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Analysis of central vs. local deviating HER2 test results in gastric cancer in the multicenter VARIANZ study

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Background

Trastuzumab is an approved targeted therapy in advanced gastric cancer (GC). It addresses HER2, a membrane-bound receptor tyrosine kinase. Indication for trastuzumab is determined by immunohistochemistry (IHC) HER2 score 3+ or in case of an intermediate score (2+) additional amplification (HER2/CEP17 ratio ≥2) by in situ hybridization (ISH). According to the ToGA-study trastuzumab is improving overall survival from 11.1 to 13.8 months in 1st-line treatment of stage IV HER2+ GC. Unfortunately not all patients (pts) respond and almost all initial responders eventually experience progression.

VARIANZ is an academic network study funded by the German Federal Ministry of Education and Research (BMBF 01ZX1310E). Patients (pts) who received medical treatment for stage IV GC were recruited in 35 sites. HER2 expression was verified centrally by two dedicated GI pathologists using IHC (DCS, HI608C0I) and chromogenic ISH (Zytomed Systems, C-3022-40). Treatment and survival outcomes were reported by investigators. In 22.3% of all pts HER2 status was not confirmed. Only pts with confirmed HER2+ status seem to benefit from trastuzumab. Here, we aimed to investigate causes of HER2 discrepancies between local and central pathologies.

Methods

VARIANZ study recruitment overview

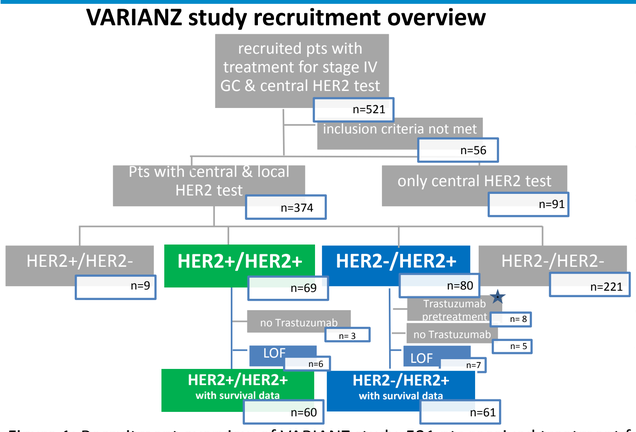


Figure 1: Recruitment overview of VARIANZ study. 521 pts received treatment for stage IV gastric cancer (GC) and were tested on HER2 test in central pathology. 374 patients were tested both centrally and locally for HER2. Groups based on central/ local HER2 test results: HER2+/HER2-, HER2+/HER2+, HER2-/HER2+ and HER2-/HER2-. ★ External HER2 test was performed with biopsy, neoadjuvant chemotherapy was applied with trastuzumab (study setting) & pretreated gastrectomy resectate was tested centrally.

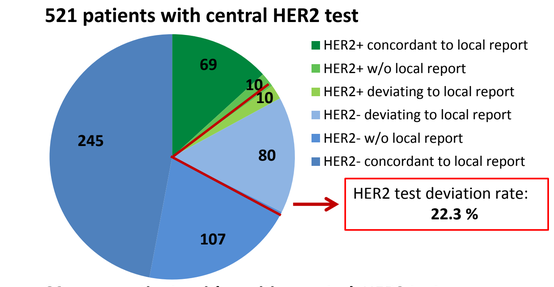
Involved local pathologies (n=105) were questioned about their HER2 test procedures, antibodies for IHC and participation in round robin tests. Tumor samples with central and local HER2 tests (n=374) were grouped: central HER2+ concordant to local HER2 status (HER2+/HER2+) and central HER2- deviating from local HER2 status (HER2-/HER2+) and analyzed with the below listed aspects.

The statistical evaluation was carried out using Chi² test. The log rank test was used for survival analysis.

Results

HER2 central test

Central HER2 test shows high deviation rate compared to local testing



89 green patients with positive central HER2 test

432 blue patients with negative central HER2 test

Fig. 2: Results of central HER2 test. Tumor samples were tested locally & retested in central study pathology using ICH and ISH. Pts with positive central HER2 test are shown in green (n=89, 17.1%) and those with negative centrall test are shown in blue (n=432, 82.9%). The red marking frames the cases in which central and local test deviate (n=90, test deviation rate of 22.3%).

