ERBB2 amplification detected in ctDNA as a surrogate for tumor tissue FISH analysis of HER2 status in a Phase 1 study with zanidatamab for the treatment of locally advanced or metastatic HER2 expressing cancers

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Abstract #CT278

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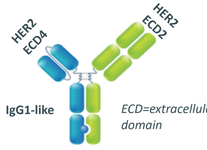
Zanidatamab monotherapy shows antitumor activity in patients with advanced HER2 expressing and *ERBB2 amplified* solid cancers

Zanidatamab (ZW25): Bispecific HER2-targeting Antibody

- Designed using the Azymetric™ bispecific platform
- Biparatopic — simultaneously binds two distinct sites of HER2, juxtamembrane ECD4 and ECD2 dimerization domain
- Unique binding results in multiple mechanisms of action[1]:
  - *Improved binding, clustering, receptor internalization & downregulation over monoclonal antibodies*
  - *Inhibition of ligand-dependent & -independent proliferation.*
  - *Activation of antibody-dependent cellular cytotoxicity & phagocytosis.*
  - *Potent complement-dependent cytotoxicity.*

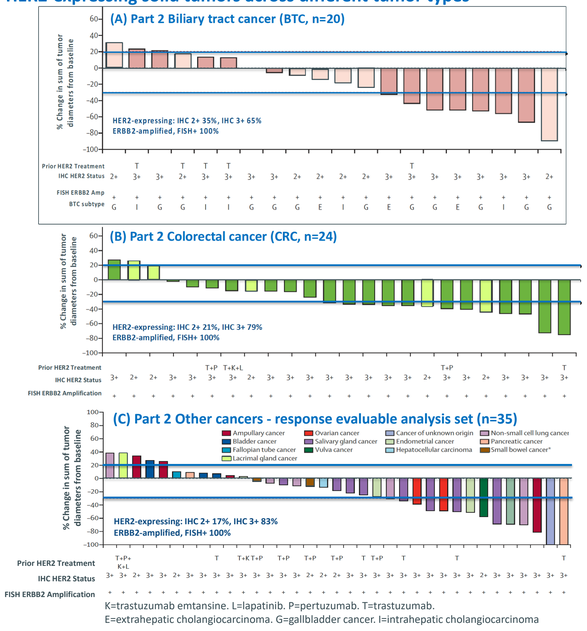


ZW25-101 (NCT02892123) is a first-in-human Phase 1 study that evaluates **zanidatamab** in HER2 expressing cancers. In Part 1 no dose-limiting toxicities were detected, and the maximum tolerated dose was not reached. The recommended dose for Part 2 was 20 mg/kg every 2 weeks. Tumor response by computed tomography or magnetic resonance imaging was determined by the investigator per RECIST every 8 weeks during treatment.[2] All HER2 assessments were performed at a central laboratory; in Part 1, enrolment based on a local assessment was permitted. Evaluating HER2 status by tissue using immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) often yield discordant status requiring multiple tissue samples to achieve robust identification of patients for clinical trials. As repeat tissue biopsies can be difficult to obtain and patients may receive mixed results, depending on the methodologies used, we sought to evaluate whether a non-invasive liquid biopsy could provide HER2 status by sequencing of the *ERBB2* gene in circulating tumor DNA (ctDNA). Unlike gene copy number in tissue analysis, the observed plasma copy number (pCN) is also a function of the tumor burden and rate of tumor shedding of ctDNA into the bloodstream[5,6].

