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Dual TIGIT and PD-1 blockade with domvanalimab plus zimberelimab in hepatocellular carcinoma refractory to anti-PD-1 therapies: the phase 2 LIVERTI trial

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T cell immunoglobulin and ITIM domain (TIGIT) is an inhibitory receptor expressed on lymphocytes and NK cells, and is a candidate compensatory immune checkpoint that may mediate anti-PD-1/L1 resistance in hepatocellular carcinoma (HCC). We conducted the phase 2 LIVERTI trial testing domvanalimab, a monoclonal Fc-silent anti-TIGIT antibody, plus zimberelimab, an anti-PD-1 antibody, in immunotherapy refractory HCC. Here, we report an analysis of the primary endpoint, the confirmed overall response rate (ORR). Secondary endpoints included rates of adverse events, progression-free survival (PFS), 6-month PFS survival, overall survival, and duration of response, of which the latter two endpoints were excluded from this analysis due to the immaturity of long-term survival data. Among the 29 patients enrolled, the confirmed ORR was 17.2% (95% CI 5.8%-35.8%) and the median PFS was 4.4 months (95% CI, 4.1–4.6 months). Treatment-related adverse events occurred in 16 patients (55.2%). Analysis of circulating tumor DNA (ctDNA) demonstrated that ctDNA dynamics may serve as pharmacodynamic markers of response to domvanalimab plus zimberelimab. Despite the primary endpoint failing to meet the protocol-specified threshold, these results indicate that targeting TIGIT in anti-PD-1/L1 therapy refractory HCC is well-tolerated, associated with anti-tumor effects, and may be guided by ctDNA assessment. ClinicalTrials.gov registration: NCT05724563.

Hepatocellular carcinoma (HCC) remains a leading cause of cancer deaths worldwide, with many patients presenting with tumor burden or advanced stages of disease which preclude curative treatment[1]. Immunotherapy regimens incorporating anti-PD-1 antibodies have emerged as the preferred frontline treatment options for advanced HCC, and are associated with significant improvements in overall survival (OS) and objective response rates (ORR) compared to vascular endothelial growth factor (VEGF) targeting tyrosine kinase inhibitors, the prior standard treatment[2–4]. Nonetheless, most HCC treated with immunotherapy will progress, and the efficacy of existing subsequent line therapies is unclear because they have only been prospectively evaluated in patients who were naïve to immunotherapy regimens[5].

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T cell immunoglobulin and ITIM domain (TIGIT) is an inhibitory receptor on lymphocytes and NK cells and has a critical role in modulating adaptive and innate immunity[6]. Preclinical studies indicate that TIGIT may act as a compensatory immune checkpoint in response to PD-1 blockade and that combining anti-TIGIT and anti-PD-1/L1 therapies may lead to synergistic eradication of HCC[7–9]. Nevertheless, clinical evidence demonstrating the efficacy of TIGIT inhibition in HCC is sparse and inconsistent. In the randomized phase 1b/2 MORPHEUS-Liver trial, tiragolumab (anti-TIGIT) plus atezolizumab (anti-PD-L1) plus bevacizumab (anti-VEGF) showed a greater ORR compared to atezolizumab plus bevacizumab (42.5% vs 11.1%) in the frontline treatment of advanced HCC[10]. However, interpretation of these results is confounded by the underperformance of the control arm, as atezolizumab plus bevacizumab in the phase 3 IMbrave150 trial was associated with an ORR of 30%[11]. In addition, the randomized phase 2 AdvanTIG-206 trial for advanced stage HCC showed that ociperlimab (anti-TIGIT) plus tislelizumab (anti-PD-1) + BAT1706 (bevacizumab biosimilar) versus tislelizumab plus BAT1706 did not result in a meaningful difference in ORR (35.5% vs 37.5%) or survival outcomes[12]. While differences in the enrolled patient population may contribute to divergent results between trials, both MORPHEUS-Liver and AdvanTIG-206 utilized randomization, tested anti-TIGIT antibodies of similar designs, and enrolled a comparable but small number of patients which makes it difficult to resolve the observed disparity in efficacy. A drawback of both studies is that the use of combination drug regimens in treatment-naïve patients presents challenges in isolating the effects of individual drugs, and thus, it remains ambiguous whether anti-TIGIT antibodies can induce synergistic anti-tumor effects in HCC. Notably, both tiragolumab and ociperlimab were designed with competent Fc domains, which permit TIGIT-directed antibody-dependent cellular cytotoxicity (ADCC)[13]. However, anti-TIGIT antibodies with inactivated Fc domains may prevent the elimination of TIGIT expressing cytotoxic cells in the tumor microenvironment and T_{reg} cells in extratumoral tissue to induce anti-tumor immunity while averting excessive toxicities[13,14]. Whether elimination of Fc effector function still enables anti-TIGIT-dependent eradication of HCC in patients while maintaining a tolerable toxicity profile is unknown.

