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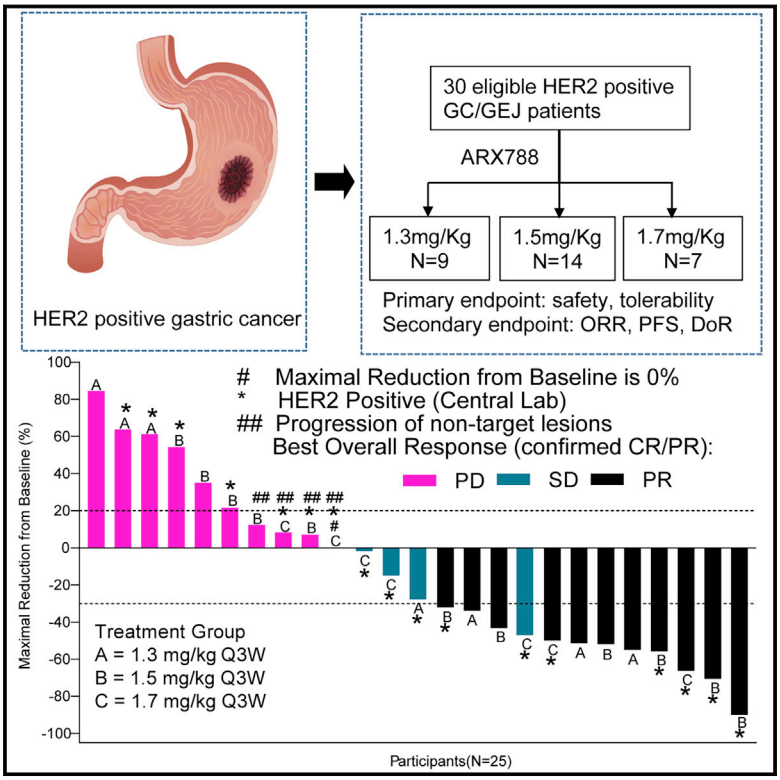
Phase 1 multicenter, dose-expansion study of ARX788 as monotherapy in HER2-positive advanced gastric and gastroesophageal junction adenocarcinoma

Graphical abstract



Highlights

- ARX788 is an anti-HER2 antibody-drug conjugate with AS269 as cytotoxic payload
- ARX788 is well tolerated and has promising anti-tumor activity in this phase 1b study
- Mutation rate of *ERBB2* was higher in the responders than in the nonresponders

Authors

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In brief

Zhang et al. demonstrate the safety, efficacy, and survival of an anti-HER2 antibody-drug conjugate, ARX788, in patients with HER2-positive advanced gastric adenocarcinoma. They show that ARX788 is well tolerated and has promising anti-tumor activities for HER2-positive advanced gastric adenocarcinoma.

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Phase 1 multicenter, dose-expansion study of ARX788 as monotherapy in HER2-positive advanced gastric and gastroesophageal junction adenocarcinoma

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SUMMARY

ARX788 is an anti-human epidermal growth factor receptor 2 (HER2) antibody-drug conjugate with AS269 as cytotoxic payload. In this phase 1 multicenter dose-expansion clinical trial, patients with HER2-positive advanced gastric/gastroesophageal junction adenocarcinoma failing to respond to prior trastuzumab-based standard treatment were enrolled. Between July 15th, 2019, and March 14th, 2022, 30 participants were enrolled. Twenty-eight (93.3%) patients experienced at least one drug-related adverse event (AE) and 13.3% experienced grade 3 ARX788-related AEs. The confirmed objective response rate is 37.9% (95% confidence interval [CI]: 20.7%–57.7%) and the disease control rate is 55.2% (95% CI: 35.7%–73.6%). With a median follow up of 10 months, the median progression-free survival and overall survival are 4.1 (95% CI: 1.4–6.4) and 10.7 months (95% CI: 4.8–not reached), respectively. The median duration of response is 8.4 (95% CI: 2.1–18.9) months. ARX788 is well tolerated and has promising anti-tumor activity in patients with HER2-positive advanced gastric adenocarcinoma (ChinaDrugTrials.org.cn: CTR20190639).

INTRODUCTION

Gastric cancer (GC) has high incidence in Eastern Asia,[1] especially in China.[2] According to the China National Cancer Center, GC was responsible for 403,000 new cases and 291,000 deaths in 2015, representing the third most common cancer in both incidence and mortality in China.[3] The frequency of human epidermal growth factor receptor 2 (HER2)-positive GC varies from 7.3% to 20.2% [4–6] and is about 13% in Chinese population.[7] HER2-positive GC was associated with poor prognosis.[8]

The ToGA trial showed that trastuzumab plus chemotherapy improved the overall survival (OS; median, 13.8 versus 11.1 months) and progression-free survival (PFS; median, 6.7 versus 5.5 months) of patients with HER2-positive gastric/gastroesophageal junction (G/GEJ) adenocarcinoma compared with chemotherapy alone.[9] These promising findings made trastuzumab plus chemotherapy the standard first-line treatment for advanced HER2-positive G/GEJ adenocarcinoma.[8–11] However, there is still no standard recommended anti-HER2 agent for patients with HER2-positive GC in second-line settings. Other anti-HER2 therapies, such as lapatinib or antibody-drug conjugate (ADC) trastuzumab-emtansine (T-DM1), showed no significant benefit as second-line chemotherapy.[12,13] Trastuzumab deruxtecan, a HER2-targeted ADC, is approved for third-line treatment in HER2-positive GC due to its significant improvement of the objective response rate (ORR) and OS in

