4102

Concordance Analysis Between Tumor Tissue Human Epidermal Growth Factor Receptor 2 Status by Immunohistochemistry and In Situ Hybridization and a Translational Analysis of Plasma Circulating Tumor DNA in Patients With Biliary Tract Cancer: An Exploratory Analysis From the Phase 2 HERIZON-BTC-01 Trial

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Background

- Human epidermal growth factor receptor 2 (HER2) protein overexpression and/or *ERBB2* amplification is observed in a subset of patients with biliary tract cancer (BTC),[1,2] making it an actionable target for precision oncology[3]
- Zanidatamab, a dual HER2-targeted bispecific antibody, is designed to bind to 2 non-overlapping domains on HER2 receptor *in trans* that drives multiple antitumor mechanisms of action including[4]:
  - Facilitation of HER2 internalization and subsequent degradation
  - Reduction of HER2 on the cell surface
  - Inhibition of HER2 signaling pathways
  - Activation of immune-mediated effects (complement-dependent cytotoxicity, antibody-dependent cellular cytotoxicity, and phagocytosis)
- Zanidatamab received accelerated approval for treatment of adults with previously treated, unresectable or metastatic HER2-positive (immunohistochemistry [IHC] 3+) BTC based on the phase 2b HERIZON-BTC-01 trial[5,6]
  - Zanidatamab monotherapy demonstrated a confirmed objective response rate of 41% in patients with HER2-positive BTC, along with manageable safety and favorable tolerability profiles[7]

Objectives

- This exploratory analysis evaluated:
  - The concordance between tissue-based IHC vs in situ hybridization (ISH) in screened patients
  - The concordance between *ERBB2* amplification status by tissue-based ISH (ratio >2.0) vs plasma circulating tumor DNA (ctDNA)-based next-generation sequencing (NGS) (copy number variation >2.2) in treated patients
  - Molecular profiling of baseline ctDNA and changes in ctDNA levels on treatment
  - Utility of ctDNA molecular response (MR) on treatment as a surrogate measure of early responses to zanidatamab

Methods

- The design of HERIZON-BTC-01 (NCT04466891) has been previously published[6]
- Patients with *ERBB2* amplification (VENTANA® HER2 Dual ISH DNA Probe Cocktail assay) were prospectively assigned to cohorts by HER2 IHC score (Ventana PATHWAY® [4B5] IHC assay): cohort 1 for IHC 2+ or 3+; cohort 2 for IHC 0 or 1+
  - Centrally confirmed HER2 status was assessed in a fresh biopsy or an archived sample per American Society of Clinical Oncology/College of American Pathologists guidelines,[8] with gastric cancer algorithm
  - Eligible patients received zanidatamab 20 mg/kg intravenously once every 2 weeks in 28-day cycles
- Plasma ctDNA samples were collected prior to the first cycle of zanidatamab (baseline) and on day 28 of cycle 2 (on-treatment) for NGS testing using Guardant360 (Guardant Health)
  - Guardant360 MR scores indicate changes in plasma ctDNA levels from baseline[9] and were calculated as:

$$MR = \frac{\text{mean on-treatment VAF}}{\text{mean pretreatment VAF}} - 1$$

MR, molecular response; VAF, variant allele frequency.

Concordance Between Tumor Tissue HER2 IHC and ISH Scores (Screened Patients)

HER2 IHC	HER2 ISH Status: Amplified	Non-Amplified	Total	Amplification n/N (%)
0	12	330	342	12/342 (4)
1+	8	94	102	8/102 (8)
2+	34	167	201	34/201 (17)
3+	104	7	111	104/111 (94)
Total	158	598	756	158/756 (21)

Green indicates concordance between tumor tissue HER2 IHC and ISH scores.

- A total of 756 screened patient tumors had central results for both HER2 IHC and ISH
- Nearly all of the HER2 IHC 3+ tumors had *ERBB2* amplification (94%) by ISH

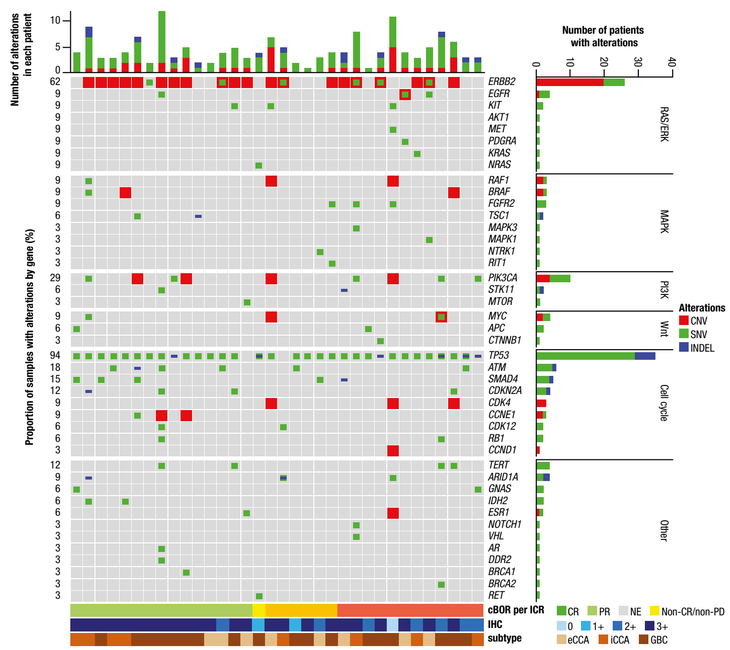
Concordance Between Tissue-based ISH and Plasma ctDNA-based NGS (Treated Patients)

	HER2 ctDNA: Amplified	Non-Amplified	Total	Total n/N (%)
HER2 ISH Interpretation				
Positive	20	14	34	20/34 (59)
Negative	0	0	0	0

Green indicates concordance between tumor-tissue HER2 ISH and ctDNA-based NGS results.

