Efficacy and biomarker analysis of nivolumab plus gemcitabine and cisplatin in patients with unresectable or metastatic biliary tract cancers: results from a phase II study

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Abstract

Background: The prognosis of patients with unresectable or metastatic biliary tract cancer (BTC) is unacceptable low. This study aimed to determine the efficacy, safety and predicting biomarkers of immune checkpoint inhibitor nivolumab in combination with chemotherapy in advanced BTCs.

Methods: In this open-label, single-arm, phase Ⅱ trial, chemo-immunology combination therapy consisting of gemcitabine 1000mg/m^2, cisplatin 75mg/m^2 plus nivolumab 3mg/kg was administered every 3 weeks for up to 6 cycles. Maintenance treatment with gemcitabine plus nivolumab was administered to patients achieving disease control following the combination therapy. The primary outcome was objective response rate (ORR). Secondary outcomes included safety, disease control rate (DCR), progression free survival (PFS) and overall survival (OS). The exploratory objectives were to assess biomarkers for predicting clinical response and prognosis.

Results: 32 patients with a median age of 60 (range 27-69) years were enrolled. As of September 31, 2019, the median follow-up was 12.8 (95% CI, 10.8-14.8) months. 27 response-evaluable patients received a median of 4 (IQR, 3-6) cycles combination therapy, of which 15 (55.6%) patients achieved objective response, including 5 (18.6%)with complete response (CR), and the DCR was 92.6%. 2 of 6 patients in cohort A who were refractory to gemcitabine or cisplatin-based chemotherapy achieved 1 CR and 1 partial response. 13 of 21 chemotherapy-naive patients (61.9%) in cohort B achieved objective response. The median PFS of all patients in cohort A+B was 6.1 months. The median OS was 8.5 months with 33.3% 12-month OS rate. The most frequent grade 3 or higher adverse events were thrombocytopenia (56%), and neutropenia (22%). Fitness might be a biomarker for predicting clinical response. On-therapy change of serum sFASL, MCP-1 and IFN-γ were correlated with prognosis.

Conclusions: Nivolumab in combination with gemcitabine and cisplatin offer promising efficacy and manageable safety profile for patients with advanced BTCs.

Background

Biliary tract cancers (BTCs) represent a diverse group of highly invasive heterogeneous epithelial cancers arising from the biliary tract with poor prognosis. Base on their anatomic location, BTCs are classified into gallbladder carcinoma (GBCA), intrahepatic cholangiocarcinoma (iCCA), perihilar cholangiocarcinoma (pCCA) and distal cholangiocarcinoma (dCCA). The incidence of BTCs increased globally over the past few decades [1], with reported prevalence of 235,900 patients diagnosed with BTCs in 2017 [2]. Surgical resection is a curative treatment option for early-stage BTCs, however, most patients with BTCs already have locally advanced or metastatic disease at the time of diagnosis. Even in cases of surgical resection, recurrence is seen in > 60% of patients within the first or the second year [3]. For patients with advanced unresectable or metastatic BTCs, gemcitabine plus cisplatin is the current standard first-line systemic therapy [4]. However, this combination regimen confers a limited efficacy, one possible reason is the rich desmoplastic stroma of BTCs, forming a barrier to the delivery of

chemotherapeutic drugs in the tumor bed, and resulting in resistance to chemotherapy. Other regimens or strategies, such as gemcitabine and oxaliplatin with or without cetuximab [5], capecitabine plus cisplatin [6], nab-paclitaxel and gemcitabine [7], and small molecule kinase inhibitors targeting FGFR, IDH, MET, Mesothelin, BRCA and other mutated genes, did not show significant improvements of efficacy and survival [8, 9].

Recently, immune checkpoint inhibitors (ICIs), exemplified by antibodies targeting programmed cell death-1 (PD-1) and programmed cell death-ligand 1 (PD-L1), have demonstrated promising anti-tumor activity in a variety of tumor types, coupled with low rates of immune-mediated toxicity [10, 11]. However, studies of anti-PD-1/PD-L1 antibodies in BTCs were few. KEYNOTE-028 trial reported 17% patients with PD-L1 positive advanced BTCs obtained a partial response (PR) from pembrolizumab monotherapy [12]. In another basket trial, pembrolizumab resulted in 100% disease control in 4 patients with tumor DNA mismatch repair (MMR)-deficient cholangiocarcinoma [13]. However, MMR deficiency occurred in only 5-10% patients with BTCs [14]. Therefore, novel strategies that could improve the efficacy of ICIs are in urgent need.

Many studies have demonstrated that ICIs could interact synergistically with chemotherapy in solid tumors [15]. However, there were few reports of this combination therapy in advanced BTCs. Here, we conducted a phase II trial to evaluate the efficacy, safety and biomarkers of nivolumab in combination with gemcitabine and cisplatin for advanced unresectable or metastatic BTCs.