Table 1. Patient demographics and baseline characteristics

Characteristic	1.3 mg/kg Q3W (n = 9)	1.5 mg/kg Q3W (n = 14)	1.7 mg/kg Q3W (n = 7)	Total (n = 30)
Median age, years (range)	61 (36–72)	55.5 (26–68)	52 (50–68)	57 (26–72)
Sex (%)				
Male	8 (88.9)	8 (57.1)	6 (85.7)	22 (73.3)
Female	1 (11.1)	6 (42.9)	1 (14.3)	8 (26.7)
ECOG performance status (%)				
0	2 (22.2)	3 (21.4)	3 (42.9)	8 (26.7)
1	7 (77.8)	11 (78.6)	4 (57.1)	22 (73.3)
Primary site (%)				
Stomach	5 (55.6)	12 (85.7)	5 (71.4)	22 (73.3)
Gastroesophageal junction	4 (44.4)	2 (14.3)	2 (28.6)	8 (26.7)
Lauren classification	4	11	4	19
Intestinal	4 (44.4)	6 (42.9)	2 (28.6)	12 (40)
Mixed	0	2 (14.3)	2 (28.6)	4 (13.3)
Diffuse	0	3 (21.4)	0	3 (10)
HER2 status (%)				
IHC 1+ FISH+	0	1 (7.1)	0	1 (3.3)
IHC 2+ FISH+	2 (22.2)	4 (28.6)	1 (14.3)	7 (23.4)
IHC 3+	7 (77.8)	9 (64.3)	5 (71.4)	21 (70)
Unknown	0	0	1 (14.3)	1 (3.3)
FISH status (%)				
Positive	2 (22.2)	7 (50)	4 (57.1)	13 (43.3)
Unknown	7 (77.8)	7 (50)	3 (42.9)	17 (56.7)
Burden of target tumor lesion (cm)				
Median (range)	3.8 (2.2–10.7)	6.3 (2.0–13.0)	2.3 (1.5–12.1)	5.4 (1.5–13)
Metastatic site (%)				
Lymph node	7 (77.8)	9 (64.3)	6 (85.7)	22 (73.3)
Liver	4 (44.4)	9 (64.3)	4 (57.1)	17 (56.7)
Bone	4 (44.4)	4 (28.6)	0	8 (26.7)
Lung	1 (11.1)	4 (28.6)	1 (14.3)	6 (20)
Peritoneum	0	4 (28.6)	2 (28.6)	6 (20)
Adrenal gland	0	2 (14.3)	2 (28.6)	4 (13.3)
Esophagus	1 (11.1)	0	1 (14.3)	2 (6.7)
Pleura	1 (11.1)	1 (7.1)	0	2 (6.7)
Brian	0	1 (7.1)	0	1 (3.3)
Others	3 (33.3)	4 (28.6)	1 (14.3)	8 (26.7)
Number of metastatic sites (%)				
1	1 (11.1)	0	2 (28.6)	3 (10)
2	5 (55.6)	4 (28.6)	1 (14.3)	10 (33.3)
>2	3 (33.3)	10 (71.4)	4 (57.1)	17 (56.7)
Number of lesions (%)				
<3	2 (22.2)	3 (21.4)	2 (28.6)	7 (23.3)
≥3	7 (77.8)	11 (78.6)	5 (71.4)	23 (76.7)
Number of previous lines of therapy (%)				
1	7 (77.8)	7 (50)	4 (57.1)	18 (60)
≥2	2 (22.2)	7 (50)	3 (42.9)	12 (40)
Previous trastuzumab treatment (%)				
Yes	7 (77.8)	13 (92.9)	7 (100)	27 (90)
No	2 (22.2)	1 (7.1)	0	3 (10)

(*Continued on next page*)

Table 1. *Continued*

Characteristic	1.3 mg/kg Q3W (n = 9)	1.5 mg/kg Q3W (n = 14)	1.7 mg/kg Q3W (n = 7)	Total (n = 30)
Adjuvant/neoadjuvant	2 (22.2)	4 (28.6)	2 (28.6)	8 (26.7)
Previous chemotherapy regimen (%)				
Platinum/fluorouracil	9 (100)	14 (100)	7 (100)	30 (100)
Taxanes	3 (33.3)	2 (14.3)	3 (42.9)	8 (26.7)
Irinotecan	1 (11.1)	1 (7.1)	1 (14.3)	3 (10)

IHC, immunohistochemistry; FISH, fluorescence in situ hybridization.

patients with HER2-positive GC compared with traditional chemotherapy.[14] Another HER2-targeting ADC, RC-48, which is approved for third-line treatment in China, demonstrated an ORR of 24.8%.[15] However, the observed incidences of adverse events (AEs) related to trastuzumab deruxtecan and RC-48 was pretty high, especially for the decrease of neutrophil count (51% of grade 3 or higher for trastuzumab deruxtecan).[14,15] Therefore, it is still necessary to develop a safe and effective HER2-targeting therapy for the unmet clinical needs.

