Poster 5148

Analysis of HER2 Prevalence by RNA Expression Across Solid Tumors

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Objectives

- To determine the correlation between HER2 IHC status and *ERBB2* mRNA expression, and between ISH and DNA amplification, by NGS, in tumor samples from patients with locally advanced or metastatic BC or G/GEJC
- To determine the prevalence of HER2 expression, based on category thresholds defined in the first objective, among patients with other RNA-sequenced key tumor types

Conclusions

- Results of this study contribute to the understanding of HER2 expression in several key tumor types for which standard HER2 IHC/ISH testing is not conducted
- A notable number of locally advanced or metastatic solid tumors other than BC and G/GEJC have *ERBB2* mRNA expression that may correspond to HER2 IHC ≥1+
- This finding opens the possibility for novel agents such as HER2-directed antibody-drug conjugates to benefit patient care for several tumor types
- Further IHC/ISH testing in these populations is needed to corroborate our results

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Introduction

- The standard methods for determining HER2 status in breast cancer (BC) and gastric/gastroesophageal junction cancer (G/GEJC) include immunohistochemistry (IHC) assay testing for HER2 protein expression and in situ hybridization (ISH) assays for determining gene amplification[1,2]
- The frequency of HER2 protein expression and gene amplification (encoded by *ERBB2*) in solid tumors other than BC and G/GEJC is not well defined, although recent evidence suggests that HER2 expression is evident in different tumor types[3,4]
- Next-generation sequencing (NGS) methods, increasingly being applied in clinical practice for molecular characterization of different cancers, have the potential to improve the understanding of HER2 status in BC and G/GEJC, as well as for other solid tumor types

Materials and Methods

STUDY DESIGN AND DATABASE

- Retrospective analysis using a deidentified clinical dataset
- Data source: Tempus multimodal database, which included approximately 600,000 patient records with linked molecular and clinical data at the time of the study[5,6]

PATIENTS

- Age ≥18 and ≤89 years at diagnosis of locally advanced or metastatic (LA/m) cancer (initial diagnosis or recurrent disease) during the 7-year study period from 2015 – 2021, including:
  - BC, G/GEJC, head and neck squamous cell carcinoma (HNSCC), non-small cell lung cancer (NSCLC), ovarian cancer (OC), and endometrial cancer (EC)
- Underwent Tempus RNA expression profiling and DNA sequencing within 90 days before or after LA/m diagnosis
  - Whole-transcriptome RNA sequencing was conducted using the Tempus RS and RS.V2 RNA assays, while Tempus xE, xT, and xO assays were used for DNA profiling
- Cohorts for correlation analyses — additional eligibility criteria:
  - For patients with BC and G/GEJC, HER2 IHC/ISH assays and RNA expression profiling used samples from the same tumor lesion and were conducted ≤30 days apart
- Exclusion criteria for patients with LA/m HNSCC, NSCLC, OC, and EC:
  - Other malignancy within 3 years of LA/m diagnosis or receipt of investigational anticancer drug during the study period
- Patients who received neoadjuvant or adjuvant therapy for the primary cancer were not excluded

CORRELATION ANALYSES

- For LA/m BC and G/GEJC, HER2 status was assessed from IHC/ISH results, with categories defined as follows:
  - HER2-zero: IHC 0
  - HER2-low: IHC 1+ or IHC 2+/ISH-negative (ISH–)
  - HER2-positive (HER2+): IHC 2+/ISH+ or IHC 3+
- A logistic regression model was fit to NGS and IHC status data for LA/m BC and G/GEJC using bootstrapped samples, and the value corresponding to the maximum Youden's J index on the receiver operating characteristics curve (ROC) was used to identify thresholds separating HER2 subgroups:
  - (1) using HER2 expression levels from IHC and *ERBB2* mRNA sequencing data, and
  - (2) using ISH and DNA amplification by NGS
- From NGS (full-transcriptome RNA data and DNA copy number), HER2 subgroups for LA/m BC and G/GEJC were categorized as:
  - mRNA-zero: IHC 0
  - mRNA-low: IHC 1+, or IHC 2+/ISH–
  - mRNA-positive: IHC 2+/ISH+, IHC 3+

APPLICATION OF HER2 SUBGROUP THRESHOLDS TO OTHER SOLID TUMORS

- Thresholds were then applied to other solid tumors to predict the distribution of HER2 expression
- HER2 amplification and mutation rates were evaluated in the Tempus NGS data for all six cancer types

Results

CORRELATION ANALYSES

- Correlation analyses included samples from 3,898 patients:
  - Of 4,242 patients with LA/m BC who had mRNA test results, 3,487 (82%) were eligible for the study
  - Of 543 patients with LA/m G/GEJC who had mRNA test results, 411 (76%) were eligible for the study
- For LA/m BC, logistic regression identified an *ERBB2* mRNA expression threshold between HER2-zero and HER2-low of 7.526, measured as $\log_2$(transcripts per million [TPM] +1), while the threshold identified between HER2-zero/HER2-low and HER2+ was 8.717 (**Figure 1A**)
- For LA/m G/GEJC, the threshold between HER2-zero and HER2-low was 7.042, and the threshold between HER2-zero/HER2-low and HER2+ was 8.093 (**Figure 1B**)
- Samples categorized as HER2+ had the highest IHC-to-mRNA agreement at 80% (BC) and 81% (G/GEJC)
  - HER2-low and HER2-zero samples ranged from 56% to 75% agreement (**Table 1**)