Here, we investigate whether domvanalimab, an Fc-silent anti-TIGIT antibody, plus zimberelimab, an anti-PD-1 antibody, may induce tumor responses in immunotherapy refractory cancers in the HCC cohort of the multicenter phase 2 basket trial LIVERTI. Patients were required to have progressed on prior immunotherapy regimens at least incorporating anti-PD-1/L1 antibodies including but not limited to combination treatments with anti-VEGF, -CTLA-4, or -LAG3 therapies. The primary endpoint was investigator determined confirmed ORR among evaluable patients. Secondary endpoints reported include disease control rate, median progression-free survival, and safety, while correlative studies assessed associations between treatment response and circulating tumor DNA (ctDNA) dynamics. Patients were treated with domvanalimab 1200 mg and zimberelimab 360 mg intravenously on day 1 of each 21-day cycle until disease progression, unacceptable toxicity, death, or discontinuation from the study treatment for any other reason.

Results

Patient characteristics

From 5 June 2023 to 12 December 2023, 29 patients were enrolled at a National Cancer Institute-designated cancer center (UT Southwestern Harold C. Simmons Comprehensive Cancer Center) and a county safety-net hospital system (Parkland Health) (Supplementary Fig. 1). The main inclusion criteria for this study included Child-Pugh A/B7-8 liver disease, ECOG score of 0 or 1, and adequate organ and bone marrow function. The exclusion criteria included severe toxicities to prior anti-PD-1/L1 therapies, history of organ transplant, specified autoimmune diseases, and chronic use of systemic immunosuppressive medications (see Methods for inclusion and exclusion criteria). Patients enrolled had a median age of 69 (IQR: 63–73), were primarily male (72.4%), had Child-Pugh A disease (82.8%), and were frequently associated with HCV cirrhosis (44.8%) (Supplementary Table 1). The number of lines of prior systemic therapy received was 1 for 20 patients, 2 for 5 patients, and 3 for 4 patients. Prior immunotherapy treatments included atezolizumab plus bevaicuzmab (19 patients), ipilimumab plus nivolumab (5 patients), tebotelimab (2 patients), durvalumab ± tremelimumab (4 patients), nivolumab (2 patients), and pembrolizumab (1 patient) (Supplementary Table 2). The total number of immunotherapy treatments exceeds the number of patients enrolled as patients could have received different multiple immunotherapies across lines of treatment including patients that were initially treated with monotherapy immunotherapy and subsequently treated with combination therapies. At the time of data cut-off, the median follow-up time was 8.8 months.

Efficacy and safety

Among the 29 enrolled patients, the investigator determined confirmed ORR of 17.2% (95% CI 5.8%-35.8%), included 1 complete response (CR) and 4 partial responses (PR) (Fig. 1a). The disease control rate including confirmed CRs, confirmed PRs, and stable disease (SD) was 62.1%. The 6-month PFS rate was 40.4% (95% CI, 21.6–58.5%) and the median PFS was 4.4 months (95% CI, 4.1–4.6 months) (Fig. 1b). Subgroup analyses indicated that tumor responses were not associated with clinical characteristics including age, race, sex, alpha-fetoprotein (AFP) levels, extrahepatic disease, or macrovascular invasion, although the relatively small sample size of patients limits statistical power to detect associations (Supplementary Fig. 2). Notably, none of the four patients who initially responded to prior anti-PD-1/L1 therapies and later experienced progression achieved an objective response to domvanalimab plus zimberelimab (Fig. 1c). Rather, all objective responses to domvanalimab plus zimberelimab were observed in patients with primary resistance to prior anti-PD-1/L1 therapy. Other prespecified secondary endpoints including overall survival and duration of response were not analyzed due to immature data at the time of the primary analysis.

