Appendix

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1. Inclusion and Exclusion Criteria

Inclusion Criteria:

1) Aged 18-70 years old, both genders.
2) Histologically or cytologically confirmed diagnosis of hepatocellular carcinoma (HCC), gastric or esophagogastric junction cancer (GC/EGJC). Diagnosed with advanced disease (not eligible for surgical and/or local regional therapies, or metastatic disease). Disease progressed or refractory to at least standard first-line therapy (had been intolerant to standard therapies, or had refused standard therapy), or lack of other effective treatment methods.
3) Eastern Cooperative Oncology Group (ECOG) performance status 0 or 1.
4) Life expectancy of at least 3 months.
5) At least one measurable targeted lesion according to RECIST v1.1 criteria.
6) Subjects with advanced HCC had Child-Pugh Class A or B (score ≤7) liver function status.
7) Adequate organ function (without blood transfusion, growth factor or blood component support within 14 days before enrollment) as determined by: Hemoglobin ≥9 g/dL; absolute neutrophil count (ANC) $\geq 1.5\times10^9$/L; platelet count $\geq 75\times10^9$/L (for patients with advanced HCC) or $\geq 100\times10^9$/L (for patients with advanced GC/EGJC); serum albumin ≥2.8 g/dL; serum total bilirubin (TBIL) ≤1.5 times the upper limit of normal (ULN); alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤2.5 times the ULN, for subjects with liver metastases or ≤5 times the ULN; calculated creatinine clearance (CrCl) >50 mL/min (Cockcroft-Gault formula will be used to calculate CrCl).
8) Females of childbearing potential (FOCBP), who are not surgically sterile or postmenopausal, must conduct a pregnancy test (serum or urine) within 7 days before enrollment, and must not be pregnant or breast-feeding. If the result is negative, the patient must agree to use adequate contraception during the experiment and for 3 months after the final study dose. Non-sterilized males who are sexually active must agree to use adequate contraception during the experiment and 3 months after the final study dose.

9) Provide written informed consent and be willing and able to comply with all aspects of the protocol.

Exclusion Criteria:

1) Subjects who have received prior treatment with SHR-1210 or any other PD-L1 or PD-1 antagonists, or who have been enrolled in the phase III study of Apatinib After Systemic Therapy in Patients With Hepatocellular Carcinoma.
2) Subjects who had any active autoimmune disease or history of autoimmune disease, including, but not limited to: hepatitis, pneumonitis, uveitis, colitis (inflammatory bowel disease), hypophysitis, vasculitis, nephritis, hyperthyroidism, and hypothyroidism, except for subjects with vitiligo or resolved childhood asthma/atopy. Patients with asthma that requires intermittent use of bronchodilators or other medical intervention should also be excluded.
3) Subjects with a medical condition requiring the use of immunosuppressive medication, or immunosuppressive doses of systemic or absorbable topical corticosteroids. Doses >10 mg/day prednisone or equivalent are prohibited within the 2 weeks before study drug administration. Corticosteroids used for the purpose of IV contrast allergy prophylaxis are allowed.
4) Known or suspected hypersensitivity to any components of the SHR-1210 formulation, or any other antibody formulation.
5) Active central nervous system (CNS) metastases with clinical symptoms (including cerebral edema, steroid requirement, or progressive disease). Subjects with treated brain or meningeal metastases must be clinically stable (magnetic resonance imaging [MRI] at least 4 weeks apart do not show evidence of new or enlarging metastases) and have discontinued immunosuppressive doses of systemic steroids (>10 mg/day prednisone or equivalent) for at least 2 weeks before study drug administration.
6) Other malignant tumors (except for cured basal cell carcinoma of the skin and cervical carcinoma).

7) Clinically significant cardiovascular and cerebrovascular diseases, including, but not limited to: severe acute myocardial infarction within 6 months prior to enrollment, unstable or severe angina, coronary artery bypass surgery, congestive heart failure (New York Heart Association (NYHA) class >2), ventricular arrhythmia requiring medical intervention, left ventricular ejection fraction (LVEF) <50%.

8) Poorly controlled hypertension within 3 months: systolic blood pressure >140 mmHg, diastolic blood pressure >90 mmHg.

9) Subjects with coagulation abnormalities (PT>16s、APTT>43s、TT>21s、Fbg<2g/L), with bleeding tendency or who are receiving thrombolytic or anticoagulant therapy.