Methods

Study design and patients

This study was a single-center, single-arm, open-label, phase trial in which key inclusion criteria were aged from 18 to 75 years, histologically confirmed unresectable or metastatic BTCs, an Eastern Cooperative Oncology Group (ECOG) performance status of 0- 2, an estimated life expectancy of at least 3 months, at least one radiographically measurable disease, adequate organ functions and ability to understand and sign a written informed consent document. Previous chemotherapy, radiotherapy, or other local ablative therapies must be completed over 4 weeks before enrollment and show radiological confirmed disease progression. Key exclusion criteria included active, known or suspected autoimmune diseases, known brain metastases or active central nervous system (CNS), being treated with either corticosteroids (> 10 mg daily prednisone equivalent) or other immunosuppressive medications within 14 days of enrollment, previously treated with anti-PD-1/PD-L1 antibody. Details of inclusion and exclusion criteria were presented in the study protocol (Additional file 1). Eligible patients were assigned to cohort A (refractory to gemcitabine- or cisplatin- based chemotherapy) and cohort B (chemotherapy naive) based on their previous systemic therapies.

This study was approved by the institutional ethics committee of Chinese PLA General Hospital and conducted in accordance with international standards of good clinical practice. Written informed consent

based on Declaration of Helsinki principles was provided by patients or their representatives before study entry.

Treatment and assessments

All enrolled patients in both cohort A and cohort B were administered the combination therapy consisting of gemcitabine 1000mg/m^2 on day 1 and day 5, cisplatin 75mg/m^2 on day 1, and nivolumab 3mg/ kg on day 3 infused intravenously every 3 weeks for up to 6 cycles. Afterwards, patients with responsive or stable disease switched to maintenance therapy in which nivolumab and gemcitabine were administered every 6- 8 weeks until disease progression, intolerable toxicity, death, withdrawal of consent, or any other reasons. Dose reductions were permitted according to the protocol. Adverse events were graded in accordance with National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0, and the causal association with study drugs was determined by investigators. Tumor responses were assessed every 2 cycles by site investigators according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 [16]. Positron Emission Tomography-Computed Tomography (PET-CT) was mandated to confirm response evaluation if targeted tumors were assessed by Computed Tomography (CT) scan with contrast or Magnetic Resonance Imaging (MRI) as complete response (CR). Patients achieving PR and progressive disease (PD) were advised to perform on-therapy site-matched tumor biopsy. Tumor cell PD-L1 expression was assessed on either archival or fresh pre-treatment study biopsy samples by immunohistochemistry using the Dako 22C3 pharmDx assay (Dako North America, Carpinteria, CA, USA). Positive tumor PD-L1 expression was defined as at least 1% tumor cells being membrane stained at any intensity in a section that contained at least 100 evaluable tumor cells.

Whole-exome sequencing

Genomic DNA isolated from tumor biopsies and matched peripheral-blood mononuclear cell samples using GeneRead DNA FFPE Kit. All sample capture libraries were prepared using the Agilent SureSelect Human All Exon kit v6 (Agilent Technologies) as the manufacturer's instructions. Libraries were sequenced on an Illumina HiSeq 6000 platform. Primary sequence data were process by filtering adaptor sequences and removing low-quality reads defined as those with >10% N rate and/or with >10% bases with a quality score of <20 using the SOAPnuke (version 1.5.6). The clean reads were mapped to hg19 using BWA-mem (version 0.7.12). Single nucleotide variants (SNVs) and small insertions and deletions (indels) were detected using VarScan (version 2.4.1). The mutations were further filtered using in-house software to remove false positive mutations. Tumor mutation burden (TMB) was determined by analyzing non-silent somatic mutations, including coding base substitution and indels per megabase. PyClone was employed to detect subclones and calculate the cancer cell fraction (CCF). The ratio of these subclones to all mutations is interpreted as the intra-tumor heterogeneity. Microsatellite instability (MSI) detection was performed by interrogating 344 available genomic microsatellites using MSI sensor. The percentage fraction of unstable sites is reported as the MSI sensor score. Human lymphocyte antigen-I (HLA-I) typing of tumor and adjacent normal sample were performed using Polysolver (version 1.0). All non-silent mutations were translated into 21-mer peptide sequences. Then, 9- to 11-mer peptide

sequences were extracted using sliding window. NetMHCpan (version 3.0) was used to predict the MHC class I binding affinity of peptides with the patient-specific HLA alleles. The predicted peptides were selected and ranked by in-house software. Peptides with scores higher than 0 were selected. Tumor neoantigen burden (TNB) was measured in those peptides per megabase. In the neoantigen fitness model, we calculated the neoantigen recognition potential (NRP) for each neoantigen using a recently developed method [17].

Cytokines

Peripheral blood samples were collected every cycles prior to the infusion of study drugs to test the concentration level of cytokines including IL-1β, IL2, IL4, IL6, IL8 (CXCL8), IL10, IL12p70, IL17A, IL18, IL23, IL33, IFN-α2, IFN-γ, TNF-α, soluble Fas, soluble FasL (sFasL), Granzyme A, Granzyme B, Perforin, Granulysin, MCP-1(CCL2), using Biolegend LEGENDplex™ bead-based immunoassays, LEGENDplex™ Human Inflammation Panel (Cat: 740118), and Human CD8/NK Panel (Cat: 740267).

Endpoints

The primary objective of this study was to assess the objective response rate (ORR) of nivolumab plus gemcitabine and cisplatin combination therapy. The second objectives included the frequency and severity of adverse events occurring up to 120 days after the last dose of study drugs, disease control rate (DCR), progression free survival (PFS), PFS at 6 months, overall survival (OS), and OS at 12 months. ORR was defined as the proportion of all treated patients with either a confirmed CR or PR per RECIST version 1.1. PFS was defined as the time from the first dose to the first documented disease progression or to death from any cause. OS was defined as time from the first dose to death from any cause. The exploratory objectives were to assess pathological, immunological or clinical predictive biomarkers for response and prognosis.

