33

Molecular circulating tumor DNA profiling from patients treated with zanidatamab + chemotherapy in first-line HER2-positive advanced or metastatic gastroesophageal adenocarcinoma

Elena Elimova[1], Geoffrey Ku[2], Keun-Wook Lee[3], Sun Young Rha[4], Sara Wienke[5], Geethika Yalamanchili[5], Phillip M Garfin[6], Emanuele Loro[7], Diana Shpektor[6], Jaffer Ajani[8]

[1]Princess Margaret Cancer Centre, ON, Canada; [2]Memorial Sloan Kettering Cancer Center, New York, NY, USA; [3]Seoul National University Bundang Hospital, Seoul National University College of Medicine, Seongnam, South Korea; [4]Yonsei Cancer Center, Yonsei University College of Medicine, Seoul, South Korea; [5]Guardant Health, Palo Alto, CA, USA; [6]Jazz Pharmaceuticals, Palo Alto, CA, USA; [7]Jazz Pharmaceuticals, Cambridge, UK; [8]The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Objective

- To identify potential predictive and prognostic biomarkers, and to define exploratory immune score signatures from plasma circulating tumor DNA (ctDNA) molecular profiling within the context of the predicted tumor microenvironment (TME) immune state (eg, desert, excluded, inflamed) correlating with durable responses or resistance for zanidatamab + chemotherapy treatment in patients with human epidermal growth factor receptor 2–positive (HER2+) advanced or metastatic gastroesophageal adenocarcinoma (mGEA)

Conclusions

- Plasma ctDNA *ERBB2* amplification may be a predictive biomarker for zanidatamab + chemotherapy benefit
- Antitumor activity was observed for zanidatamab + chemotherapy for the majority of patients with baseline *ERBB2* and *PIK3CA*-activating alterations
- On-treatment ctDNA clearance may be an early pharmacodynamic marker
- These results help support a proposed mechanism in which HER2-driven immune-accessible tumors may be primed by zanidatamab + chemotherapy, with potential conversion from immune excluded/desert to inflamed TME states favoring immuno-oncology (IO) combination (such as zanidatamab with a PD-1 inhibitor)
- Further analyses are warranted to confirm these results

References: **1.** Weisser NE, *et al. Nat Commun.* 2023;14(1):1394. **2.** Elimova E, *et al. J Clin Oncol.* 2026;44:LBA285. **3.** Elimova E, *et al. Lancet Oncol.* 2025;26(27):947-59. **4.** Lee K-W, *et al. Clin Cancer Res.* 2026;32(3):512-23. **5.** Liang S, *et al. Cancer Res Commun.* 2025;5(8):1384-95. **6.** Gordon S, et al. Presented at AACR Annual Meeting; April 17–22, 2026; San Diego, CA, USA. Abstract 100. **7.** Chen DS, *et al. Nature.* 2017;541:321-30. **8.** Mashukov A, *et al. World J Gastroenterol.* 2021;27(31):5259-71. **9.** Tiwari A, *et al. Front Immunol.* 2023;14:1084887. **10.** Zheng S, *et al. Exp Hematol Oncol.* 2024;13(1):80.

Support and Acknowledgments: This research was funded by Jazz Pharmaceuticals. Under direction of the authors, Charlotte Pettigrew, PhD, CMPP, of Red Nucleus, provided medical writing and editorial support, which were funded by Jazz Pharmaceuticals in accordance with Good Publication Practice (GPP 2022) guidelines (https://www.ismpp.org/gpp-2022). Jazz Pharmaceuticals also reviewed and edited the poster for scientific accuracy.

Scan here to access the poster on the ASCO platform

Scan here to access the Supplemental Information

Corresponding author: Elena Elimova, MD
Elena.Elimova@uhn.ca

Poster presented at the American Society of Clinical Oncology Annual Meeting; May 29–June 2, 2026; Chicago, IL, USA, and online.