Among the 29 enrolled patients, treatment-emergent adverse events of any cause were observed in 25 patients (86.2%) and serious adverse events (SAEs) of any cause were observed in 15 patients (51.7%). Treatment-emergent AEs that were grade 3 or greater occurred in 16 patients (55.2%). The most common treatment-emergent adverse events included fatigue (41.4%), abdominal pain (31%), anemia (24.1%), diarrhea (24.1%), and nausea (24.1%). Treatment-related adverse events (TRAE) defined as any adverse event that was at least possibly attributed to domvanalimab plus zimberelimab by the treating investigator and an independent safety committee occurred in 17 patients (58.6%). The most common TRAE of any grade was diarrhea (20.7%), followed by rash/pruritis (17.2%), fatigue (17.2%), nausea (6.9%), and cough (6.9%) (Supplementary Table 3). Grade 3 and 4 TRAEs occurred in 4 patients (13.8%) including pneumonitis, adrenal insufficiency (3.4%), hepatic failure (3.4%), and pancreatitis (3.4%). At the time of data cutoff, patients received a median of 5 treatment cycles, and dose delays or discontinuation due to TRAEs occurred in 3 patients. Adverse events in the safety period related to hepatic function and decompensation including worsening ascites, ALT/AST increase, hepatic failure, and hyperbilirubinemia occurred in 5 patients (17.2%), and 4 of these events were attributed to disease progression.

ctDNA biomarker analysis

Prior studies indicate that ctDNA may be a prognostic factor, and changes in ctDNA may predict treatment response in multiple cancer types[15–18]. However, the utility of ctDNA for prognostication or assessing immunotherapy treatment response in HCC has not been

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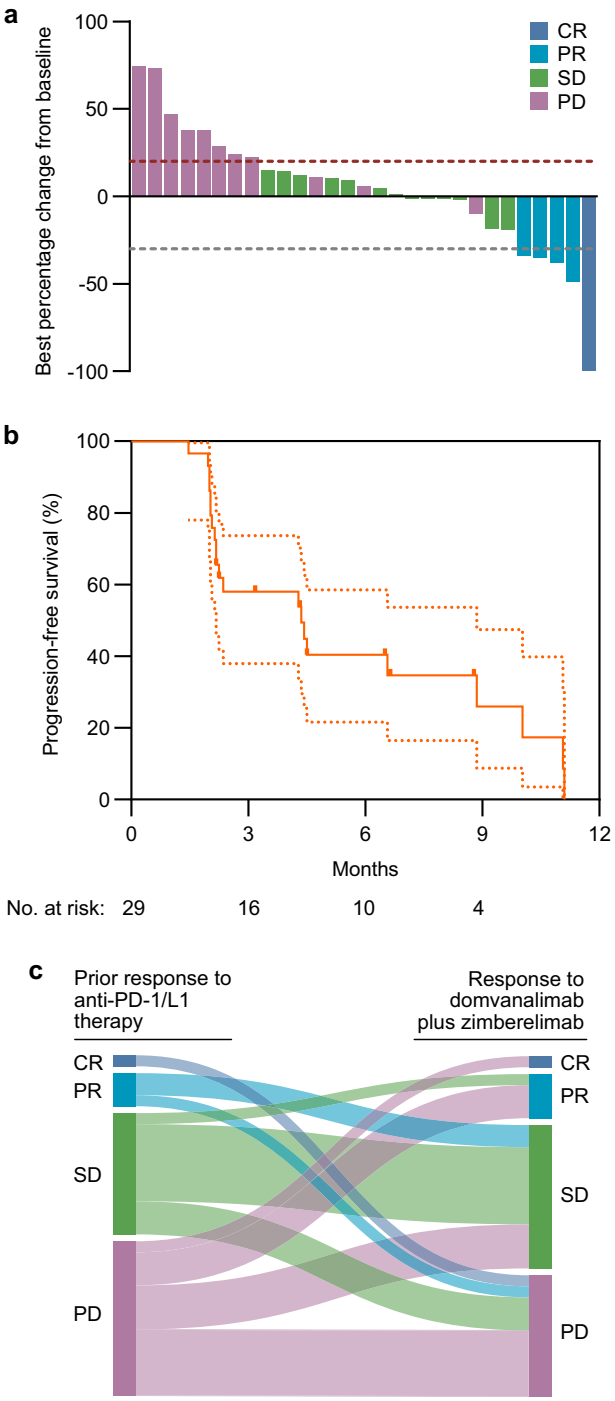

Fig. 1 | Objective response rates and progression-free survival after domvanalimab plus zimberelimab treatment in refractory HCC. a Waterfall plot of the best percentage change in tumor measurements for confirmed responses among 29 evaluable patients. CR, complete response. PR, partial response. SD, stable disease. PD, progressive disease. For one patient with an unconfirmed PR, the best percentage change prior to the unconfirmed response is displayed. The dotted red line indicates tumor growth by at least 20% and the dotted grey line indicates tumor shrinkage by at least 30%. **b** Kaplan-Meier analysis of the progression-free survival after domvanalimab plus zimberelimab. Dashed lines indicate the 95% confidence intervals. **c** Categories of response based on prior response to anti-PD-1/L1 therapy.