ARX788 is an ADC consisting of HER2-targeted monoclonal antibody (mAb) conjugated with the AS269 cytotoxic payload, a highly potent tubulin inhibitor that inhibits cancer cell growth.[16] A preclinical study showed that ARX788 was effective in T-DM1-resistant models of HER2-positive breast cancer and GC both *in vitro* and *in vivo*.[16] In a phase 1 study of ARX788 at a dose of 1.5 mg/kg (ChinaDrugTrials.org.cn: CTR20171162), the ORR for heavily pretreated patients with HER2-positive advanced breast cancer was 65.5% (19/29 participants), with a median PFS of 17 months.[17] The frequency of grade 3 or above AEs related to ARX788 was only 11.6%, including 2.9% of interstitial lung disease (ILD)/pneumonitis.[17] Grade 3 or above hematologic toxicities that are frequently observed in other HER2-targeting ADCs[18,19], only accounted for 1.4% of all AEs in ARX788.

Based on the encouraging results of the CTR20171162 trial, we initiated this phase 1 study (ChinaDrugTrials.org.cn: CTR20190639) to evaluate the safety, tolerability, and preliminary efficacy of ARX788 in patients with HER2-positive advanced G/GEJ adenocarcinoma.

RESULTS

Patients and treatments

From July 15, 2019, to January 6, 2021, a total of 30 participants were enrolled into this trial. Their baseline characteristics are shown in Table 1. There were 22 males (73.3%) and 8 females (26.7%), with a median age of 57 (range 26–72) years. Twenty-two (73.3%) had gastric adenocarcinoma, and the rest (26.7%) had GEJ adenocarcinoma. Twenty-seven patients (90%) underwent prior trastuzumab-containing therapy, eight of whom progressed within 6 months in the adjuvant or neoadjuvant phase. Twelve (40%) were treated with 2 or more lines of therapy (2–6) and eight of whom had 3 or more lines. All patients were treated with platinum-based and fluorouracil regimens, and eight with taxanes and three with irinotecan. Most participants (73.3%) had an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 1. All of them had at least one dose of ARX788, of whom 9 patients received 1.3 mg/kg, 14 received 1.5 mg/kg, and 7 received 1.7 mg/kg ARX788. The median treatment duration was 3.5 (range: 1–29) cycles.

Safety analysis

Among all 30 participants who received at least one dose of ARX788, 28 (93.3%) experienced at least one drug-related AE, mostly categorized as grade 1 to 2 (Table 2). Four (13.3%) experienced ≥grade 3 drug-related AEs, including ocular AEs (grade 3, n = 1, 1.3 mg/kg), ILD (grade 3, n = 1, 1.3 mg/kg), anemia (grade 3, n = 1, 1.7 mg/kg), and elevated glutamyltransferase level (grade 3, n = 1, 1.7 mg/kg). Two (6.7%) patients experienced drug-related serious AEs (SAEs) with one case of grade 3 pneumonitis (1.3 mg/kg) and one case of grade 3 blurred vision (1.3 mg/kg). Fourteen (46.7%) participants had dose interruptions due to ocular AEs (n = 7; including 1 participant also experiencing ILD), ILD (n = 5), decreased white blood cell count and platelet count (n = 1), reflux gastritis (n = 1), and abdominal pain (n = 1). Three (10.0%) participants had dose reductions due to dry eye. The most common AEs were dry eye (50.0%), decreased platelet count (36.7%), increased aspartate aminotransferase (AST; 33.3%) and alanine aminotransferase (ALT; 30%), decreased white blood cell count (26.7%) and neutrophil count (23.3%), corneal epithelial injury (23.3%), diarrhea (20%), and ILDs (20%). No dose-limiting toxicity (DLT) was found, and the recommended phase 2 dose was set as 1.7 mg/kg Q3W.

No participants dropped out of the study due to treatment-emergent AEs (TEAEs), and there were no drug-related deaths. Six (20%) patients had ARX788-related ILDs (grade 1 or 2, n = 5, and grade 3, n = 1) that were relieved or recovered after being administered oral prednisone. The median time to ILD onset was 148 days (range: 123–628 days). The condition of the participant with grade 3 ILD improved after treatment with prednisone combined with antibiotics and was withdrawn from the study based on the investigator's assessment due to unsuitable conditions for contuining treatment. One year after discontinuing treatment of ARX788, the follow-up assessment still showed stable disease even without further anti-cancer treatment. One participant at a dose of 1.7 mg/kg Q3W was reported to fully recover from pneumonia after treatment.

