4542

Zanidatamab modulates multiple pathways involved in tumor growth and survival and is efficacious post–T-DXd

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Objective

- To assess the specificity of zanidatamab binding to HER2 and to characterize the impact of zanidatamab on cellular signaling pathways and antitumor activity after T-DXd exposure in a xenograft model of HER2-positive breast cancer

Conclusions

- The Fab region of zanidatamab binds the ECD2 domain of HER2, and the scFv region binds the ECD4 domain
- Zanidatamab inhibited cell cycle and DNA repair pathways at early time points in a dose-dependent manner
- Zanidatamab altered profiles of kinases involved in DNA repair, cell cycle, and MAPK pathways
  - Multiomics analysis confirmed the impact of zanidatamab on multiple cellular pathways
- Zanidatamab was efficacious in tumors pretreated with T-DXd
- Zanidatamab bound to the wildtype HER2 ECD with higher affinity than the individual one-armed antibodies due to avidity effects

References: **1.** Iqbal N, et al. *Mol Biol Int*. 2014;2014:852748. **2.** ENHERTU (fam-trastuzumab deruxtecan-nxki). Prescribing Information. Daiichi Sankyo, Inc. Basking Ridge, NJ 07920. Accessed March 30, 2026. https://daiichisankyo.us/prescribing-information-portlet/getPIContent?productName=Enhertu&inline=true. **3.** Weisser N, et al. *Nat Commun*. 2023;14(1):1394.

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 was 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.

Disclosures: AK, EL, AHK, EC, RH, and **KV** are employees of Jazz Pharmaceuticals and own stock in Jazz Pharmaceuticals. **DL**, **NW**, **GD**, and **PR** are employees of Zymeworks, Inc., and own stock in Zymeworks, Inc.

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Presented at American Association for Cancer Research (AACR) Annual Meeting; April 17–22, 2026; San Diego, CA, USA

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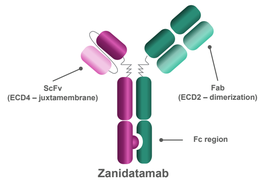
Introduction

- Human epidermal growth factor receptor 2 (HER2) signaling is a key driver in the pathophysiology of HER2-overexpressing cancers[1]
  - Signaling is mediated through receptor homo- and heterodimerization
  - HER2 overexpression leads to constitutive receptor activation and signaling, which drives tumorigenesis and progression
- Trastuzumab deruxtecan (T-DXd) is an approved second-line therapy for several HER2-positive cancers but is associated with toxicities; there is an unmet need for treatment options for patients who are intolerant to or who experience disease progression on T-DXd[2]
- Zanidatamab is an immunoglobulin G1–like bispecific antibody designed to bind 2 distinct epitopes of HER2 (extracellular domain [ECD]2 and ECD4) in a trans configuration[3]
  - Zanidatamab is asymmetric; fragment antigen-binding (Fab) and single-chain fragment variable (scFv) make up the 2 arms of the antibody
  - Crosslinking of HER2 proteins by zanidatamab induces receptor clustering and HER2 downregulation, reducing downstream signaling
  - Zanidatamab mediates effector functions, including complement-dependent cytotoxicity, antibody-dependent cellular cytotoxicity, and antibody-dependent cellular phagocytosis

Figure 1. Zanidatamab structure

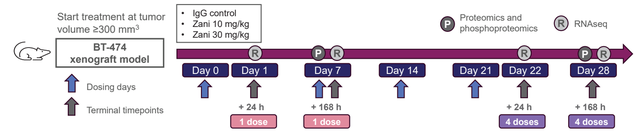


ECD, extracellular domain; Fab, fragment antigen-binding; Fc, fragment crystallizable; scFv, single-chain fragment variable.

Methods

- Surface plasmon resonance was used to determine the affinity of zanidatamab and the one-armed antibodies that comprise the Fab and scFv regions of zanidatamab to wildtype HER2 ECDs and mutein HER2 ECDs
- A multiomics analysis including whole transcriptomics, proteomics, and phosphoproteomics was performed on BT-474 *ERBB2*-amplified breast cancer xenograft tumors exposed to zanidatamab as shown in Figure 2
  - RNA sequencing (RNAseq) data were analyzed using gene set enrichment analysis (GSEA) and gene set variation analysis
  - Kinase activity was inferred from phosphoproteomics data using GSEA from samples collected 168 hours following zanidatamab treatment, and kinase-substrate relationships were obtained from the PhosphoSitePlus database
  - Multiomics integration was used to combine the experimental RNAseq, proteomics, and phosphoproteomics data with prior curated knowledge (Omnipath networks) to project the experimental data onto biologically relevant, mechanistical networks