Introduction

- Zanidatamab is a HER2-directed bispecific antibody that binds to the HER2 extracellular domains 2 and 4 in a *trans* configuration, facilitating the formation of distinct HER2 clusters on the cell surface. It has several key mechanisms of action:[1]
  - Increasing HER2 internalization
  - Reducing phosphorylation of EGFR, HER2, and HER3 and blocking downstream signaling
  - Inducing immune-mediated effects (complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity and phagocytosis)
- In the phase 3 HERIZON-GEA-01 trial, first-line (1L) zanidatamab + chemotherapy ± tislelizumab significantly prolonged PFS and, with tislelizumab, yielded significant OS benefits versus trastuzumab + chemotherapy in patients with HER2+ mGEA,[2] independent of PD-L1 baseline status
  - Consistent results were observed in prior phase 2 trials of 1L zanidatamab + chemotherapy in patients with HER2+ mGEA[3,4]
- HER2 status by tumor tissue testing (ASCO/CAP 2018) is critical for identifying patients who may benefit from HER2-targeted therapies like zanidatamab
  - Plasma ctDNA next-generation sequencing (NGS) is non-invasive, captures tumor heterogeneity, and provides a real-time assessment of tumor genomic and epigenomic landscape
  - Plasma ctDNA provides quantitative *ERBB2* copy number, co-alteration landscape, and methylation-based[5] and immune-related gene signatures[6] simultaneously from a single blood draw at baseline and during treatment
- Here, we report ctDNA molecular profiling results from patients with HER2-expressing mGEA from the phase 2 trial of zanidatamab + chemotherapy

Methods

- This phase 2 trial evaluated zanidatamab + chemotherapy as 1L treatment for patients with HER2-expressing mGEA (NCT03929666)
- Of samples used for matched ctDNA profiling, central HER2 immunohistochemistry (IHC)/fluorescence in situ hybridization (FISH) testing was performed on archival (n = 5) or fresh (n = 19) baseline tumor biopsies using the Dako HercepTest (polyclonalAb) IHC and Abbott PathVysion HER2 DNA Probe FISH kit (HER2/CEP17 ratio ≥2.0)
- **Guardant360:** A 74-gene panel detecting single nucleotide variations (SNVs), insertion-deletions (indels), copy number variations (CNVs), and fusions
- **Guardant Infinity:** Extended genomic + epigenomic panel detecting SNVs, indels, CNVs, fusions, multiexon deletions, large genomic rearrangements, promoter hypermethylation across >20,000 differentially methylated regions, microsatellite instability–high status, blood-based tumor mutational burden (bTMB), sample-level ctDNA detection, and methylation-based tumor fraction (MTF) quantification with IO module
- **bTMB cutoff:** For this analysis, TMB-high was defined by the median value (≥12.34 mut/Mb). Molecular ctDNA response was defined as a ≥99% reduction in MTF from baseline to on-treatment time point
- **H&E TME classification:** Whole-slide images from archival FFPE tumor sections were classified as desert (immune-desert/non-inflamed, tumor-infiltrating lymphocyte [TIL]-low), excluded (immune cells in stroma, but absent from tumor nests), or inflamed (intratumoral TILs, high TIL density) using established histopathological criteria

Figure 1. Study design

Key eligibility criteria
- HER2-expressing (IHC 3+ or IHC 2+) mGEA by local or central assessment (part 1) or HER2+ (IHC 3+ or IHC 2+/FISH-positive) mGEA by central assessment (part 2)
- ECOG PS ≤1
- No prior HER2-targeted agents
- No prior systemic therapy except neoadjuvant/adjuvant therapy completed ≥6 months prior to first study treatment dosing

Zanidatamab + CAPOX (21-day cycle)
Zanidatamab 30 mg/kg *or* 1800 mg (<70 kg)/2400 mg (≥70 kg) Q3W

Zanidatamab + FP (21-day cycle)
Zanidatamab 30 mg/kg *or* 1800 mg (<70 kg)/2400 mg (≥70 kg) Q3W

Zanidatamab + mFOLFOX6 (28-day cycle)
Zanidatamab 20 mg/kg *or* 1200 mg (<70 kg)/1600 mg (≥70 kg) Q2W

Primary endpoints
- Safety and tolerability (part 1)
- cORR (part 2)

PFS was a secondary endpoint

Plasma ctDNA samples were collected from all patients
- At baseline (prior to C1 of zanidatamab)
- During treatment (C2D15 for zanidatamab + CAPOX or FP; C3D1 for zanidatamab + mFOLFOX6)
- At the end of treatment