IHC results between local & central HER2 test differ widely in HER2-/HER2+ cohort

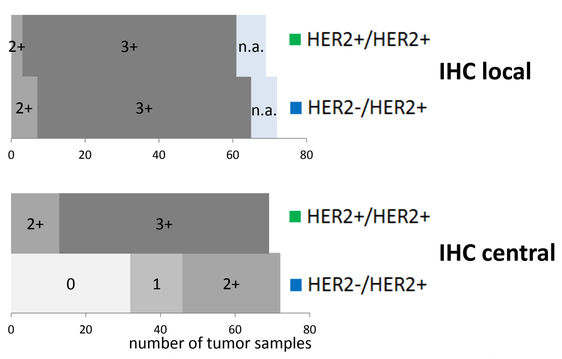


Fig. 3: Comparison of IHC results of local (above) and central (below) HER2 test within HER2+/HER2+ (n=69) and HER2-/HER2+ (n=72) cohort. IHC results are reported as negative for 0 and 1 (light grey), borderline (2+) and positive for 3+ (dark grey).

Characterization of HER2+/HER2+ vs. HER2-/HER2+

GC pts with confirmed positive HER2 test (HER2+/HER2+) do show better survival outcome than pts with deviating test results

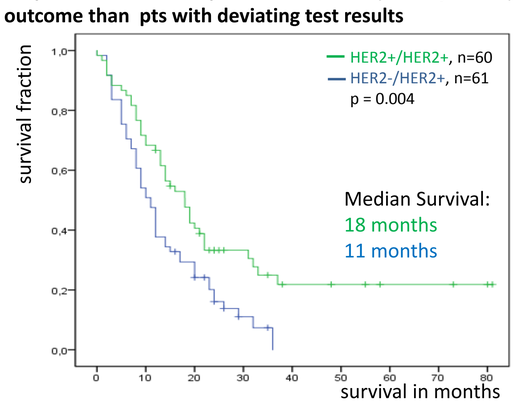


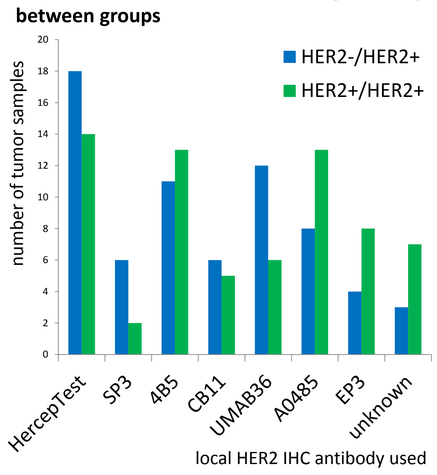
Figure 8: Survival of GC patients with confirmed positive HER2 status (HER2+/HER2+) and patients with central negative but local positive HER2 test result (HER2-/HER2+). Only pts who met inclusion criteria and received Trastuzumab treatment were included. Patients with HER2+/HER2+ status (n=60) survive an average of 18 months (95% KI: 13.5 – 22.5) while patients with HER2-/HER2+ (n=61) status survive 11 months (95% KI: 8.8 – 13.2); p=0.004 (Log Rank test).

Table 1: Characteristics of HER2+/HER2+ versus HER2-/HER2+ cohort.
Statistical evaluation with Chi²-test, * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001.

	central/local HER2 test result	
	HER2+/HER2+ (n=69)	HER2-/HER2+ (n=72)
Survival in months (95% KI)	18 (13.5 – 22.5)**	11 (8.8 – 13.2)
patients still alive; n (%)	17 (26.6%)*	7 (11.1%)
Gastric Surgery; n (%)	16 (23.5%)	21 (31.8%)
Of it with RO-Resection	15 (93.8%)*	13 (61.9%)
Initial tumor stage; n (%)		
initial metastatic	53 (76.8%)	48 (66.7%)
initial without metastases	16 (23.2%)	24 (33.3%)
Tumor localisation; n (%)		
gastric cancer	**21 (30.4%)*** **	**43 (59.7%)**
Gastroesophageal junction	48 (69.6%)	29 (40.3%)
Tumor localisation ; n (%)		
AEG I	19 (27.5%)	13 (18.1%)
AEG II	**27 (39.1%)***	**15 (20.8%)**
AEG III	2 (2.9%)	2 (2.8%)
Gastric fundus	**11 (15.9%)***	**23 (31.9%)**
Pylorus	10 (14.5%)	16 (22.2%)
Gastric stump	0 (0%)	3 (4.2%)
Type of Lauren; n (%)		
intestinal	28 (40.6%)	27 (37.5%)
diffuse	**4 (5.8%)****	**18 (25.0%)**
mixed type	2 (2.9%)	4 (5.6%)
no information	35 (50.7%)	23 (31.9%)