Efficacy analysis

One patient was considered to have a measurable lesion by the investigator at the time of enrollment but turned out to have no measurable lesion during follow up. This participant was involved in the safety analysis but not in the efficacy analysis. Among the 29 participants with measurable lesions, 4 did not receive the response evaluation due to coronavirus disease

Table 2. Most common treatment-related adverse events (any and grade 3/4) that occurred in 10% or more patients

System organ class, preferred term	1.3 mg/kg Q3W (n = 9)			1.5 mg/kg Q3W (n = 14)			1.7 mg/kg Q3W (n = 7)			Any (n = 30)		
	Grade 3	Grade 4	Any	Grade 3	Grade 4	Any	Grade 3	Grade 4	Any	Grade 3	Grade 4	Any
Any adverse event (%)	2 (22.2)	0	8 (88.9)	0	0	13 (92.9)	2 (28.6)	0	7 (100)	4 (13.3)	0	28 (93.3)
Blood and lymphatic system disorders (%)												
Anemia	0	0	0	0	0	1 (7.1)	1 (14.3)	0	2 (28.6)	1 (3.3)	0	3(10)
Platelet count decreased	0	0	5 (55.6)	0	0	4 (28.6)	0	0	2 (28.6)	0	0	11 (36.7)
White blood cell count decreased	0	0	3 (33.3)	0	0	4 (28.6)	0	0	1 (14.3)	0	0	8 (26.7)
Neutrophil cell count decreased	0	0	3 (33.3)	0	0	3 (21.4)	0	0	1 (14.3)	0	0	7 (23.3)
Eye disorders (%)												
Dry eye	0	0	4 (44.4)	0	0	10 (71.4)	0	0	5 (71.4)	0	0	19 (63.3)
Vision blurred	1 (11.1)	0	1 (11.1)	0	0	2 (14.3)	0	0	1 (14.3)	1 (3.3)	0	4 (13.3)
Visual impairment	0	0	0	0	0	0	0	0	3 (42.9)	0	0	3 (10)
Corneal epithelial injury	0	0	0	0	0	4 (28.6)	0	0	3 (42.9)	0	0	7 (23.3)
Liver disorder (%)												
Aspartate aminotransferase increased	0	0	2 (22.2)	0	0	6 (42.9)	0	0	2 (28.6)	0	0	10 (33.3)
Alanine aminotransferase increased	0	0	2 (22.2)	0	0	6 (42.9)	0	0	1 (14.3)	0	0	9 (30)
Blood bilirubin increased	0	0	0	0	0	2 (14.3)	0	0	1 (14.3)	0	0	3 (10)
γ-glutamyltransferase increased	0	0	0	0	0	2 (14.3)	1 (14.3%)	0	1 (14.3)	1 (3.3)	0	3 (10)
Gastrointestinal disorders (%)												
Diarrhea	0	0	2 (22.2)	0	0	3 (21.4)	0	0	1 (14.3)	0	0	6 (20)
Respiratory, thoracic, and mediastinal disorders (%)												
Interstitial lung disease	1 (11.1)	0	2 (22.2)	0	0	3 (28.6)	0	0	1 (14.3)	1 (3.3)	0	6 (20)
Skin and subcutaneous tissue disorders (%)												
Alopecia	0	0	2 (22.2)	0	0	2 (14.3)	0	0	1 (14.3)	0	0	5 (16.7)
Rash	0	0	0	0	0	1 (7.1)	0	0	2 (28.6)	0	0	3 (10)
Pruritus	0	0	0	0	0	1 (7.1)	0	0	2 (28.6)	0	0	3 (10)
General disorders (%)												
Pyrexia	0	0	0	0	0	3 (21.4)	0	0	1 (14.3)	0	0	4 (13.3)
Weight decreased	0	0	0	0	0	1 (7.1)	0	0	1 (14.3)	0	0	3 (10)

2019. One patient had tumor shrinkage greater than 30% but the response was not confirmed 4 weeks after the first evaluation, so the response for this patient was stable disesase (SD). In total, 11 of the 29 evaluable participants were confirmed to have a partial response (PR). Of them, 3 received 1.3 mg/kg, 6 received 1.5 mg/kg, and 2 received 1.7 mg/kg ARX788. The overall ORR was 37.9%, with the highest in 1.5 mg/kg (46.2%) and 33.3% in 1.3 mg/kg and 28.6% in 1.7 mg/kg, respectively. It seems there is no significant dose-response relationship for ORR, presumably due to fewer participants in the 1.7 mg/kg group. However, the disease control rate (DCR) increased with dose and is the highest in the 1.7 mg/kg cohort (85.7%). With a median follow up of 10 (95% CI: 6.5–15.9) months, the median PFS was 4.1 (95% CI, 1.4–6.4) months, with the highest in 1.7 mg/kg (4.1 months), and the median OS was 10.7 (95% CI, 4.8–not reached) months (Figure 1; Table 3). The median duration of response (mDOR) was 8.4 (95% CI: 2.1–18.9) months. Although the median OS and mDOR were not reached in the 1.7 mg/kg group, both were higher than patients in the 1.5 mg/kg and 1.3 mg/kg groups.

When the 3 participants who had not received prior trastuzumab therapy was excluded, the confirmed ORR, PFS, and OS were 34.6% (9/26), 4.1 (95% CI, 1.4–6.4) months, and 10.7 (95% CI, 4.6–not reached) months, respectively.