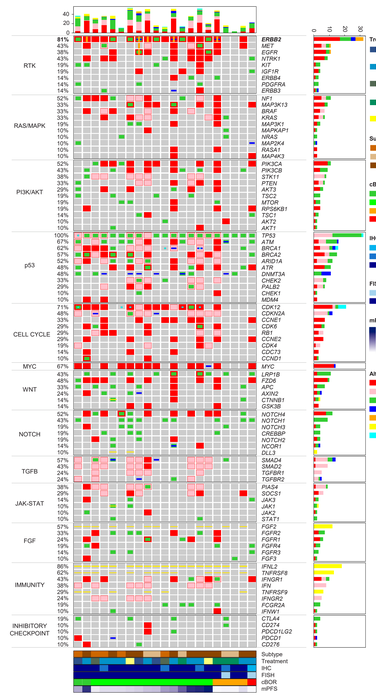
C, cycle; CAPOX, capecitabine + oxaliplatin; cORR, confirmed objective response rate; ctDNA, circulating tumor DNA; D, day; ECOG PS, Eastern Cooperative Oncology Group performance status; FISH, fluorescence in situ hybridization; FP, 5-FU + cisplatin; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; mFOLFOX6, modified leucovorin + 5-FU + oxaliplatin; mGEA, advanced or metastatic gastroesophageal adenocarcinoma; PFS, progression-free survival; Q2W, every 2 weeks; Q3W, every 3 weeks.

Results

Patient characteristics and ctDNA evaluability

- A total of 46 patients were enrolled across 3 chemotherapy cohorts: zanidatamab + mFOLFOX6 (n = 24), CAPOX (n = 20), and FP (n = 2)
- Most patients (n = 41; 89%) had centrally confirmed HER2+ mGEA (IHC 3+ or IHC 2+/FISH+); 5 patients were enrolled based on local HER2 testing only (IHC 2+ without central FISH confirmation or IHC 1+), representing the broader eligibility criteria in part 1
- Baseline ctDNA was evaluable by Guardant360 or Guardant Infinity in 39/46 patients. The Guardant Infinity methylation module was evaluable in a subset of 19–21 patients depending on the analysis (MTF, TMB, IO response score)

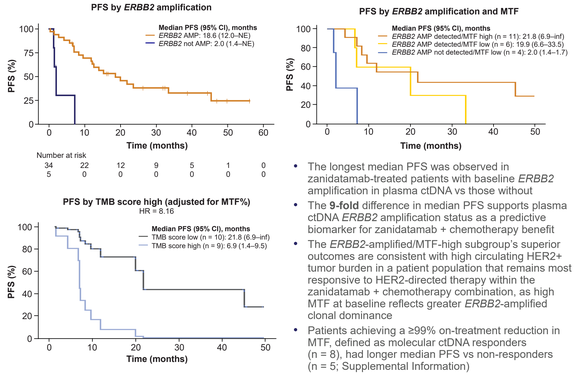
Figure 2. Baseline biomarkers of response from ctDNA NGS analysis



C, cycle; CAPOX, capecitabine + oxaliplatin; cBOR, confirmed best overall response; CNG, copy number gain; CNL, copy number loss; CR, complete response; ctDNA, circulating tumor DNA; D, day; FISH, fluorescence in situ hybridization; FP, 5-FU + cisplatin; GEJ, gastroesophageal junction; IHC, immunohistochemistry; Indel, insertion-deletion; LGR, large genomic rearrangement; mFOLFOX6, modified leucovorin + 5-FU, + oxaliplatin; mPFS, median progression-free survival; NGS, next-generation sequencing; PD, progressive disease; PR, partial response; PrMet, promoter methylation; Q2W, every 2 weeks; Q3W, every 3 weeks; SD, stable disease; SNV, single nucleotide variation.