- Of 87 zanidatamab-treated patients, 34 patients had available plasma ctDNA samples for testing, of whom 20 had *ERBB2* amplification detected in plasma ctDNA

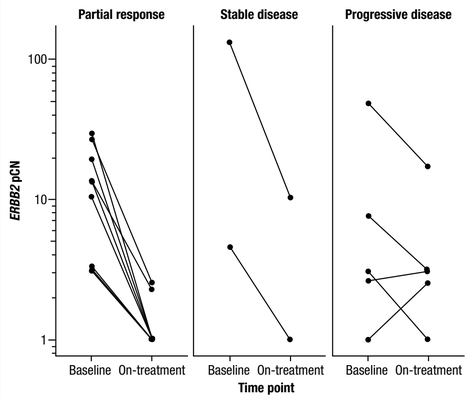
Mutational Landscape of Baseline ctDNA Samples in Zanidatamab-Treated Patients by Selected Pathway

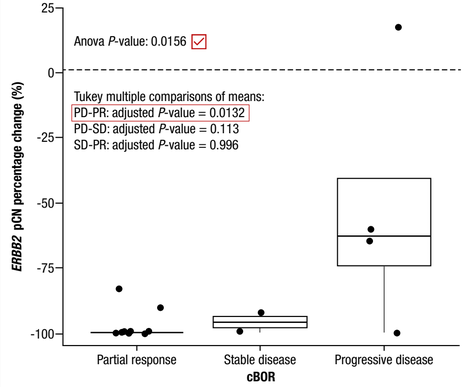


- *TP53*, *ERBB2*, and *PIK3CA* were the most frequently altered genes at baseline
- Co-mutations occurring in >10% of patients included *KRAS* (n=1 [3%]), *ERBB2* S310F (n=3 [9%]), and *PIK3CA* (n=6 [18%])
- In patients with a CR or PR, co-alterations (>2) included *ERBB2*, *CCNE1*, *IDH2*, *TSC1*, and *CDK12*
- In non-responders, co-alterations (>2) included *TERT*, *CDK4*, *KRAS*, *PIK3A*, and *MET*
- *PIK3CA* mutations may contribute to the activation of the PI3K–AKT pathway in BTC and are the second most common co-alteration in patients without a CR or PR
- Mutated HER2 (*ERBB2* S310F) can form active heterodimers with EGFR; 2 patients harboring this mutation responded to zanidatamab

AKT, protein kinase B; cBOR, confirmed best overall response; CNV, copy number variation; eCCA, extrahepatic cholangiocarcinoma; EGFR, epidermal growth factor receptor; GBC, gallbladder carcinoma; iCCA, intrahepatic cholangiocarcinoma; ICR, independent central review; IHC, immunohistochemistry; INDEL, insertion-deletion; NE, not evaluable; PI3K, phosphatidylinositol 3-kinase; SNV, single nucleotide variant.

Changes in *ERBB2* Plasma Copy Number Correlate With Tumor Responses After Zanidatamab Treatment

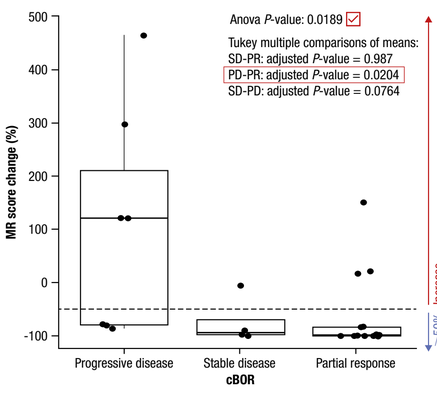




Percent change in *ERBB2* pCN was calculated as a ratio of on-treatment levels of *ERBB2* pCN vs the baseline levels. Red checkmark and box indicate statistical significance.
pCN, plasma copy number.

- Of the 25 patients, 18 (72%) had a decrease in ctDNA levels from baseline to on-treatment, including 12 (48%) patients with a PR to zanidatamab treatment
- Decreases of >90% in *ERBB2* plasma copy number were observed in patients with a best response of PR (*P*-value = 0.0132)

Changes in MR Scores Correlate With Tumor Responses After Zanidatamab Treatment



MR assesses changes in on-treatment ctDNA levels from baseline with a cutoff of >50% decrease in mean VAF.[9] Red checkmark and box indicate statistical significance.

- Differences in MR scores between patients with a PR and those with progressive disease were statistically significant
- Patients with a ⩾50% decrease in on-treatment MR score had a numerically greater progression-free survival vs patients with a <50% decrease (5.6 vs 2.9 months), although the difference between these patient groups was not significant

Conclusions

- In this exploratory analysis, there was a high concordance of 94% observed between tumor tissue HER2 IHC 3+ status and *ERBB2* amplification by ISH of all (n=756) screened patients from HERIZON-BTC-01
- In treated patients, 59% concordance was observed between *ERBB2* amplification in plasma ctDNA by NGS and tumor tissue by ISH
- Translational analysis shows that after 2 treatments with zanidatamab, a decrease in both plasma ctDNA levels and MR score may be predictive of response in the majority of patients
- ctDNA testing offers a non-invasive way to detect tumor alterations, but its limitations may include low sensitivity in early stage disease and variability in shedding
  - These findings are also limited by the small sample size

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