prospectively evaluated to date. We therefore examined whether ctDNA could serve as biomarkers in HCC treated with domvanalimab plus zimberelimab. ctDNA analysis using a tumor agnostic 83 gene panel assay was conducted on peripheral blood specimens obtained at baseline for all 29 enrolled patients and then approximately 6–8 weeks (post cycle 2) after treatment initiation for 28 patients (see Methods). The most common baseline ctDNA alterations included mutations in the *TERT* promoter (73%), *TP53* (60%), and *CTNNB1* (40%) (Supplementary Fig. 3a). Genetic alterations in these genes were not associated with objective radiographic responses to domvanalimab plus zimberelimab, although the limited sample size of patients limits statistical power to detect associations (Supplementary Fig. 3b). Moreover, baseline ctDNA variant allele frequency (VAF) did not differ between patients with objective (CR/PR) and other (SD/PD) responses (Supplementary Fig. 4a). Stratifying patients by ctDNA levels using the median VAF also did not demonstrate a significant association between ctDNA levels and objective radiographic responses or difference in PFS among individuals with low ctDNA levels versus high ctDNA levels (Supplementary Fig. 4b and c).

We next assessed whether early changes in ctDNA levels corresponded with objective responses to domvanalimab plus zimberelimab in the 28 patients with serial blood collections. Among 5 patients with a CR or PR to domvanalimab plus zimberelimab, 4 patients had a decline in ctDNA levels including 3 patients with a decrease of greater than 50% (Fig. 2a). Among 13 patients with SD, ctDNA levels were increased in 10 patients, while among 10 patients with PD, ctDNA levels were increased in 9 patients (Fig. 2a). In two patients who had an initial PR followed by disease growth, ctDNA levels initially declined but subsequently increased at the time of progression, showing that ctDNA levels in these patients tracked closely with disease status (Fig. 2b). Dynamics of VAF for individual genes that were recurrently mutated in our cohort showed considerable genetic evolution after treatment with domvanalimab plus zimberelimab regardless of tumor response (Fig. 2c). Enrichment and depletion of ctDNA alterations (defined as a greater than 20% difference from the baseline VAF) after domvanalimab plus zimberelimab treatment occurred across all recurrently mutated genes, with the exception of *PIK3CA*, *FGFR2*, *NRAS*, and *KRAS* where ctDNA levels demonstrated a uniform decline across patients (Fig. 2c).

AFP is a commonly used diagnostic and pharmacodynamic marker for HCC, although limitations in its specificity and sensitivity are well documented. There were weak positive correlations between baseline AFP and ctDNA levels (Spearman $r = 0.24$, p value = 0.21) and changes in AFP and ctDNA (Spearman $r = 0.18$, p value = 0.36), but neither of these associations were statistically significant (Supplementary Fig. 5). This analysis is likely underpowered due to the limited sample size of the trial. Nonetheless, among the 5 objective responses to domvanalimab plus zimberelimab, 3 patients had normal baseline and post-treatment AFP levels, but exhibited declines in ctDNA levels, suggesting that ctDNA may have greater pharmacodynamic utility in HCC with normal AFP levels.

Discussion

Results from the LIVERTI trial demonstrate that dual blockade of TIGIT and PD-1 is well-tolerated in many patients, and may lead to anti-tumor responses in anti-PD-1/L1 therapy refractory HCC. While the confirmed ORR rate of 17.2% falls short of the protocol-defined threshold, the ORR associated with domvanalimab plus zimberelimab compares favorably to the ORR observed with existing second line HCC treatments. Confounding the comparison of our results with historic data is that all currently approved second line drugs were tested in immunotherapy-naïve patients. Nonetheless, in recent prospective trials testing cabozatinib and regorafenib in immunotherapy refractory HCC demonstrated unconfirmed ORRs of 6.1% and 7.4%, respectively, which is substantial less than the ORR associated with domvanalimab plus zimberelimab[19,20]. In addition, rates of AEs and TRAEs associated with domvanalimab plus zimberelimab in the post-frontline setting across both Child-Pugh A and B disease were less than those observed with tyrosine kinase inhibitors and approved