Statistical analysis

Safety analysis was performed in patients received at least one dose of nivolumab in combination with gemcitabine and cisplatin and efficacy analysis in patients underwent one or more post-treatment scans. The proportion of patients with objective response and adverse events was summarized by descriptive statistics with 95% Wilson CIs. Response differences among clinical subgroups were assessed with Fisher's exact test. For progression-free survival, patients without disease progression were censored at the time of last radiological imaging. For overall survival, patients still surviving were censored at the time of data cutoff. Survival analysis was performed using Kaplan-Meier method and compared using the log-rank test. Immune biomarker changes were detected by paired t test between pre- and post-treatment, and differences among groups were evaluated by t test or Mann-Whitney U test. All statistical analyses were completed using Stata/SE 15.1.

Results

Patient Population

Between November 16, 2017, and December 31, 2018, 32 eligible patients with advanced unresectable or metastatic BTCs were enrolled, of which 7 patients were refractory to gemcitabine- or cisplatin-based chemotherapy and 25 patients were chemotherapy- naive (fig.1). All enrolled patients, including 1 (3%) patient with regional unresectable disease, 6 (19%) with metastatic disease, and 25 (78%) with recurrent disease (defined as patients who had regional relapsed disease or distant metastases after complete resection or locoregional or systemic therapies), were administered at least one cycle of nivolumab plus gemcitabine and cisplatin combination therapy (Table 1). Patients who did not meet inclusion criteria or were participating other trials were excluded (n=9). At the time of data cutoff (September 31, 2019), all patients in cohort A and cohort B were eligible for safety analyses, of which 6 in cohort A and 21 in cohort B were qualified for efficacy analyses, 5 patients discontinued treatment within the first cycle due to rapidly deteriorated tumor-related complications (n=4) or adverse events unrelated to study drugs (n=1). Detailed baseline demographics and characteristics of all enrolled patients were summarized in Table 1. The median age was 60 years (range 27 -69). 14 patients (44%) had target lesion larger than or equal to 5 cm. Liver metastases were detected in 28 patients (88%) , while abdominal lymphatic metastases in 21 patients (66%). PD-L1 status was evaluable in 26 tumor samples (81%), of which 12 (37%) were detected positive PD-L1 expression and 14 (44%) negative.

Treatment-Related Toxicity

Safety data from cohort A and cohort B were summarized and analyzed together. Of the 32 enrolled patients, all patients experienced at least one treatment-related adverse event. The most frequent adverse events were nausea in 29 patients (91%), neutropenia in 26 patients (81%), fatigue in 21 patients (66%), thrombocytopenia in 20 patients (62%), and anemia in 19 patients (59%) (Table 2). The most common grade 3 or higher treatment-related adverse events were thrombocytopenia, reported in 18 patients (56%), and neutropenia in 7 patients (22%). Other severe adverse events included elevated alanine aminotransferase in 1 patient (3%, grade 3), elevated aspartate aminotransferase in 1 patient (3%, grade 4), elevated lipase in 1 patient (3%, grade 3), hyponatremia in 1 patient (3%, grade 3), and hypertension in 2 patients (6%, grade 3). 1 (3%) patient had immune-related adverse event (rash, grade 1). There were no treatment-related deaths at the time of analysis.

Clinical response and biomarkers

After a median follow-up of 12.8 months (95% CI, 10.8-14.8), 27 response-evaluable patients received a median of 4 cycles nivolumab plus gemcitabine and cisplatin combination therapy (IQR, 3-6). 15 (55.6%) patients in total achieved confirmed objective response, including 5 (18.6%) CR and 10 (37%) PR (Table 3, fig. 2A). The disease control was achieved in 25 patients (92.6%), including 10 (37%) patients who had SD as their best response. Radiological changes of each response-evaluable patient were summarized in Additional file 2. In cohort A, 6 of 7 patients who were refractory to gemcitabine- or cisplatin-based regimens were response evaluable, of which 1 patient achieved CR and 1 patient obtained PR, the ORR and DCR was 33.3%, 83.3%, respectively. In cohort B, 13 of 21 chemotherapy-naive patients (61.9%)

achieved CR or PR and the proportion of patients with disease control was 95.2%. Responses were ongoing at the time of data cutoff in 2 patients with CR and 1 patient with PR (fig. 2B, fig. 2C). Analysis of 27 response evaluable patients found that PD-L1 expression level could not be used as a biomarker for predicting clinical response (p=0.395, Additional file 2: fig. S1A). Whole-exome sequencing was performed on patients' biopsied tumor samples and their paired peripheral-blood mononuclear cells, which were allocated to respond group (CR+PR) and non-respond group (SD+PD) according to their clinical response. TMB and TNB were generally low in this study (Additional file 2: fig. S2). However, the median value of TMB, TNB, and fitness was higher in respond group than that in non-respond group, while the median value of heterogeneity was lower in respond group, of which fitness had statistical difference (p= 0.041, fig. 3A). Mutations of RYR2, MUC4, APOB were detected only in samples from respond group (fig. 3B). We did exploratory analysis to study the association between the activation of peripheral T cells and clinical antitumor activity. Evaluation of T cells in peripheral blood showed that the baseline percentage of CD3+ cells in responders were higher than those in non-responders (p=0.046, Additional file 2: fig. S3A). The proportion of HLA-DR+CD3+ cells in patients' peripheral blood increased after the start of the combination therapy, especially in patients with objective response (p=0.009). However, statistic difference was not observed between responders and non-responders (Additional file 2: fig. S3B and 3C). The association between change of peripheral serum cytokines and chemokines at baseline (C1D0) and C3D0 (the day before the first dose of the 3rd cycle) and clinical response was also assessed. The concentration of serum sFasL and Granzyme A were higher in non-responders than responders after 2 cycles of combination treatment (p = 0.042 and 0.048), while the concentration of IL-2, IL-18, sFasL and CCL2 dropped significantly in responders than non-responders (P=0.036, 0.047, 0.012 and 0.042, Additional file 2: fig. S4A and4B).