- High concordance (36/38 patients; 94.7%) was observed for *ERBB2* amplification status in plasma ctDNA by NGS vs in tumor tissue by FISH
- Of 19 patients with baseline *ERBB2* mutations (SNV, indel, and/or fusion), 14 showed responses to zanidatamab + chemotherapy
  - Both patients with baseline *PIK3CA* SNVs showed a response

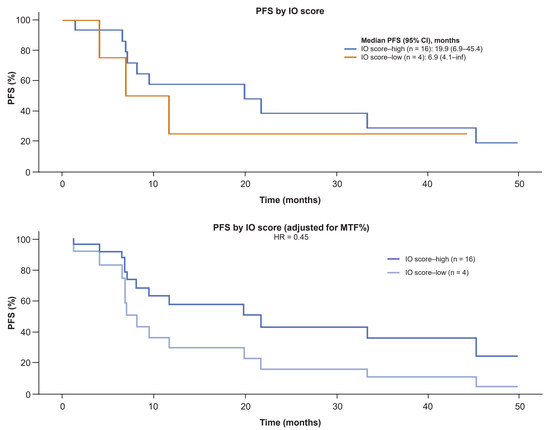
Figure 3. Predictive biomarkers derived from baseline ctDNA



- The longest median PFS was observed in zanidatamab-treated patients with baseline *ERBB2* amplification in plasma ctDNA vs those without
- The **9-fold** difference in median PFS supports plasma ctDNA *ERBB2* amplification status as a predictive biomarker for zanidatamab + chemotherapy benefit
- The *ERBB2*-amplified/MTF-high subgroup's superior outcomes are consistent with high circulating HER2+ tumor burden in a patient population that remains most responsive to HER2-directed therapy within the zanidatamab + chemotherapy combination, as high MTF at baseline reflects greater *ERBB2*-amplified clonal dominance
- Patients achieving a ≥99% on-treatment reduction in MTF, defined as molecular ctDNA responders (n = 8), had longer median PFS vs non-responders (n = 5; Supplemental Information)

TMB score cutoff was set at >16 mut/Mb. TMB adjusted Kaplan-Meier analysis by MTF (n = 19) was performed to stratify patients by the median bTMB identified in the cohort (12.34 mut/Mb). AMP, amplified; bTMB, blood-based TMB; ctDNA, circulating tumor DNA; MTF, methylation-based tumor fraction; PFS, progression-free survival; TMB, tumor mutational burden.

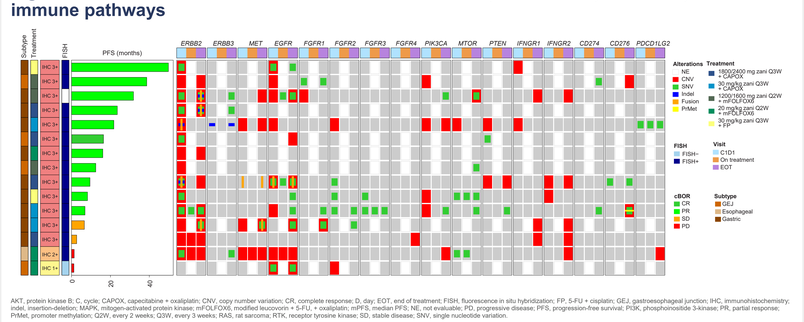
Figure 4. PFS by exploratory IO response score



IO response score was based on the methylation patterns of 41 genes associated with IO response as well as plasma-derived TMB and MSI status.
ctDNA, circulating tumor DNA; HR, hazard ratio; IO, immuno-oncology; MSI, microsatellite instability; MTF, methylation-based tumor fraction; PFS, progression-free survival.

- Numerically longer median PFS was observed in patients with IO score–high versus IO score–low; however, patient numbers are small
- Further optimization on a larger number of GEA tumor–specific samples will be needed

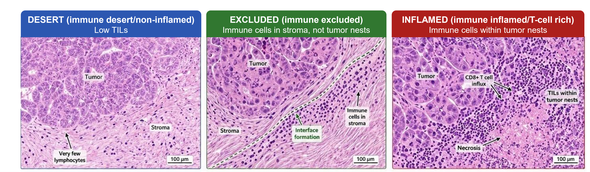
Figure 5. On-treatment ctDNA co-alterations across RTK, RAS/MAPK, PI3K/AKT, and immune pathways