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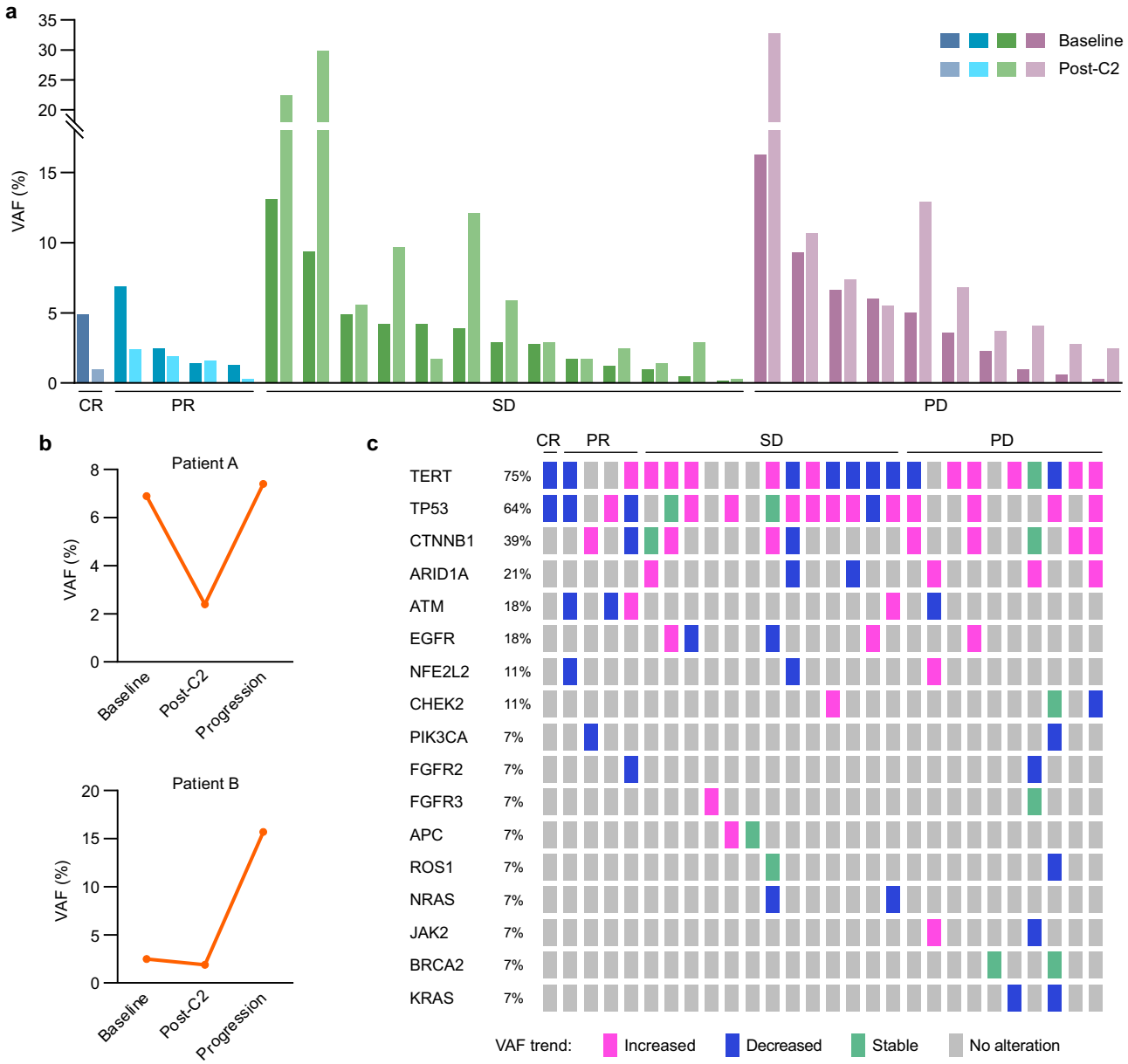

Fig. 2 | ctDNA dynamics associated with domvanalimab plus zimberelimab treatment in refractory HCC. a ctDNA levels at baseline and post-cycle 2 among patients grouped by response categories. VAF, variant allele frequency. CR, complete response. PR, partial response. SD, stable disease. PD, progressive disease. **b** Longitudinal monitoring of ctDNA levels among two patients who attained a PR followed by eventual progression. **c** Heatmap depicting ctDNA dynamics for recurrent genetic alterations in individual patients. Each column depicts a patient with serial ctDNA testing organized by response category. Each row indicates changes in the VAF of ctDNA collected post-cycle 2 compared to baseline. Only genes mutated in more than one patient were analyzed. A 20% change from the baseline VAF was used to determine whether ctDNA levels of individual alterations were increased, decreased, or stable.

combination immunotherapy regimens. For instance, serious or grade 3–4 TRAEs were reported in 44%, 57%, 53%, and 43% of patients treated with regorafenib, lenvatinib, ipilimumab plus nivolumab, and atezolizumab plus bevacizumab[2,21–23]. In contrast, the rate of grade 3–4 TRAEs observed with domvanalimab plus zimberelimab was only 10.3%, which is more in line with rates of high grade TRAEs observed in prior monotherapy trials of nivolumab (16%) and pembrolizumab (24%)[24,25]. A caveat to the safety data of the LIVERTI trial is that patients with high grade toxicities to prior immunotherapies were excluded, and the safety of domvanalimab plus zimberelimab in a broader patient population remains to be further clarified. Nonetheless, combined targeting of TIGIT and PD-1 may represent a promising therapeutic strategy for HCC, particularly as safe alternatives to other immunotherapy combinations or second line VEGF inhibitors.