PFS and biomarkers

Median PFS in this study was 6.1 months (95% CI, 3.4 -8.2), and the proportion of patients who were progression free at 6 months and 12 months were 51.9 % (95% CI, 31.9-68.6) and 18.5% (95% CI, 6.8-34.8), respectively (fig. 2D). Comparison between cohort A and cohort B showed chemotherapy-naïve patients could obtain longer median PFS, however, there was no statistical difference. Further analysis found that patients who were administered more than 4 cycles of combination treatment had longer PFS (p= 0.024), and PD-L1 expression status could not been established as a biomarker in predicting PFS (p= 0.125, Additional file 2: fig. S1B). We also analyzed the impact of TMB, TNB, and fitness on PFS in this study. However, there was no correlation between the above four biomarkers and PFS (fig. 3C; Additional file 2: fig. S5). Analysis of peripheral serum cytokines found that patients whose concentration of IFN-γ decreased following the combination therapy could obtain longer PFS (p=0.033, fig. 3D), similar association was found between the decrease of MCP-1 and PFS (p=0.019, fig.3D).

OS and biomarkers

Median OS was 8.5 months (95% CI, 5.0-12.5), the 12-month OS rate and 18-month OS rate were 33.3% (95% CI, 16.8- 50.9) and 24.7% (95% CI, 10.2- 42.4), respectively (fig. 2D). There was no statistical

difference between the median OS from cohort A and that from cohort B. 4 cycles or more combination therapy was a parameter that could be correlated with longer OS (HR 0.595, [95% CI, 0.398-0.89], p=0.012; Additional file 2: fig. S1C), while the correlation between PD-L1 expression and OS was not established (p=0.499, Additional file 2: fig. S1C). Whole-exome sequencing results showed that 1.37Neos/Mb as cutoff value of TNB in this study could be a prognostic biomarker, and patients with TNB of greater than 1.37Neos/Mb had significant longer OS (p=0.048, fig. 3C). Analysis of serum cytokines detected the concentration of sFASL or IFN-γ dropped significantly in patients with longer OS (p=0.00076, p=0.032; fig. 3E). The change of Granulysin, MCP-1, IL-17a, IL-23, TNF-α and Granzyme B in serum following the combination therapy had no statistical influence on OS (fig. 3E; Additional file 2: fig. S6).

Discussion

We assessed the efficacy and safety of nivolumab in combination with gemcitabine and cisplatin in patients with advanced BTCs in this study. The most frequent especially severe adverse events in this study were from hematologic toxicities, which were mainly attributed to chemotherapy. However, we observed that the incidence of grade 3 or higher thrombocytopenia was much higher than currently reported gemcitabine and cisplatin chemotherapy [4, 5, 7], whether the addition of nivolumab to chemotherapy deteriorated thrombocytopenia remained unclear. Indeed, thrombocytopenia was a common toxicity of immune checkpoint inhibitors [18- 20]. One study reported that the amount of PD-L1 expressing platelets diminished in the blood of four lung cancer patients treated with anti-PD-L1 antibody atezolizumab in the first 7 days of therapy [21]. Another study reported that the average time to onset of thrombocytopenia induced by immune checkpoint inhibitors was 70 days, and the average platelet count was 61,000/uL with an average decrease of 70% from baseline [19].

Currently, there were several reports of ICIs alone or in combination with chemotherapy treating advanced BTCs in small sample size [12, 13, 22]. There was one unresolved question of whether the addition of ICIs had potential to reverse the resistance of gemcitabine- or cisplatin-based chemotherapy. The cohort A in this study enrolled 7 patients who were previously treated with gemcitabine- or cisplatin-based chemotherapy and obtained 1 CR and 1 PR, indicating that nivolumab was capable to refuel gemcitabine and cisplatin chemotherapy. For chemotherapy naive patient, the combination of nivolumab and cisplatin plus gemcitabine could result in better tumor shrinkage and disease control when compared with historical reports [4- 6]. The improvement of clinical response may due to the synergistic interaction between chemotherapy and ICIs, in which gemcitabine reduced the amount of circulating MDSCs, favoring the reprogramming of TAMs toward an immunostimulatory phenotype, boosting cross-priming and increasing the antigenicity of cancer cells [23, 24], and ICIs in return neutralized the unwarranted immunosuppressive effects of anticancer drugs and maximized the immunostimulatory effects of chemotherapy [15]. The immunostimulatory potential of gemcitabine has been identified in experimental tumor models with ipilimumab in combination, and in patients with metastatic solid tumors when combined with adoptive cell transfer therapy [25, 26].