Exploration analysis

To explore the genetic alterations before and after the ARX788 treatment, we performed a 108-gene panel sequencing assay on tissue samples and blood samples in this clinical trial. The genetic variations in blood and tissue samples were analyzed at baseline level, including 28 blood samples and 17 tissue samples. No mutation was found in baseline blood samples of cases 0110 and 0117 as well as in baseline tissue samples of cases 0105, 0116, and 0501, so there was a total of 26

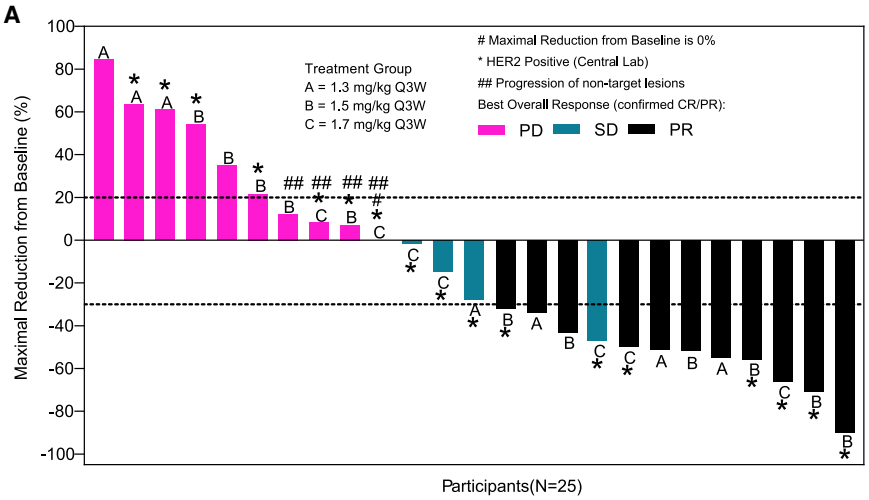

Figure 1. Best percentage of change to date from baseline in sum of diameters in target lesions

(A) Waterfall plot of best confirmed objective response assessed by investigator.

(B) Spider plot of best objective response.

baseline blood samples and 14 baseline tissue samples for analysis. As shown in Table S2 and Figure S1, we found the highest frequency of *TP53* mutation in both baseline blood samples (69%) and tissue samples (86%). In addition, frequency of *ERBB2* mutation was also high in both blood (19%) and tissue samples (14%) at baseline level. Next, we mapped gene mutations in all sequenced samples (58 samples including 40 baseline samples and 18 sequencing blood samples). Furthermore, we assigned the samples to response group (11 PR + 5 stable disease [SD]) and nonresponse group (9 progression disease [PD]) to compare the difference of gene variations. We observed that *ERBB2* was more recurrently mutated in the response group than in the nonresponse group ($p = 0.022$; Figure 2A). Moreover, we found that genes such as *ERBB2* and *ERBB4* were more frequently mutated in the response group, and the predominant mutation type was missense mutations, whereas genes including *CDH1* and *ABL1* were more frequently altered in the nonresponse samples, with a nonsense mutation in *ABL1* and multihits in *CDH1* (Figure 2B). Collectively, these results provide clues for us to further explore the mechanism of ARX788.

DISCUSSION

The study showed that ARX788 was well tolerated and associated with sustained anti-tumor activity in participants with metastatic HER2-positive G/GEJ adenocarcinoma. The confirmed ORR was 37.9%, and the median PFS was 4.1 months, similar to trastuzumab deruxtecan and better than RC48.[14,15] Most AEs experienced were mild or moderate, with only 4 patients (13.3%) experiencing grade 3 AEs related to ARX788. Moreover, no grade 4 or 5 AEs occurred. There was no difference in safety among the three dosage groups.

In the present study, HER2 status was re-evaluated in a central lab, and we found that the consistency of HER2 status between local and central labs was 75%. The potential reason for this observation could be the degradation of HER2 protein in the specimen, the differences among laboratories, and the heterogeneity of GC. The confirmed ORR was 41.2% (7/17) in the HER2-positive patients who were confirmed by the central lab.

In a phaseIdose-escalation clinical study of ARX788 in breast cancer,[20] the most commonly reported AEs related to ARX788 were increasing AST and ALT levels, corneal epitheliopathy, alopecia, hypokalemia, ILD, and pneumonitis, which are consistent with the findings in this study. ARX788 has significantly lower hematological toxicities. No grade 3 or higher neutropenia was found for ARX788, while the incidence rate of grade 3 or higher neutropenia was as high as 51% for trastuzumab deruxtecan.[14] This might be due to the high stability of ARX788 in blood circulation. ARX788 is the first site-specifically conjugated ADC developed with the proprietary EuCODE technology platform, enabling site-specific incorporation of nonnatural amino acids in eukaryotic cells in clinical study settings.[21]

In this trial, ILD occurred in 20% of the treated participants. The underlying mechanism remains unclear. Lung toxicity has been observed with trastuzumab,[22,23] pertuzumab,[24] lapatinib,[25] trastuzumab deruxtecan,[26] and T-DM1[27] with variable frequency (0.2%–17.4%) and severity (0.8%–14.8% for grade 1–2, 0%–7.1% for grade 3 or above). A systematic review that assessed 9,886 patients who received anti-HER2 agents summarized that the overall incidence of ILD was 2.4% for all grades and 0.5% and 0.2% for grades 3–4 and 5, respectively.[28] The mechanism of anti-HER2-related lung injury may be theoretically associated with HER2 expression on type II pneumocytes and with involvement in alveolar wall repair, so agents targeting HER2 may impair alveolar repair mechanisms.[29]