Figure 2. Multiomics experimental design



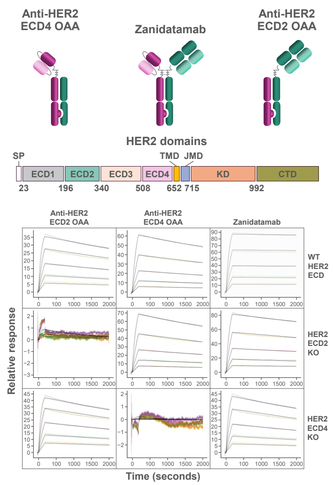
IgG, immunoglobulin G; RNAseq, RNA sequencing; Zani, zanidatamab.

Efficacy evaluation of zanidatamab following T-DXd

- The efficacy of zanidatamab following T-DXd exposure was assessed using the BT-474 xenograft model
  - Female NSG mice were implanted with BT-474 xenografts and received T-DXd 6 mg/kg intravenously (IV) on days 17, 27, and 37 post-implant and zanidatamab 10 mg/kg IV on days 70, 74, 78, and 82 post-implant, and tumor volume was assessed
  - HER2 status was assessed using immunohistochemistry (IHC) based on 2023 American Society of Clinical Oncology/College of American Pathologists guidelines (IHC 0, 1+, 2+, or 3+)

Results

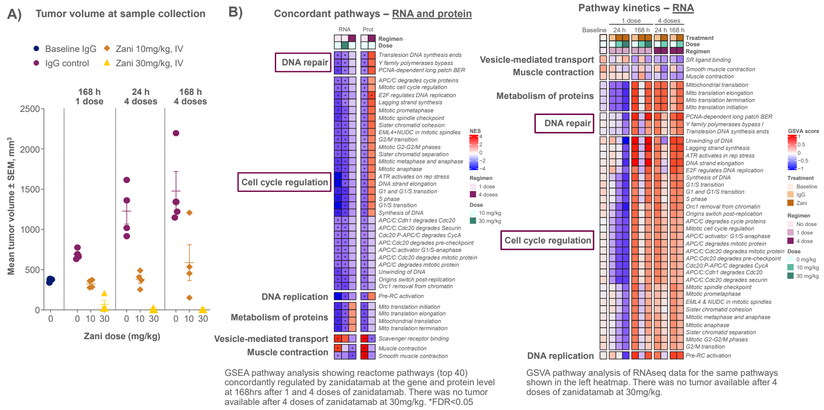
Figure 3. Zanidatamab specifically binds to ECD2 and ECD4



Target protein	Mean equilibrium dissociation constant, K_d (M), ± SD		
	Anti-HER2 ECD2 OAA	Anti-HER2 ECD4 OAA	Zanidatamab
WT HER2 ECD	2.74E−10 (± 2.79E−11)	4.85E−10 (± 3.69E−11)	4.67E−11 (± 7.60E−11)
HER2 ECD2 KO	No binding	4.80E−10 (± 3.98E−11)	3.12E−10 (± 1.14E−10)
HER2 ECD4 KO	2.46E−10 (± 3.10E−11)	No binding	2.60E−10 (± 2.19E−11)

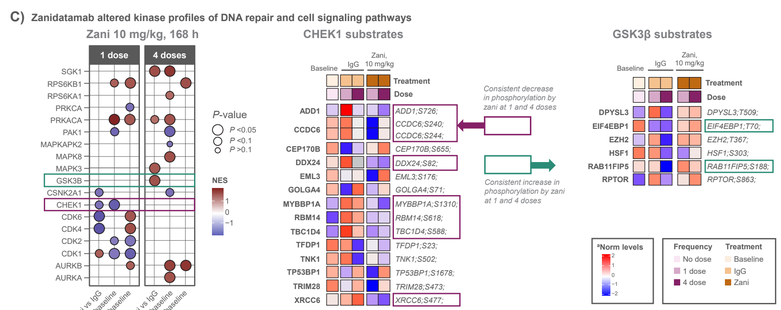
CTD, C-terminal domain; ECD, extracellular domain; HER2, human epidermal growth factor receptor 2; JMD, juxtamembrane domain; K_d, equilibrium dissociation constant; KD, kinase domain; KO, knockout; M, molar; OAA, one-armed antibody; SD, standard deviation; SP, signal peptide; TMD, transmembrane domain; WT, wild type.