Though PD-L1 expression as biomarkers in predicting the efficacy of ICIs have been extensively studied in various types of cancers [27- 29], however, contradictory results indicated that PDL1 expression remains an imperfect predictor, because some studies established a positive correlation between PD-L1 and ICI response, while others had detected no association [30, 31]. Our data found that the efficacy of nivolumab in combination with gemcitabine and cisplatin was independent of PD-L1 expression level. Meanwhile, we also evaluate the potent of other biomarkers in predicting the response of nivolumab in combination with gemcitabine and cisplatin, including TMB, TNB, and fitness, which are current hotspots in predicting the association between clinical response and ICI monotherapy or in combination with chemotherapy [32- 37, 17]. Despite no statistical significance, which was probably caused by limited sample size, we observed that higher TMB, TNB and lower heterogeneity may result in better clinical response, which may be potent response predicting biomarkers in the future. Recently, there is growing interest in developing blood- derived or serum- derived predictive biomarkers of ICI response across a variety of cancer types [38- 39], especially on-therapy biomarkers [40]. We analyzed the early on-therapy change of peripheral serum cytokines and circulating T cell levels and found that higher percentage of baseline CD3+ cells and decrease of IL-2, IL-18, sFasL and CCL2 level in peripheral blood could predict better outcome of ICI-based combination therapy.

Despite of exciting data of clinical response, the survival data in this study, such as median PFS, PFS at 6-month, OS, and OS at 12-month, was disappointing, which did not present significant survival benefit when compared with the data achieved by chemotherapy alone in the UK-ABC-01 trial and BINGO trial [41, 5]. One possible cause was the high incidence of grade 3-4 hematologic toxicities, which resulted in dose reduction of study drugs or treatment suspension. To assess the correlation between prognostic biomarkers and chemo-immunology combination therapy, we found that patients with higher pre-treatment TNB load seemed had better OS. Disappointingly, correlations between other prognostic biomarkers, including PD-L1 expression level, and OS were not established in this study.

Conclusion

Our study may be susceptible to research bias because of the non-randomized design. A larger randomized trial is needed to confirm the results of this preliminary study about the activity of nivolumab in combination with chemotherapy in BTCs. In summary, our study suggested that nivolumab in combination with gemcitabine and cisplatin had promising antitumor efficacy and manageable safety profile in advanced unresectable or metastatic BTCs, providing a potential treatment option and supporting further study of this combination therapy in patients with this cancer.

Abbreviations

BTCs, biliary tract cancers; GBCA, gallbladder carcinoma; iCCA, intrahepatic cholangiocarcinoma; pCCA, perihilar cholangiocarcinoma; dCCA, distal cholangiocarcinoma; ICIs, immune checkpoint inhibitors; PD-1, programmed death-1; PD-L1, programmed death-1 ligands; PR, partial response; MMR, mismatch repair; ECOG, Eastern Cooperative Oncology Group; ULN, upper limit of normal range; CNS, central nervous

system; CR, complete response; SD, stable disease; CTCAE, Common Terminology Criteria for Adverse Events; CT, contrast-enhanced computed tomography; MRI, magnetic resonance imaging; RECIST 1.1, Response Evaluation Criteria in Solid Tumors version 1.1; PET-CT, positron emission tomography-computed tomography; PD, progression of disease; CCF, cancer cell fraction; TMB, tumor mutation burden; HLA, human lymphocyte antigen; TNB, tumor neoantigen burden; NRP, neoantigen recognition potential; sFasL, soluble FasL; ORR, objective response rate; PFS, progression-free survival; OS, overall survival;

Declarations

Ethics approval and consent to participate

This study was approved by the institutional ethics committee of Chinese PLA General Hospital and conducted in accordance with international standards of good clinical practice. Written informed consent based on Declaration of Helsinki principles was provided by patients or their representatives before study entry.

Consent for publication

Not applicable

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

All authors declared no competing interests.

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Authors’ contributions

All authors made substantial contributions to the manuscript. Conception and design: KCF and WDH; Collection and assembly of data: KCF, YL, YTZ, QMY, LD, JJL, XL, ZKZ, and QM; Data analysis and

interpretation: KCF, YL, YTZ and QM; Manuscript writing: All authors. All authors reviewed iterations of the report and approved the final version for submission.

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Tables

Table 1. Baseline demographics and characteristics of all enrolled patients

	Patients (n=32)
Median age, years	60 (27-69)
Sex	
Male	18 (56%)
Female	14 (44%)
Stage at enrollment	
Unresectable	1 (3%)
Primary metastatic	6 (19%)
Recurrent/ metastatic	25 (78%)
Histology	
GBCA	6 (19%)
Intra-CCA	11 (34%)
Perihilar-CCA	6 (19%)
Distal-CCA	9 (28%)
ECOG performance status	
0-1	30 (94%)
2	2 (6%)
Diameter of the largest target lesion (cm)	
< 5	18 (56%)
≥ 5	14 (44%)
Sum of target lesions (cm)	
< 10	20 (62%)
≥ 10	12 (38%)
Sites of metastases	
Liver	28 (88%)
Lung	5 (16%)
Abdominal lymph node	21 (66%)
Previous treatment	
Surgery	21 (66%)
Locoregional therapy	10 (31%)
Chemotherapy	7 (22%)
None	7 (22%)
Tumor PD-L1 expression	
< 1%	14 (44%)
≥ 1%	12 (37%)
Not assessable	6 (19%)

Data are n (%), unless otherwise specified. Histology was categorized according to the WHO Classification of Tumors, GBCA= gallbladder carcinoma, CCA=cholangiocarcinoma. ECOG=Eastern Cooperative Oncology Group.