10) Subjects who have received prior systemic chemotherapy, radiotherapy, immunotherapy, hormonal therapy, surgery or target therapy within 4 weeks (or equivalent to 5 half-lives, whichever is greater) prior to study drug administration, or who have had any unresolved AEs > Common Terminology Criteria for Adverse Events (CTCAE) Grade 1 (stable chronic toxicities that are not expected to resolve are permitted).

11) Ascites or pleural effusion with clinical symptoms requiring therapeutic puncture and drainage.

12) Previous digestive tract bleeding history within 3 months or evident gastrointestinal bleeding tendency, such as: esophageal varices, local active ulcerative lesions, gastric ulcer and duodenal ulcer, ulcerous colitis or gastrointestinal diseases such as portal hypertension or resection of tumor with bleeding risk, etc.

13) Serious hemorrhage (bleeding >30 ml within 3 months), haemoptysis (>5 ml within 4 weeks) or thromboembolic events (within 12 months, including stroke events and/or transient ischemic attack).

14) Active infection or an unexplained fever >38.5 ℃ during screening visits or on the first scheduled day of dosing (subjects with tumor fever are eligible at the discretion of the investigator).

15) Prior abdominal fistula, diverticulitis, gastrointestinal perforation, or abdominal abscess within 4 weeks.

16) Objective evidence of previous or current pulmonary fibrosis, interstitial pneumonia, pneumoconiosis, radiation pneumonitis, drug-related pneumonia, severely-compromised pulmonary function etc.

17) History of immunodeficiency including human immunodeficiency virus (HIV) positive, or other acquired or congenital immune-deficient disease, or active hepatitis (transaminase does not meet the inclusion, hepatitis B virus (HBV) DNA $\geq 10^4$/ml or hepatitis C virus (HCV) RNA$\geq 10^3$/ml (or higher). Chronic hepatitis B virus carriers with HBV DNA<2000 IU/ml ($<10^4$/ml) must receive anti-viral treatment throughout the study.

18) Subjects who have participated in other clinical trials, or have completed other clinical trials within 4 weeks.

19) Subjects who may receive other antitumor systemic therapy during the study.

20) Subjects with bone metastasis and those who have received palliative radiotherapy (radiotherapy area >5% marrow).

21) Subjects who may receive a vaccination during the study period, or who have had a previous had vaccination within 4 weeks.

22) History of mental disorders or psychotropic drug abuse.

23) Any other medical, psychiatric, or social condition deemed by the investigator to be likely to interfere with a subject's rights, safety, welfare, or ability to sign informed consent, cooperate, and participate in the study or would interfere with the interpretation of the results.

2. Study endpoint definitions

Primary endpoints:

Physical examinations, vital signs, 12-lead electrocardiograms, laboratory examination, adverse events (AEs) and SAEs will be used to assess safety and tolerance.

The recommended phase II dose (RP2D) will be determined based on safety and tolerance.

Efficacy endpoints:

1) The overall response rate (ORR) is defined as the percentage of participants who achieve confirmed complete response (CR) or partial response (PR) based on RECIST 1.1 criteria.
2) The disease control rate (DCR) is defined as the percentage of participants who achieved confirmed response (CR or PR) or stable disease (SD) based on RECIST 1.1 criteria.
3) Progression-free survival (PFS) is defined as the time from first dose of study drug until documented disease progression or death due to any cause.
4) Overall survival (OS) is defined as the time between the first dose of study drug to the occurrence of death regardless of the cause. Remaining patients will be censored on last date of follow-up.

Exploratory objectives:

1) Evaluate tumor mutation burden (TMB) analysis and correlate the TMB with response, PFS and OS.
2) Identify PD-L1 expression levels on circulating tumor cells (CTCs) at baseline and correlate with ORR, PFS and OS.

3. Appendix Table 1.

Tumor Response in HCC Patients to Differing Doses of Apatinib

Patient ID	Apatinib 125 mg, n=4		Apatinib 250 mg, n=14		Apatinib 500 mg, n=2	
	First response evaluation	Best response	First response evaluation	Best response	First response evaluation	Best response
001*	SD	SD	PR	PR		
002†	SD	SD	SD	PR		
004	SD	PR				
003	PD					
009			SD	PR		
006			SD	PR		
018			SD	SD		
019			SD	SD		
021			SD	SD		
027			SD	SD		
033			PR	PR		
035			PR	PR		

032	Not available	Not available		
037	SD	PR		
042	SD	SD		
043	SD	SD		
014			SD	SD
015			Not available	Not available

HCC, hepatocellular carcinoma; PD, progressive disease; PR, partial response; SD, stable disease.