Figure 4. Zanidatamab inhibited cell cycle and DNA repair pathways



GSEA pathway analysis showing reactome pathways (top 40) concordantly regulated by zanidatamab at the gene and protein level at 168hrs after 1 and 4 doses of zanidatamab. There was no tumor available after 4 doses of zanidatamab at 30mg/kg. *FDR<0.05

GSVA pathway analysis of RNAseq data for the same pathways shown in the left heatmap. There was no tumor available after 4 doses of zanidatamab at 30mg/kg.



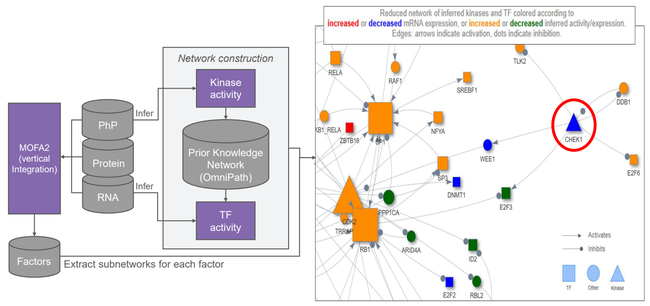
Kinase activity inferred from phosphoproteomics data using GSEA analysis 168 hours following zanidatamab treatment. Kinase-substrate relationships were obtained from the PhosphositePlus database. There was no tumor available after 4 doses of zanidatamab at 30mg/kg.

Heatmaps representing the substrate phosphorylation status for selected kinases, as measured experimentally. There was no tumor available after 4 doses of zanidatamab at 30mg/kg.
[a]Levels normalized to total protein.

CHEK1, checkpoint kinase 1; FDR, false discovery rate; GSEA, gene set enrichment analysis; GSK3β, glycogen synthase kinase 3 beta; GSVA, gene set variation analysis; IgG, immunoglobulin G; IV, intravenous; NES, normalized enrichment score; Norm, normalized; RNAseq, RNA sequencing; SEM, standard error of the mean; Prot, protein; zani, zanidatamab.

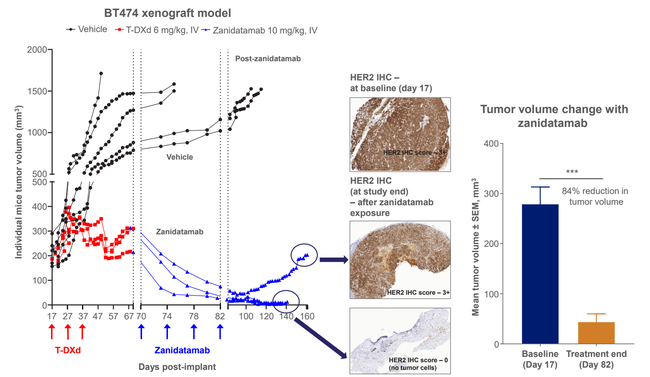
- In the BT-474 xenograft model, zanidatamab inhibited cell cycle and DNA repair pathways and altered kinase profiles associated with DNA repair and cell signaling pathways

Figure 5. Multiomics integration confirmed inhibition of cell cycle and DNA repair pathways by zanidatamab



Multiomics integration paradigm and example subnetwork of nodes related to cell proliferation and DNA damage response, where node color indicates activation status. CHEK1 is a critical component of the DNA damage repair mechanism by ATR and is activated by replication stress-related DNA damage.
ATR, ataxia telangiectasia and Rad3-related; CHEK1, checkpoint kinase 1; MOFA, multiomics factor analysis; mRNA, messenger RNA; PhP, phosphoprotein; TF, transcription factor.

Figure 6. Zanidatamab showed antitumor activity in T-DXd–exposed tumors



Initially, 20 animals were treated with T-DXd; 17/20 animals regressed to non-measurable tumors and were unavailable for further treatment. The remaining 3 animals post–T-DXd treatment were allowed to escape
T-DXd– induced stasis and commenced treatment with zanidatamab.**/***P <0.01, Student's t-test.
HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; IV, intravenous; SEM, standard error of the mean; T-DXd, trastuzumab deruxtecan.