Table 2. Treatment-related adverse events in 32 patients

Treatment-related events	Any Grade	Grade 1-2	Grade 3	Grade 4
Anemia	19 (59%)	18 (56%)	1 (3%)	-
Neutropenia	26 (81%)	19 (59%)	6 (19%)	1 (3%)
Thrombocytopenia	20 (62%)	2 (6%)	7 (22%)	11 (34%)
Nausea	29 (91%)	29 (91%)	-	-
Vomit	4 (13%)	4 (13%)	-	-
Constipation	7 (22%)	7 (22%)		
Fatigue	21 (66%)	21 (66%)	-	-
Rash	1 (3%)	1 (3%)	-	-
Fever	11 (34%)	11 (34%)	-	-
Elevated alanine aminotransferase	9 (28%)	8 (25%)	1 (3%)	-
Elevated aspartate aminotransferase	9 (38%)	8 (25%)	-	1 (3%)
Elevated amylase	1(3%)	1 (3%)	-	-
Elevated lipase	2 (6%)	1 (3%)	1 (3%)	-
Hyponatremia	1 (3%)	-	-	1 (3%)
Peripheral neuropathy	2 (6%)	2 (6%)	-	-
Hypertension	2 (6%)	-	2 (6%)	-

Data are n (%), unless otherwise specified. No patients had fatal treatment-related adverse events.

Table 3 Clinical antitumor activity

	Overall (n=27)	Cohort A (n=6)	Cohort B (n=21)
Confirmed objective response	15 (55.6%)	2 (33.3%)	13 (61.9%)
Best overall response			
Complete response	5 (18.6%)	1 (16.7%)	4 (19.0%)
Partial response	10 (37%)	1 (16.7%)	9 (42.9%)
Stable disease	10 (37%)	3 (50.0%)	7 (33.3%)
Progressive disease	2 (7.4%)	1 (16.7%)	1 (4.8%)
Disease control	25 (92.6%)	5 (83.3%)	20 (95.2%)

Data are n (%), unless otherwise specified. Responses were assessed in accordance with the Response Evaluation Criteria in Solid Tumors version 1.1.