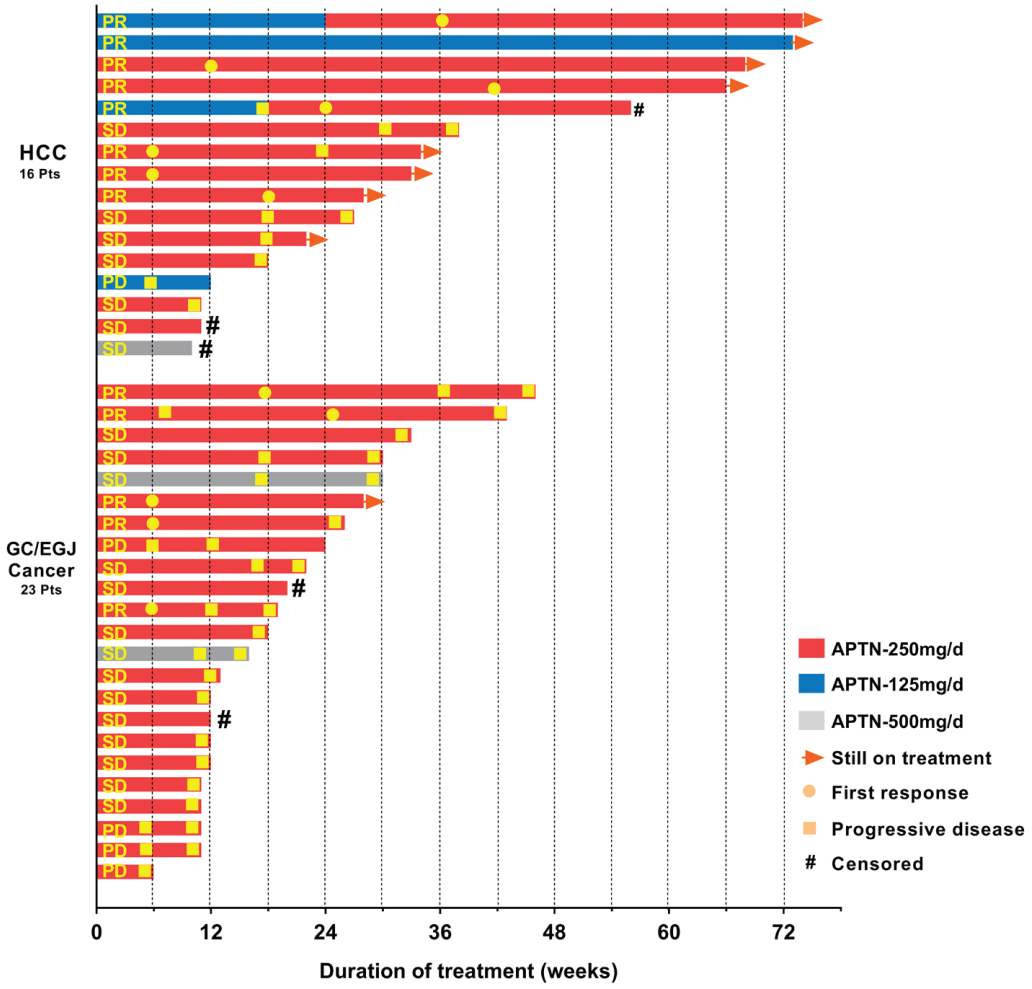
* Patient 001 had SD within 6 cycles of treatment and experienced PD after 9 cycles of apatinib 125 mg plus SHR-1210. Following dose escalation of apatinib to 250 mg, the patient had PR after 3 cycles of combination treatment.

† Patient 002 had SD with non-target enlargement after 12 cycles of apatinib 125mg plus SHR-1210. Following dose escalation of apatinib to 250 mg, the patient had PR after 6 cycles of combination treatment.