Although the ocular toxicity of ARX788 is mostly mild to moderate, as the most common AE, more attention is required. The ocular toxicity of ARX788 including dry eye, blurred vision, and corneal abnormalities, which can be improved or resolved with cessation of treatment or treatment interruption. Only one participant had grade 3 blurred vision, and the patient recovered after interruption of ARX788 therapy. Currently, the underlying toxicological mechanism and pathogenesis are not well understood. The possible mechanism could be related to the HER2 target and cytotoxin. It has been shown that HER2 is preferentially expressed by superficial differentiated ocular surface epithelia.[30] In a phase II study of T-DM1,[31] ocular AEs were reported in 31.3% of patients (commonly dry eye, increased lacrimation, vision blurred/visual impairment). Another possible reason could be

Table 3. Summary of confirmed responses of the 30 participants who received at least one dose of ARX788

Overall response (%)	1.3 mg/kg Q3W (n = 9)	1.5 mg/kg Q3W (n = 13)	1.7 mg/kg Q3W (n = 7)	Total (n = 29[a])
CR	0	0	0	0
PR	3 (33.3)	6 (46.2)	2 (28.6)	11 (37.9)
SD	1 (11.1)	0	4 (57.1)	5 (17.2)
PD	3 (33.3)	5 (38.5)	1 (14.3)	9 (31)
NE[b]	2 (22.2)	2 (15.4)	0	4 (13.8)
ORR (95% CI)	33.3 (7.5–70.1)	46.2 (19.2–74.9)	28.6 (3.7–71)	37.9 (20.7–57.7)
DCR (95% CI)	44.4 (13.7–78.8)	46.2 (19.2–74.9)	85.7 (42.1–99.6)	55.2 (35.7–73.6)
PFS				
Median (95% CI) (months)	3.6 (1.3–NE)	2.4 (1–12.5)	4.1 (1.4–NE)	4.1 (1.4–6.4)
OS				
Median (95% CI) (months)	10.1 (1.8-NE)	10.7 (2.6– NE)	NE (7.1– NE)	10.7 (4.8–NE)
DOR				
Median (95% CI) (months)	4.2 (3.3–NE)	9 (2.1–18.9)	NE (NE)	8.4 (2.1–18.9)

Tumor response was confirmed by investigators. ORR includes complete response and partial response. Disease control rates include complete response, partial response, and stable disease $\geq$12 weeks.
[a]1 participant at 1.5 mg/kg Q3W had no measurable lesion.
[b]4 participants could not be evaluated due to lack of efficacy evaluation data.

related to cytotoxins. One review summarizing 13 ADCs targeting different targets reported that most ADCs (12/13) employed tubulin inhibitor (8 with maytansinoids and 4 with auristatins),[32] and there was a strong association between the cytotoxins and ocular AEs. The cytotoxic payload of ARX788 is a highly potent tubulin inhibitor (microtubule-disrupting monomethyl auristatin F [MMAF]), identical to belantamab mafodotin (belamaf), which is an ADC approved by the US Food and Drug Administration (FDA) for the treatment of relapsed and refractory multiple myeloma. The most common AE of belantamab mafodotin was keratopathy (73%), and the most common symptoms were blurred vision and dry eye.[33]

Yang et al.[34] performed a meta-analysis and showed that cell-free DNA (cfDNA)-based copy-number variation (CNV) detection may have limitations such as low sensitivity and low accuracy, and the effect is not as good as SNVs. Therefore, we did not include data of CNVs in this study, and this issue remains to be further explored in the future.

In conclusion, ARX788 demonstrated promising anti-tumor activity with a confirmed ORR of 37.9%. The median PFS and OS were 4.1 and 10.7 months, respectively. Based on these encouraging results, ARX788 was granted as an orphan drug for the treatment of HER2-positive GC by FDA on March 18, 2021. A randomized controlled, open-label phase 2/3 study to evaluate the efficacy of ARX788 as second-line treatment with HER2-positive advanced G/GEJ adenocarcinoma globally is ongoing (Chinadrugtrials.org.cn: CTR20211583).

Limitations of the study

There are several limitations of this study. The major limitations were small sample size and patient selection (one patient without measurable lesion, and 3 patients did not receive prior trastuzumab therapy). In addition, dose escalation does not follow the 3 + 3 principle. Though exploration analysis with blood and tissue samples is carried out, some specimens are missing.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xcrm.2022.100814.