Figure 1. Logistic regression model results for *ERBB2* mRNA expression in locally advanced/metastatic (A) breast cancer and (B) gastric cancer

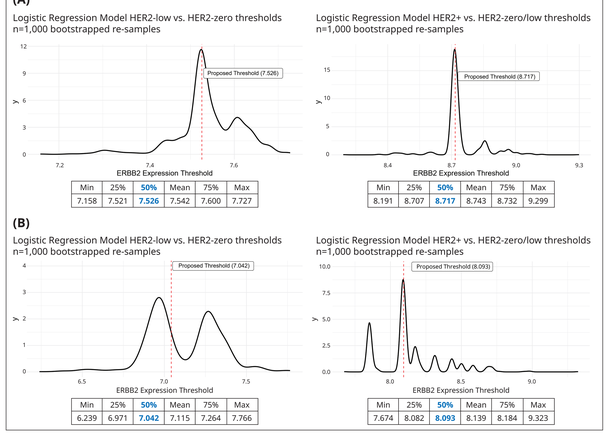

Table 1. Correlation between *ERBB2* mRNA expression and HER2 IHC/ISH status in locally advanced/metastatic breast and gastric cancer

		HER2 IHC/ISH status, n (%)[a]		
		HER2-zero	HER2-low	HER2+
Breast cancer	**N=3,487**	**1,101 / 32%[b]**	**2,016 / 58%**	**370 / 11%**
NGS thresholds	*ERBB2* $\log_2$(TPM+1)			
mRNA-zero	<7.526	**829 (75.3)**	695 (34.5)	17 (4.6)
mRNA-low	≥7.526–<8.717	262 (23.8)	**1,166 (57.8)**	58 (15.7)
mRNA-positive	≥8.717	10 (0.9)	155 (7.7)	**295 (79.7)**
HER2 amplified, n (%)	–	4 (0.4)	11 (0.5)	250 (67.6)
HER2 mutated	–	36 (3.3)	74 (3.7)	20 (5.4)
G/GEJC cancer	**N=411**	**149 / 36%[b]**	**169 / 41%**	**93 / 23%**
NGS thresholds	*ERBB2* $\log_2$(TPM+1)			
mRNA-zero	<7.042	**84 (56.4)**	54 (32.0)	4 (4.3)
mRNA-low	≥7.042–<8.093	60 (40.3)	**100 (59.2)**	14 (15.1)
mRNA-positive	≥8.093	5 (3.4)	15 (8.9)	**75 (80.6)**
HER2 amplified	–	3 (2.0)	2 (1.2)	64 (68.8)
HER2 mutated	–	5 (3.4)	4 (2.4)	6 (6.5)

Percentages may not add up to 100% because of rounding. [a]HER2-zero was defined as IHC 0; HER2-low was defined as IHC 1+ or IHC 2+/ISH-negative; and HER2-positive (HER2+) was defined as IHC 2+/ISH+ or IHC 3+. [b]Row percentages are depicted for total numbers of patients with LA/m BC and G/GEJC categorized by HER2 IHC/ISH status.
G/GEJC, gastric/gastroesophageal cancer; LA/m, locally advanced/metastatic; NGS, next-generation sequencing; TPM, transcripts per million.

APPLICATION OF *ERBB2* (HER2) SUBGROUP THRESHOLDS TO OTHER SOLID TUMORS

- Thresholds were then applied to a total of 4,725 other solid tumor samples, including LA/m HNSCC, NSCLC, OC, and EC
- Using LA/m BC thresholds identifying *ERBB2* mRNA-low and mRNA-positive samples, the models predicted that up to 33% of tumors express HER2 at levels that may be detectable (**Table 2**)
- Using LA/m G/GEJC thresholds, the models predicted that up to 56% of tumors express HER2 at levels that may be detectable (**Table 2**)

Table 2. *ERBB2* (HER2) categories among other locally advanced/metastatic solid tumors

HER2 Status	HNSCC N=630	NSCLC N=2945	OC N=855	EC N=295
NGS BC thresholds, n (%)				
mRNA-zero	589 (93.5)	1,974 (67.0)	626 (73.2)	205 (69.5)
mRNA-low	33 (5.2)	853 (29.0)	214 (25.0)	64 (21.7)
mRNA-positive	8 (1.3)	118 (4.0)	15 (1.8)	26 (8.8)
mRNA-low + mRNA-positive	41 (6.5)	971 (33.0)	229 (26.8)	90 (30.5)
NGS G/GEJC thresholds, n (%)				
mRNA-zero	492 (78.1)	1,345 (45.7)	395 (46.2)	130 (44.1)
mRNA-low	124 (19.7)	1,190 (40.4)	384 (44.9)	127 (43.1)
mRNA-positive	14 (2.2)	410 (13.9)	76 (8.9)	38 (12.9)
mRNA-low + mRNA-positive	138 (21.9)	1,600 (54.3)	460 (53.8)	165 (55.9)
DNA amplified, n (%)	8 (1.3)	34 (1.2)	12 (1.4)	18 (6.1)
DNA mutated, n (%)	7 (1.1)	29 (1.0)	5 (0.6)	8 (2.7)

Percentages may not add up to 100% because of rounding. BC, breast cancer; G/GEJC, gastric/gastroesophageal cancer; NGS, next-generation sequencing; HNSCC, head and neck squamous cell carcinoma; NSCLC, non-small cell lung cancer; OC, ovarian cancer; EC, endometrial cancer.