4. Appendix Figure & Figure Legends

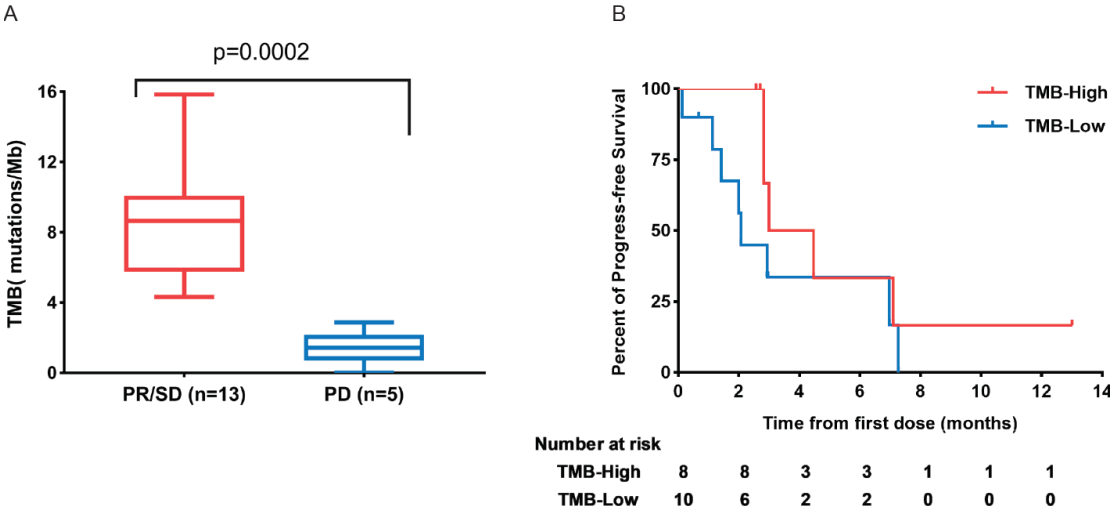
Appendix Figure 1. Tumor swimmer plot for the response-evaluable population (n=38)

Duration of combination treatment (weeks) in HCC and GC/EGJ cancer patients. Two HCC patients were started on apatinib 125 mg and achieved a PR after dose escalation to 250 mg. APTN, apatanib; d, day; EGJC, esophagogastric junction cancer; GC, gastric cancer; HCC, hepatocellular carcinoma; PR, partial response; Pts, patients.



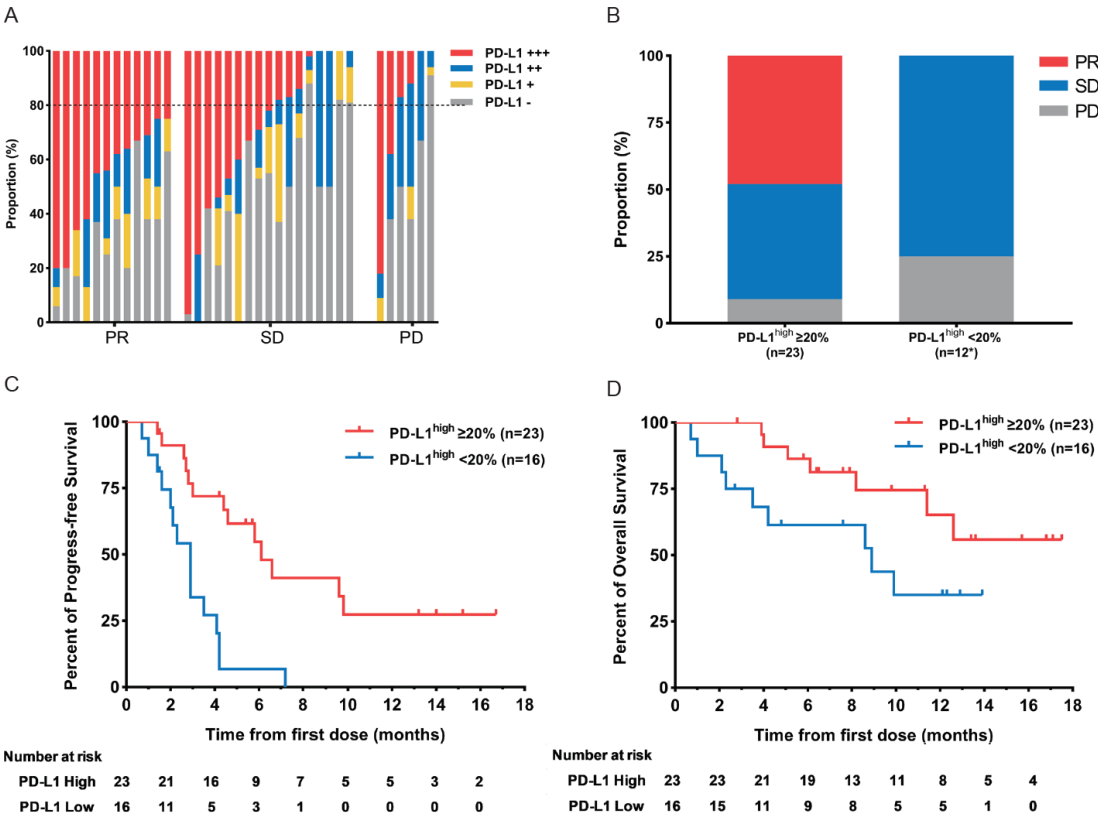
Appendix Figure 2. Clinical outcomes of patients with high and low TMB.