Influence of local HER2 test procedure

Local HER2 IHC antibodies do not significantly vary between groups



Participation in round robin tests does not explain HER2 test deviation rate

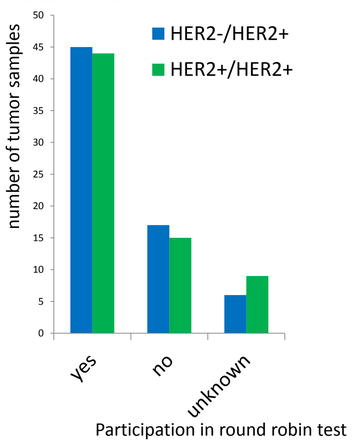


Fig. 4: Locally used HER2 Immunhistochemistry (IHC) antibodies (left) and participation in round robin tests (right). Involved pathologies (n=105) were questioned about their HER2 test procedure. The commerciall antibodies available are diverse: HercepTest (Dako), SP3 (Labvision), 4B5 (Ventana), CB11 (Novocastra), UMAB36 (Zytomed), A0485 (Dako) and EP3 were reported. Antibody used in central study pathology: CB11 (DCS).

Analysis of tested tumor material

Within HER2-/+ slightly more surgical specimens were tested

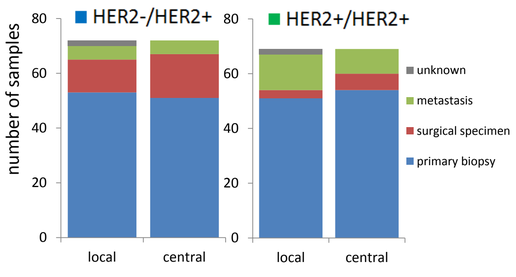


Fig. 5: Tested tumor material in local and central HER2 test within HER2-/HER2+ (n=72; left) & HER2+/HER2+ (n=69; right) cohort. Primary biopsies (blue), gastrectomy resectates (red) & metastases (green) were tested in local & central pathologies. For the most part, biopsies were tested on HER2 (both groups), whereby centrally surgical specimens were tested more often in the HER2-/+ group than in HER2+/+ ($p<0.05$, Chi²-test).

The large part of surgical specimens tested in VARIANZ were pretreated with neoadjuvant chemotherapy

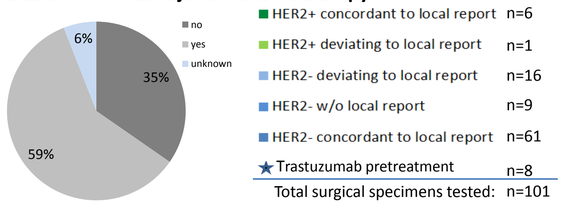


Fig. 6: Percentage of chemotherapy pretreated gastrectomy resectates within VARIANZ study. 101 resectates were centrally tested, of whom 59 % were pretreated with neoadjuvant chemotherapy (light grey).

Chemotherapy pretreatment does not influence HER2 test deviation

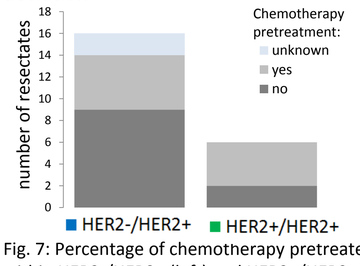


Fig. 7: Percentage of chemotherapy pretreated surgical specimens within HER2-/HER2+ (left) and HER2+/HER2+ (right) cohort. Number of cases wihtin groups are too small for statistical evaluation.

Conclusion

Identification of patients who benefit from HER2 targeting therapy remains challenging. No leading cause for the high HER2 test deviation rates within VARIANZ study could be identified.

Within HER2-/HER2+ cohort many tumors with borderline HER2 expression were found and patients do not benefit from Trastuzumab treatment. To prevent overtreatment of patients it seems important to distinguish these borderline cases from highly HER2 expressive cases. This can be done by increasing the thresholds for the HER2 test and by choosing high quality tumor material. The use of surgical resection specimens for assessment of HER2 seems to lead to less robust results compared to the use of biopsy material. In order to find the optimal threshold for HER2 test (percentage of positive tumor cells in IHC) further investigations are necessary.

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