Figures

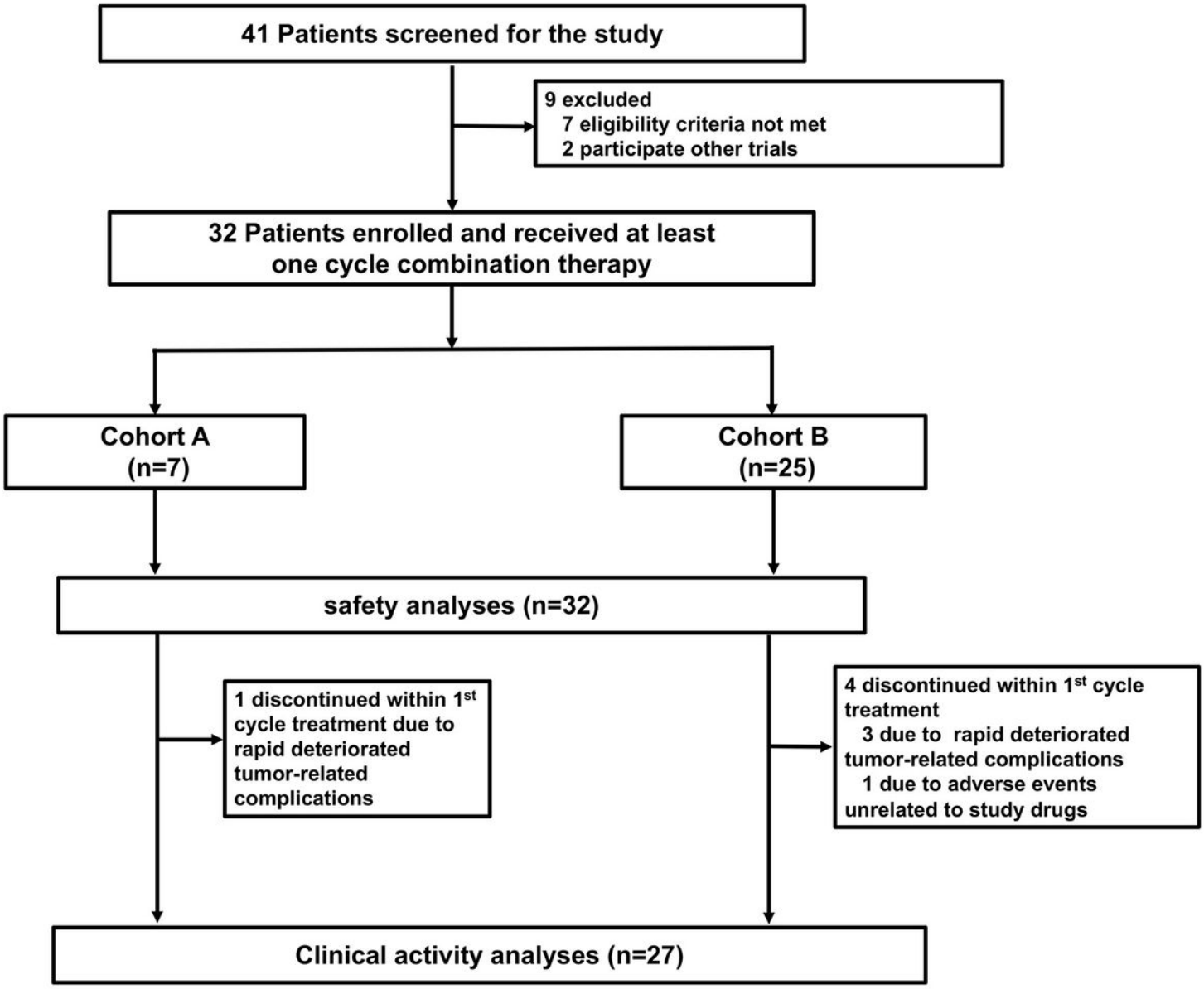

Figure 1

Trial profile.

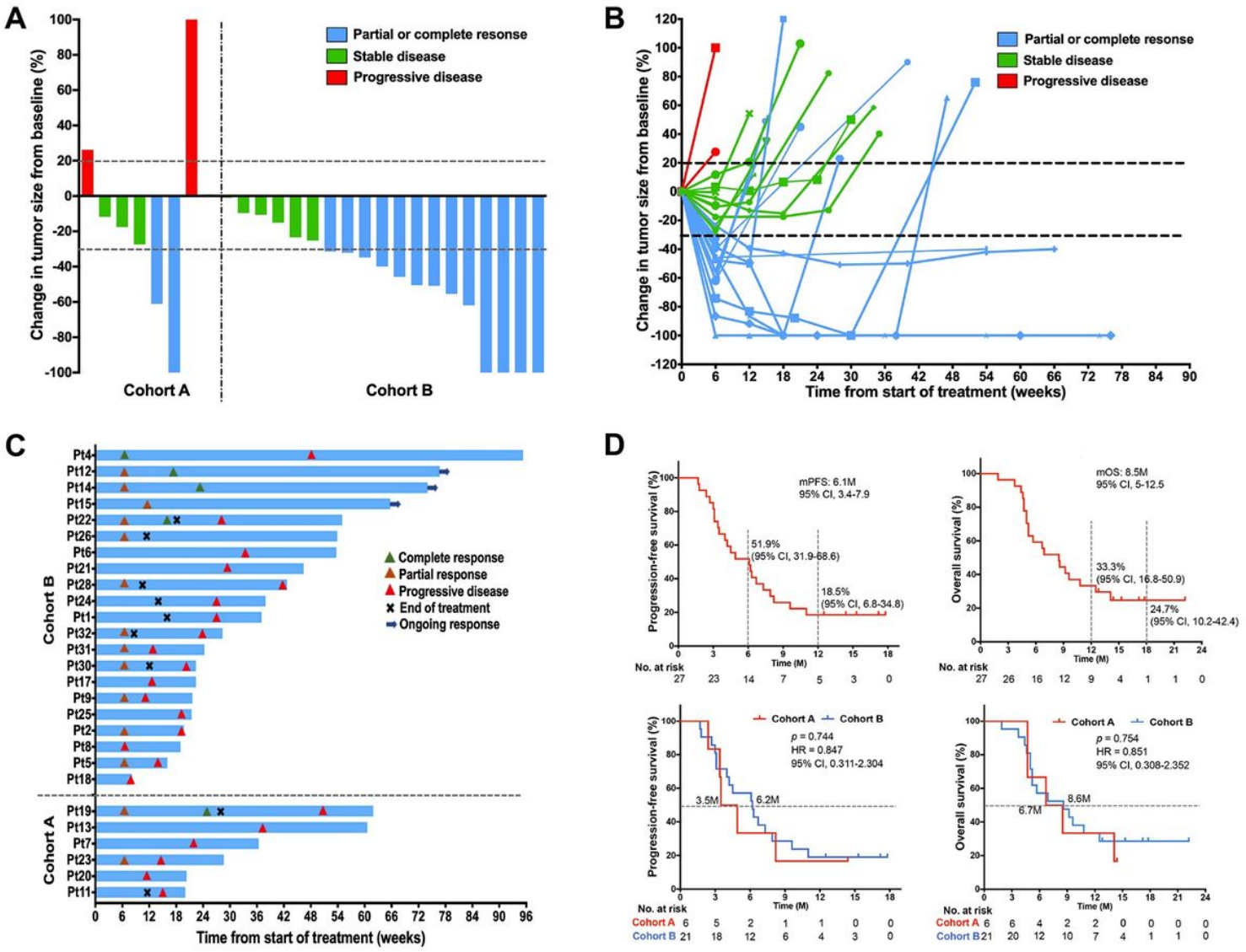

Figure 2

Characteristics of clinical response and survival. (A) Best percentage change in sum of target lesion size from baseline in patients from cohort A and cohort B, horizontal dotted lines denote 30% decrease and 20% increase indicating objective response and progressive disease, respectively, as per RECIST version 1.1. (B) Percentage change in target lesion tumor size from baseline over time for all evaluable patients, defined as those with baseline tumor assessments and at least one post-treatment assessment, the upper horizontal dotted line indicates disease progression at 20% increase in size of target lesions and the lower dotted line represents objective response at 30% decrease in size of target lesions. (C) Time to response and duration of response in patients from cohort A and cohort B. (D) Kaplan-Meier curves of investigator-assessed progression-free survival in all evaluable patients (upper left). Kaplan-Meier curves of investigator-assessed overall survival in all evaluable patients (upper right). Comparison of median progression-free survival between cohort A and cohort B (low left). Comparison of median overall survival between cohort A and cohort B (low right).