AKT, protein kinase B; C, cycle; CAPOX, capecitabine + oxaliplatin; CNV, copy number variation; CR, complete response; D, day; EOT, end of treatment; FISH, fluorescence in situ hybridization; FP, 5-FU + cisplatin; GEJ, gastroesophageal junction; IHC, immunohistochemistry; Indel, insertion-deletion; MAPK, mitogen-activated protein kinase; mFOLFOX6, modified leucovorin + 5-FU, + oxaliplatin; mPFS, median PFS; NE, not evaluable; PD, progressive disease; PFS, progression-free survival; PI3K, phosphoinositide 3-kinase; PR, partial response; PrMet, promoter methylation; Q2W, every 2 weeks; Q3W, every 3 weeks; RAS, rat sarcoma; RTK, receptor tyrosine kinase; SD, stable disease; SNV, single nucleotide variation.

- Patients with complete response or partial response showed an on-treatment reduction in detectable *ERBB2* CNVs
- At EOT/progression, emergent bypass RTK alterations were identified across multiple genes, including *EGFR* amplification (most frequent), *MET* focal amplification, *FGFR1/2/3/4* amplification, *PIK3CA* activating mutations, and *MTOR* amplification
- Few patients with absent baseline signal showed EOT alterations in *CD274* (PD-L1) and *PDCD1LG2* (PD-L2) and changes in promoter methylation status of immune genes (Supplemental Information), which suggests that zanidatamab + chemotherapy may induce PD-L1 expression changes after treatment

Figure 6. Proposed hypothetical mechanism of action based on observed gene alterations and expected pathway engagement across TME states in gastric cancer[7-10]



	DESERT (immune desert/non-inflamed) Low TILs	EXCLUDED (immune excluded) Immune cells in stroma, not tumor nests	INFLAMED (immune inflamed/T-cell rich) Immune cells within tumor nests
Zani Mechanism of Action (Predominant)	• Limited pre-existing immune cells; zani acts primarily through direct tumor targeting • HER2 clustering → internalization and signaling inhibition • Complement activation → CDC • Minimal ADCC/ADCP due to low number of effector cells **Role: Immune initiator – starts tumor killing and antigen release**	• Immune cells present in stroma but excluded from tumor nests • Zani engages FcγR on NK cells and macrophages at tumor-stroma interface • ADCC (NK cell–mediated killing) • ADCP (macrophage phagocytosis) • Antigen release and chemokine induction drive immune cell recruitment into tumor nests **Role: Immune recruiter – converts excluded TME to inflamed**	• Pre-existing T cells and innate immune cells in tumor nests • Strong ADCC (NK) and ADCP (macrophage) amplify tumor killing • Extensive tumor cell death and antigen spreading • Synergy with PD-1 blockade to release adaptive immune resistance **Role: Immune amplifier – maximizes Fc-effector and T-cell–mediated killing**
Expected Downstream Effects	↓ IFNγ: Low to modest increase ↓ T-cell infiltration: Low to modest increase (delayed) ↑ PD-L1 expression: Low to modest increase **Immune activation begins slowly**	↑ IFNγ: Moderate increase ↑ T-cell infiltration: Moderate increase (progressive) ↑ PD-L1 expression: Moderate increase (adaptive response) **Progressive immune activation; T-cell influx increases**	↑ IFNγ: High increase ↑ T-cell infiltration: High increase (sustained) ↑ PD-L1 expression: High increase (strong adaptive resistance) **Robust immune activation and tumor killing**

AI-generated representation of gastric H&E TME immune types informed by The Cancer Genome Atlas.
ADCC, antibody-dependent cellular cytotoxicity; ADCP, antibody-dependent cellular phagocytosis; AI, artificial intelligence; CD, cluster of differentiation; CDC, complement-dependent cytotoxicity; IFN, interferon; H&E, hematoxylin and eosin; NK, natural killer; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; TIL, tumor-infiltrating lymphocyte; TME, tumor microenvironment; zani, zanidatamab.

- ZW25-201 Guardant Infinity ctDNA integration: HER2-driven, immune-accessible tumors may be primed by zanidatamab + chemotherapy, with potential conversion from immune excluded or desert TME immune state to inflamed favoring the IO combination