Our results clarify ambiguities on the therapeutic utility of anti-TIGIT antibodies in HCC. First, given that the LIVERTI trial only enrolled anti-PD-1/L1 therapy refractory cancers, the ORR associated with domvanalimab plus zimberelimab may possibly be ascribed to the synergy of combined TIGIT and PD-1 inhibition given that anti-TIGIT antibodies are not associated with tumor responses when used as monotherapies[26,27]. However, corroboration of anti-TIGIT and -PD-1 inhibition synergy would require disambiguation of additive or independent action effects in a confirmatory study. Second, while prior studies suggest that intact Fc domains contribute to anti-tumor responses in anti-TIGIT antibodies, results from a prior randomized trial in non-small cell lung cancer and from the LIVERTI study provide evidence that the Fc-silent antibody domvanalimab is capable of enhancing anti-tumor immunity[28,29]. Whether the functionality of the

Fc domain induces differential effects in human tumors and is tissue context dependent remains to be elucidated by a direct comparison of antibodies of varying designs in a future clinical study.

Correlative studies of the LIVERTI trial demonstrate that ctDNA levels may be prognostic in advanced stage HCC, and dynamic changes in ctDNA VAF may represent early pharmacodynamic markers of response to immunotherapies. No baseline genetic alterations were identified to be predictive of domvanalimab plus zimberelimab response, indicating that the clinical use of domvanalimab plus zimberelimab may not require specific genetic markers for patient selection. The VAF of several driver genes such as in *PIK3CA*, *FGFR2*, *N/KRAS* were consistently decreased after domvanalimab plus zimberelimab treatment in multiple patients, suggesting that cancer clones harboring these mutations may be vulnerable to dual TIGIT and PD-1 blockade. Given the exploratory nature of our analysis with a sample size powered by clinical endpoints rather than biomarker measurements, an analysis of a larger patient cohort is warranted to corroborate these findings. Other tumor and immune determinants of anti-TIGIT therapy efficacy in HCC remains to be resolved, including the identity of the specific immune cells that mediate anti-tumor effects after TIGIT inhibition.

There are several limitations to this study, including its small sample size, lack of randomization to a control treatment, and the exploratory nature of the clinical findings and correlative analyses. In addition, the number of patients with Child Pugh B disease enrolled in the LIVERTI trial was small, which may confound the analysis of PFS and precludes definitive interpretation on the efficacy or lack thereof in this patient population. Nonetheless, a notable aspect of the LIVERTI trial which may underlie its generalizability to heterogenous populations is the high enrollment of underrepresented demographics, particularly in HCC where trials predominantly include Asian and White patients, and a high diversity of disease etiologies represented among enrolled patients.

Methods

Study design and endpoints

The study was approved by the UT Southwestern institutional review board, and all patients provided written informed consent prior to enrollment. This study was an investigator-initiated phase 2, open-label basket trial conducted at 2 clinical sites (UT Southwestern Harold C. Simmons Comprehensive Cancer Center and Parkland Health). One arm of the study enrolled patients with advanced stage biliary tract cancers, and one arm of the study enrolled patients with advanced stage HCC, which is reported here. The study design and conduct complied with all relevant regulations regarding the use of human study participants and was conducted in accordance with the criteria set by the Declaration of Helsinki. This study was registered at Clinicaltrials.gov on February 2, 2023 (https://clinicaltrials.gov/study/NCT05724563). The trial protocol is available as a Supplementary Note in the Supplementary Information file. There were no deviations that affected the trial design.

The primary endpoint was the confirmed objective response rate (ORR) per Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 of all evaluable patients receiving at least one dose of domvanalimab and zimberelimab. ORR was defined as number of patients with a complete response (CR) or partial response (PR) that was confirmed on imaging greater than or equal to 4 weeks after initial response. Secondary endpoints included safety, 6-month progression-free survival, overall survival, and duration of response, of which the latter two will be reported in a subsequent analysis due to limited follow-up time at the time of analysis for the primary endpoint. Exploratory endpoints included correlative studies between treatment response and outcomes with clinical and laboratory studies. This study followed the Transparent Reporting of Evaluations With Nonrandomized Designs (TREND) reporting guideline, and was conducted in compliance with the trial protocol.