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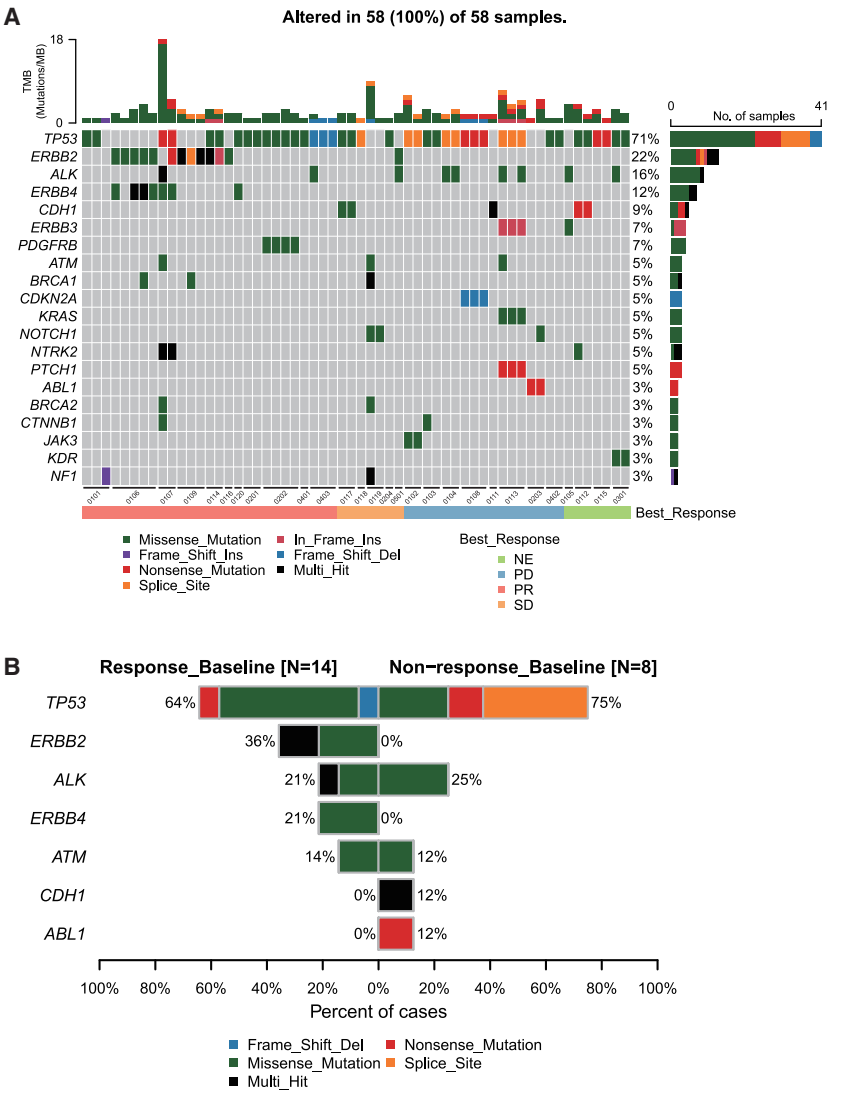

Figure 2. The 108-gene panel analysis results of ACE-Gastric-01

(A) The oncoplot map of top 20 frequently mutated genes.

(B) Difference of gene variations between response group and nonresponse group. PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluable.

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AUTHOR CONTRIBUTIONS

R.-H.X. conceived, designed, and supervised the study. Y.Z., M.-Z.Q., and G.-Z.X. wrote original draft. A.S. and Q.Z. performed data analyses. X.-L.W., H.-Y.Z., Y.-P.J., X.-J.L., and G.X. contributed to data collection. R.-H.X., M.-Z.Q., J.-F.W., Y.-Q.Z., X.-L.Y., T.Z., and D.-S.W. contributed to enrolling patients. R.-H.X. and M.-Z.Q. reviewed and edited the manuscript. All authors have read and approved the manuscript.

DECLARATION OF INTERESTS

G.-Z.X., Y.-P.J., X.-J.L., and G.X. are employee of Novocodex Biopharmaceuticals.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Blood	Patients in this study	N/A
Tumor tissue	Patients in this study	N/A
Chemicals, peptides, and recombinant proteins		
ARX788	Novocodex Biopharmaceuticals	N/A
xGen Custom Hybridization Capture Panels	Integrated DNA technologies (IDT)	Customized panel
Rapid Plus DNA Lib Prep Kit for illumina	Abclonal	RK20208
Deposited data		
Raw data of 108-gene panel sequencing	Genome Sequence Archive database	HRA003266
Software and algorithms		
SAS version 9.4	SAS 9.4 Software Overview for the Customer	https://support.sas.com/software/94

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Rui-Hua Xu (xurh@sysucc.org.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

The raw data of 108-gene panel sequencing reported in this paper have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2021) in National Genomics Data Center (Nucleic Acids Res 2022), China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (Database accession number: HRA003266) that are publicly accessible at https://ngdc.cncb.ac.cn/gsa. Due to restrictions on patients privacy, the data of patients in this study is not publicly available. There was no new code developed as part of this study. Any additional information required to reanalyze the data reported in this work paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Ethics statement

The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines after approval by the ethics board in Sun Yat-sen University Cancer Center (ID: A2019-001-01).

Human subjects

Chinese adults, both male and female, with histologically confirmed metastatic or advanced gastric/gastroesophageal junction adenocarcinoma refractory to or intolerant of trastuzumab based systemic treatment, were enrolled in the study. Demographic information (i.e., age and gender) was provided in Table 1. Informed consent was obtained from all subjects.