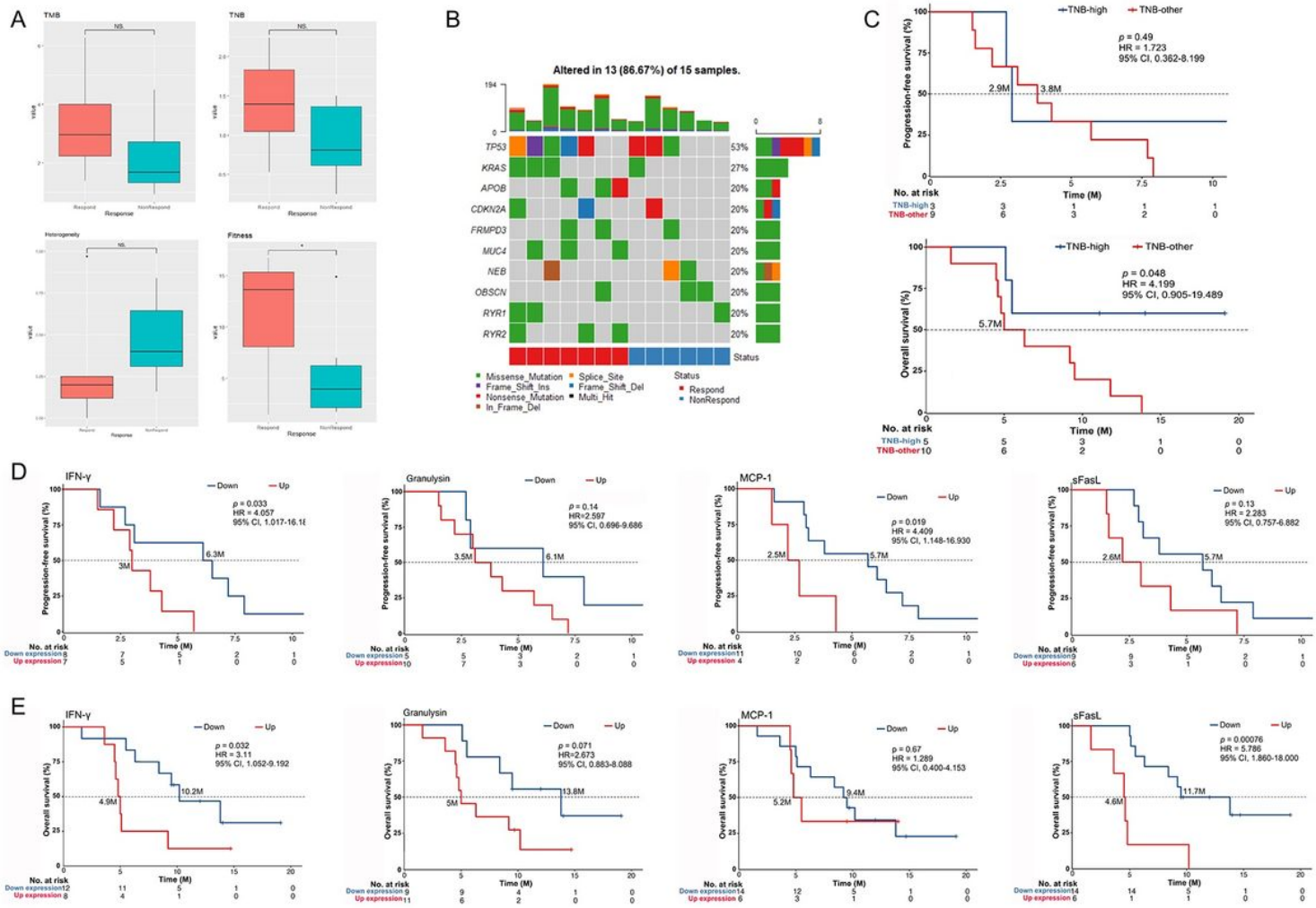

Figure 3

Biomarkers for response and prognosis. (A) The correlation of TMB, TNB, heterogeneity, fitness and clinical response between respond group and non-respond group. (B) Mutated genes detected by whole-exome sequencing. (C) Kaplan-Meier curves of progression free survival and overall survival between patients who were TNB-high and TNB-other. (D) Kaplan-Meier curves of progression free survival of patients who were IFN-γ-up versus IFN-γ-down, Granulysin-up versus Granulysin-down, MCP-1-up versus MCP-1-down, and SFASL-up versus SFASL-down. (E) Kaplan-Meier curves of progression overall survival of patients who were IFN-γ-up versus IFN-γ-down, Granulysin-up versus Granulysin-down, MCP-1-up versus MCP-1-down, and SFASL-up versus SFASL-down.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1ProtocolV2.doc
- Additionalfile2Supplementarydata.docx