7:818–31.
23. Loibl S, Poortmans P, Morrow M, Denkert C, Curigliano G. Breast cancer. Lancet 2021;397:1750–69.
24. Zhu Y, Zhu X, Wei X, Tang C, Zhang W. HER2-targeted therapies in gastric cancer. Biochim Biophys Acta Rev Cancer 2021;1876:188549.
25. Varadan V, Gilmore H, Miskimen KL, Tuck D, Parsai S, Awadallah A, *et al.* Immune signatures following single dose trastuzumab predict pathologic response to preoperativetrastuzumab and chemotherapy in HER2-positive early breast cancer. Clin Cancer Res 2016;22:3249–59.
26. Yamashita K, Iwatsuki M, Yasuda-Yoshihara N, Morinaga T, Nakao Y, Harada K, *et al.* Trastuzumab upregulates programmed death ligand-1 expression through interaction with NK cells in gastric cancer. Br J Cancer 2021;124:595–603.
27. Janjigian YY, Kawazoe A, Yañez P, Li N, Lonardi S, Kolesnik O, *et al.* The KEYNOTE-811 trial of dual PD-1 and HER2 blockade in HER2-positive gastric cancer. Nature 2021;600:727–30.

Figures

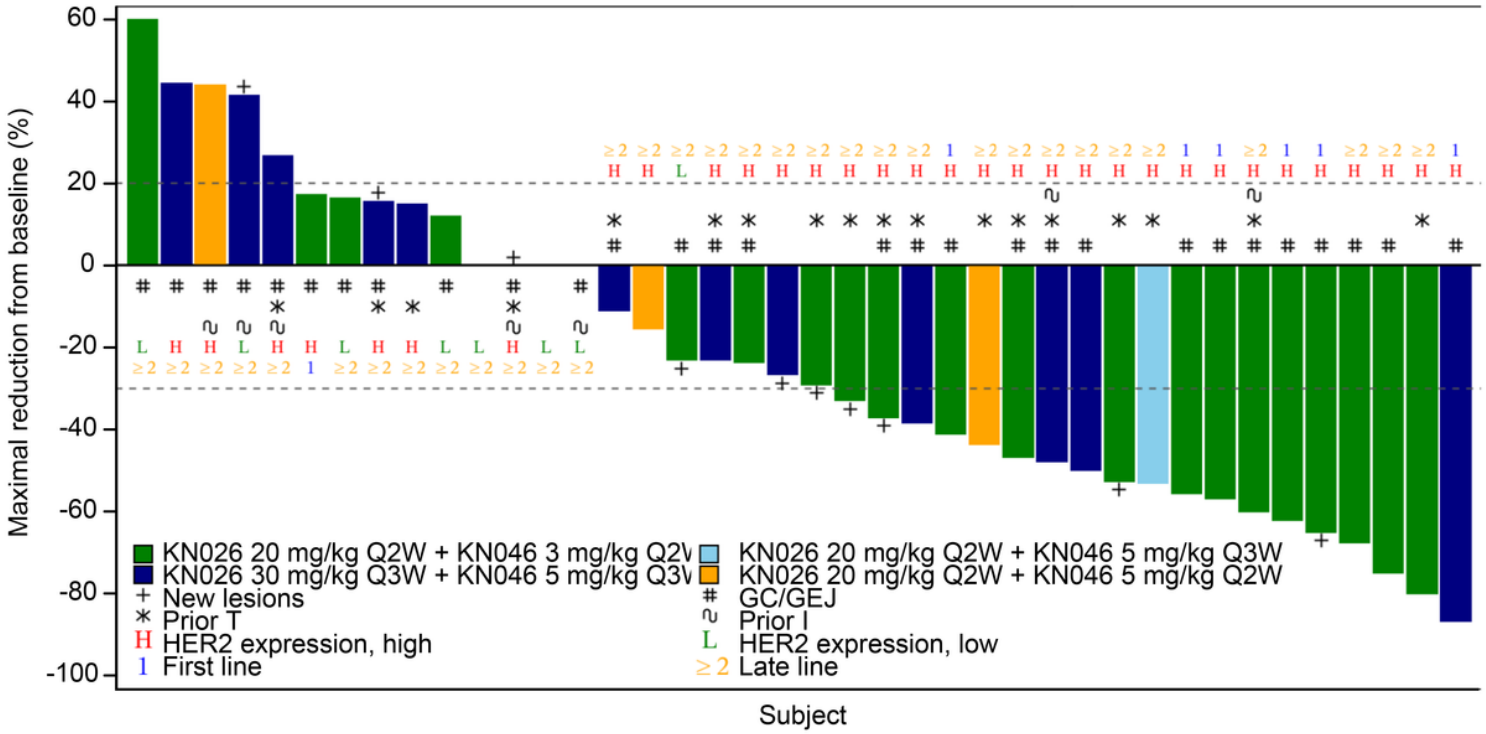

Figure 1

Maximal change in tumor size from baseline assessed by the investigators according to RECIST version 1.1 (N=40). The length of the bar represents the maximal decrease or minimal increase in the size of the target lesion(s).

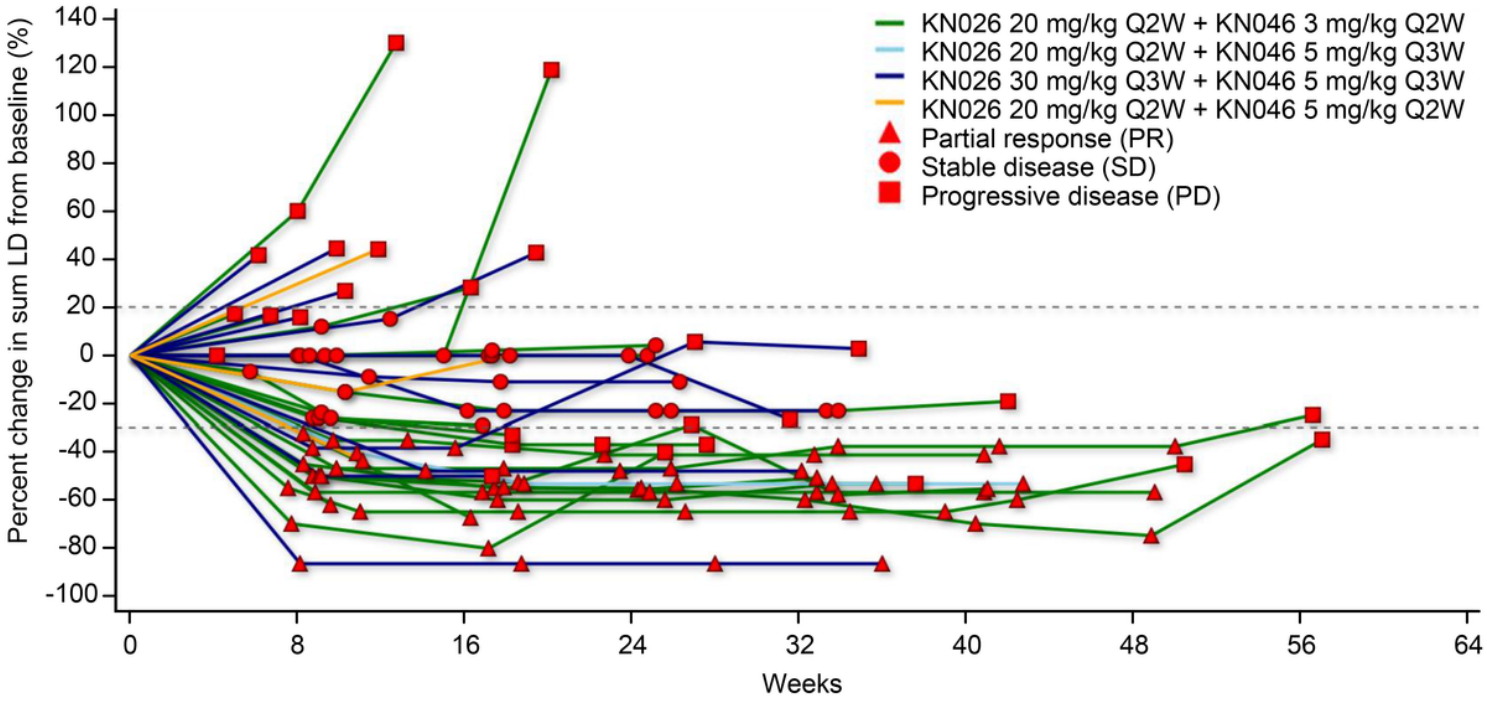

Figure 2

Change in individual tumor burden over time from baseline assessed by the investigators according to RECIST version 1.1 (N = 40).