Methods and Results

In the Phase 1 study, HER2 status was determined from a fresh tumor biopsy or in archival FFPE tumor tissue samples by **IHC** (HercepTest, Dako) and/or **FISH** (HER2 IQFISH pharmDx, Dako) according to ASCO-CAP guidelines. Plasma samples were collected prior to the first cycle of zanidatamab and on-treatment at cycle 2 (day 14) for testing by Guardant360, a 74 gene **ctDNA NGS**-based assay. Guardant360 is a CLIA-certified, CAP accredited, FDA-approved NGS assay (Guardant Health, CA). Plasma isolation, cfDNA extraction, library construction, sequencing, quality-control assessments were performed as previously described[3,4]. ctDNA fraction in patient plasma was measured by the maximum variant allelic frequency (VAF) (Figures 2 & 3). The maxVAF is the allele frequency of the alteration with the greatest allelic fraction in the sample. Since amplifications are not quantified by VAF, it is typically a clonal alteration in a tumor suppressor gene such as *TP53*. Antitumor activity was assessed by best overall response (BOR, defined as the best response per RECIST and confirmed best overall response (cBOR), which required confirmation of complete response (CR) or partial response (PR), with a minimum duration between the initial and confirmatory responses (CR or PR) of 28 days. Antitumor activity is shown for BTC, CRC, other cancers excluding breast and GEA. Baseline plasma sample and matching tumor biopsy were available for testing with ctDNA NGS and tumor FISH methods from 135 patients; BTC (n=21), CRC (n=27), all other cancers (n=33), breast (n=38), GEA (n=16).

Figure 1. Antitumor activity of zanidatamab observed in patients with HER2-expressing solid tumors across different tumor types



K=trastuzumab emtansine. L=lapatinib. P=pertuzumab. T=trastuzumab.
E=extrahepatic cholangiocarcinoma. G=gallbladder cancer. I=intrahepatic cholangiocarcinoma

Figure 1: Waterfall plot of greatest percentage change in the sum of the longest diameters of measurable tumors in patients with biliary tract cancer **(A)**, colorectal cancer **(B)**, and other cancers **(C)**. Blue lines in Figure 1 represent 20% increase or 30% decrease in tumor size. All patients were FISH-amplified and had HER2 expression of either IHC3+ or IHC2+ based on central assessment. (Figure adapted from [2])

Table 1. *ERBB2 a*mplification status by tumor tissue FISH and plasma ctDNA NGS is 82.4 % concordant

		FISH Interpretation		
		Positive	**Negative**	**Total**
Discordant: (21 + 2) / 131 = 17.6%	**ctDNA**** HER2 Amp +	101	2	103
Concordant: (101 + 7) / 131 = 82.4%	**ctDNA**** HER2 Amp -	21	7	28
	Total	122	9	**131***

** 4 samples had failed FISH test*
*** ctDNA amplification (from Guardant) is assumed negative if an amplification is not detected*

Table 1: Samples from 135 patients were tested with both screening assays, ctDNA NGS and tumor FISH. A high concordance rate of 82% between the methods supports the potential use of plasma ctDNA testing as a surrogate for tumor tissue FISH analysis.

Utility of plasma ctDNA quantitation

Figure 2. (A) Lower ctDNA fraction levels at baseline correlate with fewer number of screening target lesions. (B) CRC and BTC have higher median ctDNA fraction than other cancer groups.

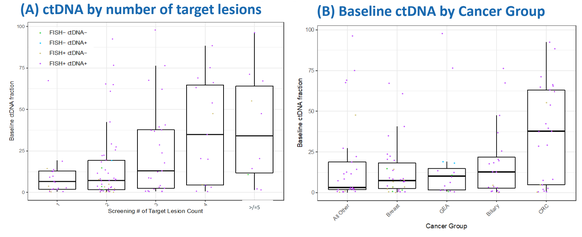


Figure 2: Lower tumor volume as well as low-shedding tumor subtypes are known to be associated with low ctDNA levels[5,6]. Similar pattern was observed in our dataset.