(A) TMB in patients with PR/SD (n=13) and PD (n=5) following combination treatment. Significantly higher TMB was observed in PR/SD patients compared with PD patients at first response (p=0.0002). (B) TMB-high patients were identified with ≥7.2 mutations/MB (upper quartile of Geneplus data). The median PFS of high TMB patients was numerically longer than that of low TMB patients, without significant difference. HR, hazard ratio; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease; TMB, tumor mutation burden.



Appendix Figure 3. Predicative value of PD-L1 expression levels on CTCs.

(A) Stacked bar plot of PD-L1 expression levels on CTCs and their correlation with response. PD-L1 expression was categorized as negative (PD-L1-), low (PD-L1+), medium (PD-L1++), and high (PD-L1+++), based on mean fluorescence intensity. (B) Response rates of patients with ≥20% and <20% PD-L1^{high} CTCs at baseline were 47.8% and 0%, respectively (P=0.002). Significantly longer PFS (C) (P=0.0002) and numerically longer OS (D) (P=0.0601) were observed in patients with baseline PD-L1^{high} CTCs of ≥20% as compared with patients that had PD-L1^{high} CTCs <20%. CTCs, circulating tumor cells; OS, overall survival; PD, progressive disease; PR, partial response; PD-L1, programmed death-ligand 1; PFS, progression-free survival; SD, stable disease; TMB, tumor mutation burden. * (B) excludes four patients without response evaluation.



5. Method of TMB Analysis

DNA isolation

All tumor tissues for TMB analysis were from new biopsy samples including 13 GC/EGJ cancer patients (5 in primary lesions, 8 in metastatic sites) and 5 HCC patients (1 in primary lesions, 4 in metastatic sites). DNA from new biopsy tumor tissues and buffy coat blood (germline DNA) were isolated using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions.

Hybridization capture sequencing

Prior to library construction, 1 μg each of tissue and buffy coat DNA was sheared to 300-bp fragments with a Covaris S2 ultrasonicator. Indexed libraries were prepared using the KAPA Library Preparation Kit (Kapa Biosystems, Wilmington, MA, USA). Hybridization enrichment was performed using custom-designed biotinylated oligonucleotide probes (Integrated DNA Technologies, Iowa, IA, USA) according to the manufacturer's protocol. Sequencing was carried out using Illumina 2×100 bp paired-end reads on an Illumina HiSeq 3000 instrument according to the manufacturer's recommendations, using a TruSeq PE Cluster Generation Kit v3 and a TruSeq SBS Kit v3 (Illumina, San Diego, CA, USA). Hybridization capture sequencing revealed a mean effective depth of coverage of 1,047 ×in tissues and 296 ×in buffy coat DNA samples.

TMB analysis

Sequencing data of paired tumor-germline samples were used to identify somatic mutations. Single nucleotide variants were identified using MuTect (version 1.1.4) and NChot[1,2]. Small insertions and deletions were determined by GATK[3]. Mutations with a variated allele frequency ≥3 % were used to calculate TMB. To analyze the TMB per megabase, the total number of mutations counted is divided by the size of the coding region of the targeted panel. TMB-high patients were identified as those with ≥7.2 mutations/MB (upper quartile of geneplus data).

References

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