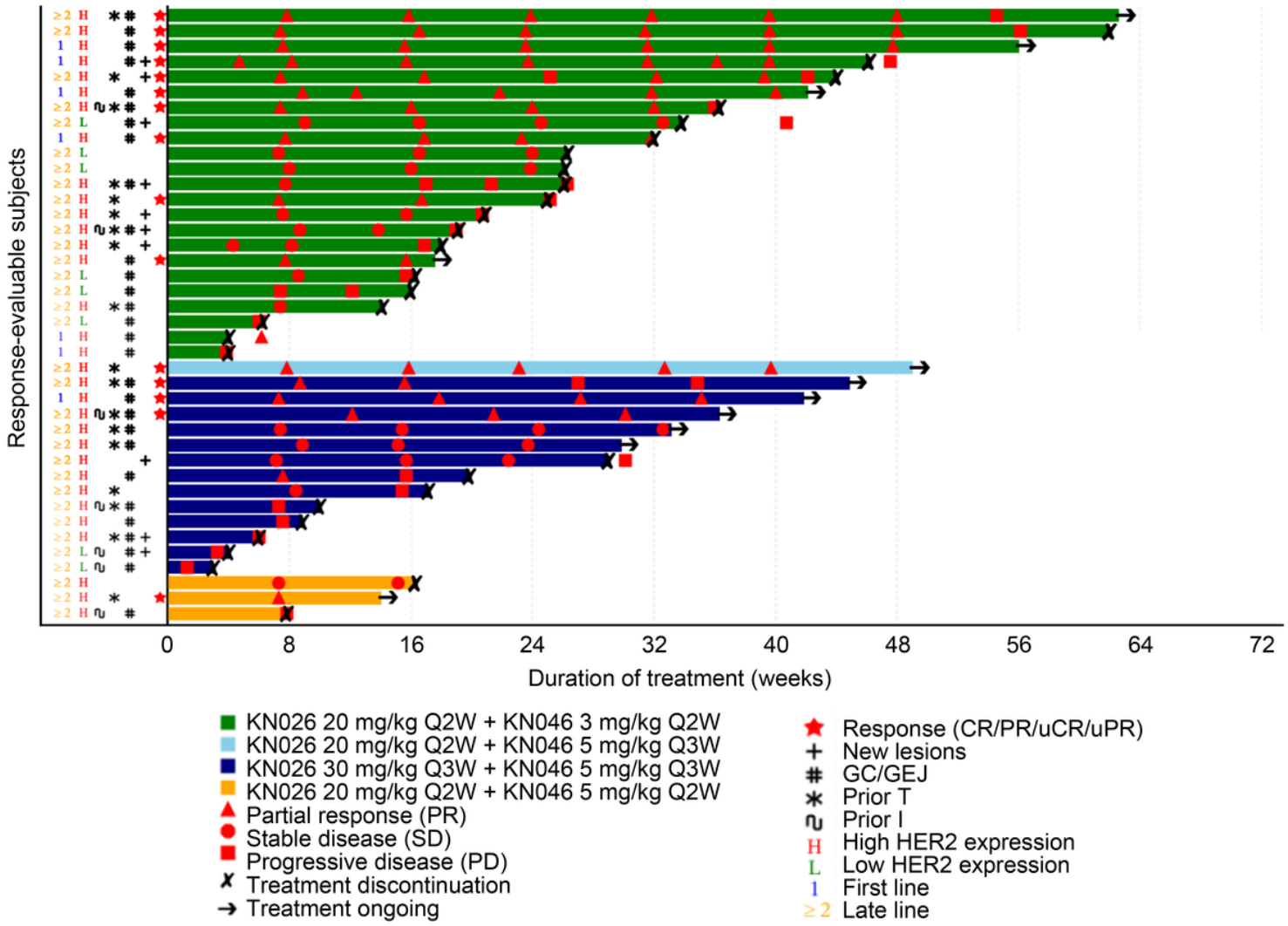

Figure 3

Exposure and duration of response according to RECIST version 1.1 (N = 40).

Supplementary Files

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- SupplementaryAppendix20220417.docx