Other models

This study did not use any other models of animals, plants, cell lines, or primary cell cultures.

Subject allocation

The current phase I clinical trial was a one-arm study, with no control group, and thus all the patients were enrolled in one group.

METHOD DETAILS

Study population and criteria

This is a single-arm, open-labeled phase 1 clinical trial. Patients aged between 18 and 75 years old with incurable, histologically confirmed, locally advanced or metastatic HER2-positive (immunohistochemistry IHC 3 + or IHC 2+ and FISH positive) G/GEJ adenocarcinoma whose disease had progressed despite first-line or later treatments were eligible for this study. Additional criteria for inclusion were: (1) had at least one measurable lesion (according to the RECIST version 1.1); (2) an ECOG PS score of 0 or 1; (3) a predicted life expectancy of at least 12 weeks; (4) no radiotherapy for pulmonary diseases including lung parenchyma; (5) normal left ventricular ejection fraction and adequate organ function; (6) no history of ILD or other significant lung diseases; (7) no history of keratitis, corneal disease or active ocular infection, and; (8) no history of hypersensitivity to trastuzumab or any component of ARX788. Considering that there is no standard recommended later lines of treatment for HER2-positive GC in China, patients who could not obtain trastuzumab due to financial or other reasons as their previous first-line treatment and progressed were allowed to participate in this trial for the determination of potential treatment benefits.

Treatment protocol

ARX788 was administered intravenously once every 3 weeks (Q3W). Based on the dose-escalation and expansion study protocol, the starting dose of ARX788 was at 1.3 mg/kg Q3W and followed by 1.5 mg/kg and 1.7 mg/kg Q3W. All participants received treatment until the emergence of intolerable toxicity, disease progression, death, voluntary withdrawal, or end of the study (defined as study completion or early study termination). At the end of this study, participants who benefited from uninterrupted treatment were continuously given ARX788 without additional collection of safety and efficacy data.

Safety and efficacy assessment

All participants were assessed for treatment safety following protocol guidelines. Clinical and laboratory AEs were graded using the National Cancer Institute Common Terminology Criteria for AEs (NCI-CTCAE, version 5.0). Medical histories were obtained. Physical and laboratory evaluations were conducted before and during the study treatment. Comprehensive ophthalmic examinations (including any topical corneal changes) were obtained at screening as per protocol. The association of an AE to ARX788 was assessed by the investigator following protocol-specified assessment criteria.

All participants were evaluated for antitumor response every 6 weeks by Computer Tomography (CT) imaging examination by the investigators, following the Response Evaluation Criteria in Solid Tumors (RECIST, version 1.1). The primary endpoints were the safety and tolerability of ARX788. Secondary endpoints included objective response rate (ORR), progression-free survival (PFS) and duration of response (DOR).

Analysis of 108-gene panel sequencing assay

Archival tissue samples were obtained from the department of pathology at the respective diagnosed institution prior to enrollment. Peripheral blood samples were collected predose on day 1 of cycle 1 for every 2 cycles. 108 gene markers were assessed on the tissue and blood samples to investigate their association with response as an exploratory objective. The list of gene markers was provided in Table S1.

We annotated the .vcf files of each sample using The Ensembl Variant Effect Predictor (VEP) software[35] according to the hg19 reference genome, converted these files into .maf files through the vcf2maf v1.6.20 software (https://doi.org/10.5281/zenodo.593251), and merged them together in one .maf file for further analysis. First, we applied the ''subsetMaf'' function in the R package ''maftools''[36] to extract baseline tissue and baseline blood samples (a total of 40 samples, Table S2), and the ''oncoplot'' function was performed to visualize the top 20 frequently mutated genes. Secondly, we also used the ''oncoplot'' function to plot top 20 genes for all sequenced 58 samples (including baseline samples and subsequent time point samples), then sorted and displayed the samples according to the response. Within each broad category, all samples from each corresponding patient were also shown together. Finally, we excluded those patients with ''NE (not evaluable)'' efficacy evaluation, classified blood samples at baseline level of all PR and SD patients into the response group, and blood samples at baseline level of all PD patients into the non-response group. We extracted the subsets of the .maf file corresponding to response samples and non-response samples, respectively. Then we used the ''mafCompare'' function for differential gene variation analysis. The parameter minimum number of mutations (minMut) was set as 3, and the results were visualized with the ''coBarplot'' function.

QUANTIFICATION AND STATISTICAL ANALYSIS

We summarized demographic and safety data by descriptive statistics. Description statistical assessments were conducted to analyze the safety and efficacy (ORR, PFS, OS and DOR). Safety and tolerance of ARX788 were assessed by characterizing adverse events and laboratory abnormalities. The efficacy endpoints of ORR, PFS, OS and DOR were assessed per RECIST 1.1. The PFS, OS

and DOR were estimated using Kaplan-Meier method. The best percentage change of target lesions from screening for each subject was analyzed and presented as a waterfall plot. All analysis were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC).

ADDITIONAL RESOURCES

This study has been registered on Chinadrugtrials.org.cn identifier CTR20190639.