Statistical considerations

This study is exploratory because of an absence of data on the efficacy of treatments in hepatobiliary cancers previously treated with immunotherapies and on the efficacy of anti-TIGIT therapies used in any line of treatment for hepatobiliary cancers at the time of study development. Thus, a sample size of 29 evaluable patients was determined based on statistical considerations and feasibility. This design yields a type I 1-sided error rate of 5.5% and power of 85.9% if the observed response rate is 20% compared to a null benchmark of 5%. Justification for the null hypothesis in HCC is based on the objective response rate of 4% with cabozatinib in the second line setting among patients previously treated with sorafenib in a phase 3 trial.

Participants

Patients were enrolled between 5 June 2023 to 12 December 2023 at a National Cancer Institute-designated cancer center (UT Southwestern Harold C. Simmons Comprehensive Cancer Center) and a county safety-net hospital system (Parkland Health). Major inclusion criteria included age 18 years or older, histologically confirmed diagnosis of HCC (excluding fibrolamellar and combined subtypes), locally advanced or metastatic disease not amenable to surgical resection, transplantation, or locoregional therapies, refractory to or relapsed after prior anti-PD-1/L1 therapy as any line of therapy (patients who discontinued prior anti-PD-1/L1 therapy due to toxicities were not eligible), Child-Pugh A or B7/8 disease, ECOG status of 0 or 1, adequate laboratory values (total bilirubin ≤3.0 mg/ml, international normalized ratio ≤1.7, hemoglobin ≥8.5 g/dl, aspartate transaminase and alanine transaminase ≤5 times upper limits of normal, platelet count ≥50,000/mm3, serum creatinine ≤1.5 mg/dL or creatinine clearance ≥50 mL/min, albumin ≥2.0 g/dl, absolute neutrophil ≥1000 cells/mm3), agreed to use an adequate method of contraception through the course of the study and 120 days after the last dose of study medication (male and female subjects of child bearing potential), and a negative pregnancy test if the subject was a woman of child bearing age. Major exclusion criteria included a prior liver transplant, recent history of a major surgery, underlying medical conditions that would make treatment hazardous, active autoimmune disease that requires systemic treatment within the past 2 years, active infections, medical conditions requiring systemic treatment with either corticosteroids (>10 mg/day prednisone equivalent) or other immunosuppressive medications within 14 days of study administration (inhaled or topical steroids and adrenal replacement doses >10 mg/day prednisone equivalents are permitted in the absence of autoimmune disease), symptomatic or clinically active brain metastases, and pregnancy (defined as state of a female after contraception and until the termination of gestation, confirmed by a positive hCG laboratory test) or nursing (lactating) women.

Procedures

Domvanalimab (1200 mg) and zimberelimab (360 mg) were administered as consecutive 60-minute IV infusions on Day 1 of each 21-day cycle. Treatment was administered until disease progression, discontinuation due to toxicity, withdrawal of consent, or study termination. No dose reductions were allowed. Dose interruptions were permitted for immune-related toxicities, and treatment was resumed once the event resolved (grade 1 or less) and any corticosteroids used for toxicity management had been tapered. Prohibited concomitant medications or treatments during the screening and treatment phase of this trial included other antineoplastic systemic chemotherapy or biological therapy, other immunotherapy agents, other investigational agents, radiation therapy of target lesions, live vaccines, and systemic glucocorticoids for any purpose other than to

modulate symptoms from an event of clinical interest of suspected immunologic etiology.

Response and progression were evaluated using RECIST 1.1 as determined by the investigator. Patients were evaluated by imaging for progression every 9 weeks following the initial study drug administration. Imaging consisted of computed tomography scans, or magnetic resonance imaging scans of the abdomen and pelvis with triple-phase acquisition. CT Chest or additional imaging was at the discretion of the investigator based on clinical suspicion of progressive disease.