Figure 3. Better correlation observed between *ERBB2* plasma copy number and FISH ratio in tumors with higher levels of ctDNA shedding

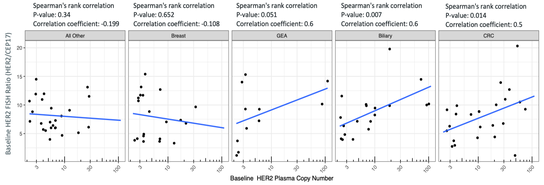


Figure 3: Highest correlation coefficients between *ERBB2* pCN and *HER2* FISH ratio were observed in CRC, BTC and GEA. These 3 cancer groups had the highest baseline median ctDNA fraction (Figure 2B).

Figure 4. Majority of the patients experienced a decrease in the ERBB2 plasma copy number post-treatment with zanidatamab

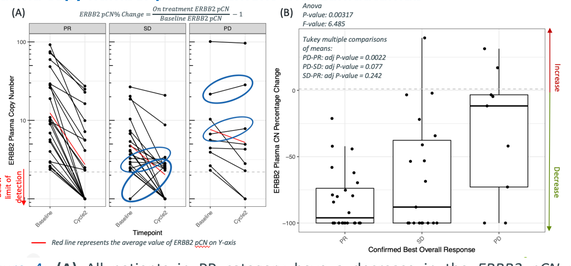
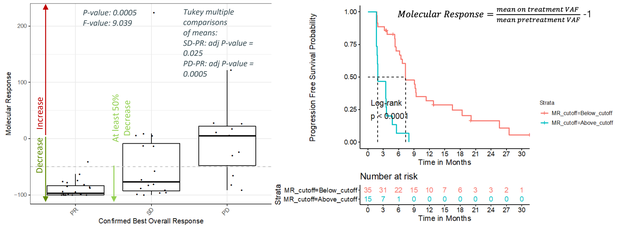


Figure 4: **(A)** All patients in PR category have a decrease in the *ERBB2 pCN* post-treatment. Two patients in SD and two patients in PD category have an increase in the *ERBB2* plasma copy number at cycle 2 (**circled in blue**). **(B)** In total 23 patients had 100% decrease (from detectable to non-detectable) in the *ERBB2* pCN. The 21 PR patients had the largest changes in ctDNA fraction (maxVAF), while the 9 PD patients had the least. The boxes represent 25th–75th percentiles; center lines indicate the median; whiskers extend to the maximum and minimum values within 1.5× of the interquartile range.

Molecular response (MR)

Figure 5. ctDNA molecular response enables the prediction of progression free survival (PFS) and patient outcomes

MR score	ctDNA	N	Events	Median PFS	0.95LCL	0.95UCL
< 50%	> 50% decrease	35	28	7.36	6.44	12.75
≥ 50%	Increase or <50% decrease	15	15	1.94	1.84	4.76



** Including any change to ctDNA level (not considering -50% cutoff)*

Figure 5: To calculate MR, SNVs, indels, and fusion events with sufficient mutant molecule count were averaged to generate the mean VAF values from baseline and on-treatment plasma samples for all patients. Sample pairs that do not have any detectable variants or variants that meet the inclusion criteria are not evaluable for molecular response analysis. Confidence in the molecular response score was determined by a statistical model of VAF precision based on the number of variants that meet the inclusion criteria, panel-wide molecular coverage, and ctDNA shedding as approximated by the maxVAF.[7,8]

Conclusions

- A high concordance of 82% was observed in *ERBB2* amplification between the Guardant360 NGS ctDNA and FISH tumor tissue methods, supporting that *ERBB2* amplification detected in ctDNA could be used as a surrogate for FISH analysis.
- Lower ctDNA fraction (maxVAF) suggests less shedding of ctDNA into the bloodstream which may be associated with lower tumor burden, fewer distant metastases or slower growing tumors.
- 21 PR patients had statistically significant decrease in the median percent change of *ERBB2* pCN as compared to 9 PD patients, post-treatment with zanidatamab.
- Patients with < 50% ctDNA MR score post-treatment showed longer PFS of 7.36 months vs 1.94 months for patients with MR score of >/= 50%.

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