All patients receiving at least one dose of study drugs were evaluated in the safety analysis regardless of their evaluability for the primary ORR endpoint. Treatment-related adverse events were defined as any adverse event at least possibly attributed to study drugs per the protocol that occurred between the first dose of study drugs through 30 days after the last dose of study drugs. Adverse events occurring beyond 30 days at least possibly attributed to study drugs per the investigator was also reported. Adverse events were graded according to National Cancer Institute Common Terminology Criteria for AEs, v.5.0. All serious AE occurrences, attribution, and management were reviewed by the internal gastrointestinal oncology research committee composed of a multidisciplinary team of oncology providers and the UTSW Data and Safety Monitoring Committee.

ctDNA analysis

Peripheral blood specimens were collected in Streck Cell-Free DNA tubes prior to starting treatment (baseline) and then approximately 6–8 weeks (post-cycle 2) after treatment initiation. Baseline specimens were obtained on all 29 enrolled patients, while serial specimens were available for 28 patients as one patient missed their second blood draw collection. Samples were shipped to a Clinical Laboratory Improvement Act (CLIA)-certified, College of American Pathologists-accredited laboratory (Guardant Health, Redwood City, CA). ctDNA was assessed using a targeted high throughput hybridization-based capture technology for the detection of single nucleotide variants, insertions, and deletions in 83 genes by paired-end synthesis-sequencing using the NextSeq 500 and/or HiSeq 2500 platforms (Illumina, Inc.)[30]. Putative germline mutations including variants identified by allele fractions between 40% and 60%, prior annotation as germline mutations, and manual review were excluded from analyses. ctDNA was detected in all 29 patients based on the baseline specimen harboring any alteration with a VAF of at least 0.01%, which is the limit of detection for the assay used. TMB was calculated by counting all somatic nonsynonymous and single nucleotide variants and delins across 1 Mb of coding regions and then algorithmically adjusted to correct for confounding biological and technical sample features as previously described[30]. Changes in ctDNA variant allele frequencies (VAF) for specific gene alterations over time were classified as increased (rise in VAF by 20% or greater(not detectable at baseline but detectable at any VAF at progression, increasing (detectable at baseline with rise in VAF by 20% or greater at progression), stable (detectable at baseline with less than 20% increase or decrease in VAF at progression), decreasing (detectable at baseline with decline in VAF by 20% or greater at progression), or lost (detectable at baseline at any VAF but not detectable at progression). CNAs were first categorized on a semi-quantitative scale as low (below the 50th percentile of amplifications detected by the assay), medium (between the 50th and 90th percentile) or high (above the 90th percentile). CNAs were then classified as emergent (not detectable at baseline but detectable at any amplification at progression), increasing (detectable at baseline with an increase in amplification category), stable (detectable at baseline with no change in amplification category), decreasing (detectable at baseline with a decrease in amplification category), or lost (detectable at baseline but not detectable at progression). Due to the qualitative measurement of CNAs and inability to directly convert copy gains or loses into VAF, CNAs were not assessed in determining changes in VAF dynamics.

Statistical analysis

ORR was defined as the percentage of patients with CRs and PRs that were subsequently confirmed on follow-up restaging scan. The disease control rate was defined as the percentage of patients with SD or confirmed CR/PRs as their best response per RECIST1.1. PFS was estimated using the Kaplan-Meier method. 95% confidence intervals for the median PFS and 6-month PFS rate were calculated using the Greenwood method. Descriptive analyses were used to describe treatment-related adverse events. To assess the association between ORR and clinical characteristics, odds ratio confidence intervals were calculated using the Baptista-Pike method. Fisher's exact tests were used to assess statistically significant associations between tumor response and baseline molecular alterations. Due to the small sample size of patients within each response category, ctDNA dynamics were descriptively characterized among patients with CR/PR, SD, and PD. Statistical analyses were performed using SPSS 28 (IBM); p values were from 2-sided tests and results were deemed statistically significant at $p < 0.05$. Because correlative studies conducted were exploratory, analyses were not corrected for multiple-testing.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The trial protocol is available as a Supplementary Note in the Supplementary Information file. Individual de-identified clinical data may be requested for research purposes only through the corresponding author David Hsiehchen (David.hsieh@utsouthwestern.edu), which will require the approval of the institutional review board. Written requests that include data required and study purpose will be responded to within 6 weeks. Circulating tumor DNA profiling data are provided in the source data. Remaining data are available in the manuscript and Supplementary Information. Source data are provided with this paper.

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Author contributions

D.H. conceived the study. D.H. supervised the study. R.K., H.K., E.S., H.Z., and D.H. participated in patient enrollment and treatment. C.A. contributed to the statistical considerations and design of the study. All authors participated in the revision of the manuscript and approved the paper.

Competing interests

D.H. has served as a consultant for AztraZeneca. H.Z. is a co-founder of Quotient Therapeutics and Jumble Therapeutics, is an advisor for Newlimit, Alnylam Pharmaceuticals, and Chroma Medicines. H.Z. receives research support from Chroma Medicines and owns stock in Ionis and Madrigal Pharmaceuticals. The remaining authors declare no competing interests.

Additional information

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