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ORIGINAL ARTICLE

Local and central testing for HER2, PD-L1, MSI/MMR, EBV, and CLDN18.2 in advanced gastric cancer: results from the SAPHIR registry☆

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Background: Predictive biomarkers guide treatment selection of patients with advanced gastric and gastroesophageal junction adenocarcinoma (GAC/GEJAC). However, real-world data on testing frequencies and prevalence of biomarkers in Europe are limited.

Methods: The SAPHIR registry, a prospective, observational cohort study of patients with metastatic GAC/GEJAC, collects longitudinal clinical data, patient-reported outcomes as well as tumor tissue samples from routine diagnostics. Here, we focus on biomarker testing and test results in routine clinical practice. In addition, central pathology assessment was performed for HER2, PD-L1, dMMR/MSI, EBV, and CLDN18.2.

Results: From December 2019 until March 2022, a total of 473 evaluable patients were enrolled at 109 study sites in Germany. Their median age was 66.8 years, 73.6% were male, and 76.7% had an ECOG performance status of 0/1. In clinical routine, testing rates for HER2, PD-L1, MSI, EBV, and MMR were 78.6%, 31.3%, 15.6%, 4.9%, and 2.5%, respectively. CLDN18.2 was not tested during the respective period. By central testing, the positivity rates were 11.8% for HER2, 75.2% for PD-L1 (CPS $\geq$1, TPS/IC $\geq$1%), 2.5% for dMMR/MSI-H, 0.6% for EBV, and 26.7% for CLDN18.2. Deviations between local and central testing were 12.4% for HER2 and 22.4% for PD-L1.

Conclusions: This study provides valuable insights on molecular testing in patients with GAC/GEJAC in Germany. In real-world, patients were frequently tested for actionable alterations, but there is room for improvement. Deviating test results of HER2 and PD-L1 by local and central pathology may reflect limitations in testing methodologies. In addition, these patients might not receive the most effective treatment.

Key words: biomarker, gastric cancer, HER2, PD-L1, real-world data, test deviation

INTRODUCTION

In recent years, biomarker testing has revolutionized cancer treatment to provide each patient with a more personalized treatment and, thus, optimize clinical outcomes.[1] For the treatment of advanced gastric and gastroesophageal junction adenocarcinoma (GAC/GEJAC), several targeted drugs have already been approved, and numerous ongoing studies are focused on the identification of new targets and the development of novel treatment options.[2,3] GAC, including GEJAC, are among the most frequently diagnosed cancer entities worldwide and are associated with a poor prognosis.[4-6] Systemic chemotherapy with platinum—fluoropyrimidine combinations represents the recommended treatment option in the first-line setting.[5-8] As GAC/GEJAC is known to be a heterogeneous disease with different molecular subtypes and relevant clinical implications, molecular testing has become a key for treatment selection, especially for newly diagnosed patients with stage IV disease.[5-8]

In the first-line setting, the predictive biomarker human epidermal growth factor receptor 2 (HER2) is of special importance for treatment selection because patients with

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HER2 expressing GAC/GEJAC benefit from chemotherapy plus trastuzumab.[9-11] The expression of programmed death-ligand 1 (PD-L1) is a further predictive biomarker for GAC/GEJAC associated with a better response to immunotherapy.[3,10] In Europe, nivolumab and pembrolizumab were the first immune checkpoint inhibitors (ICIs) that were approved in combination with chemotherapy as first-line treatment for patients with HER2-negative, advanced GAC/GEJAC, whose tumor or immune cells have a specific threshold of PD-L1 protein expression.[7,12-16] Recently, based on KEYNOTE-811 (NCT03615326), the pembrolizumab first-line approval in combination with trastuzumab and chemotherapy was amended to HER2-positive GAC/GEJAC with PD-L1 combined positive score (CPS) $\geq$1.[16,17] Furthermore, based on RATIONALE-305 (NCT03777657), tislelizumab plus chemotherapy was approved for patients with unresectable or metastatic HER2-negative GAC/GEJAC with a PD-L1 tumor area positivity (TAP) score of at least 5%.[18] However, still several patients do not benefit from immunotherapy, and further research is crucial to select suitable patients for immunotherapy.

Microsatellite instability (MSI) has become a prognostic and predictive biomarker in GAC/GEJAC, and high MSI (MSI-H) is known to be associated with a good prognosis, particularly in localized stages. The majority of the molecular subtypes that have been distinguished by The Cancer Genome Atlas [Epstein—Barr virus (EBV) associated, MSI, genomically stable, and chromosomal instability] currently have only a limited impact on the selection of appropriate treatment options for patients with GAC/GEJAC.[15,19] MSI-H, which is associated with mismatch repair deficiency (dMMR), represents well-established biomarkers for response to ICI, and patients with dMMR/MSI-H GAC/GEJAC benefit from anti-PD-1 immunotherapy.[2,7,15,20,21] EBV correlates with increased expression of PD-L1 and PD-L2 in GAC/GEJAC, which indicates the potential of EBV as a biomarker for immunotherapy.[2] Further predictive biomarkers for GAC/GEJAC are currently evolving, such as Claudin18 isoform 2 (CLDN18.2), which now represents a new target for cancer treatment.[10,22] Zolbetuximab, a monoclonal antibody directed against CLDN18.2, has currently been approved by the European Medicines Agency.[23-25]

The rapid and precise determination of an individual patient's biomarker status is of great relevance for the selection of the most effective treatment strategy for advanced GAC/GEJAC. However, this is a challenging task that depends on multiple variables and requires a certain degree of experience.[26,27] Inherent difficulties resulting in deviating test results regarding HER2 and its impact on patient outcome were recently demonstrated in the prospective multicenter VARIANZ study.[28]

Real-world data (RWD) on testing frequencies and the prevalence of relevant biomarkers in advanced GAC/GEJAC in the European population are limited. In this study, we present comprehensive RWD from the tumor registry platform SAPHIR[29] on biomarker testing frequency in clinical routine, test results from local and central pathologies as well as potential deviations thereof for patients with metastatic GAC/GEJAC treated in clinical routine in Germany.

PATIENTS AND METHODS

Study design and cohort definition

SAPHIR is a national, prospective, observational, multicenter tumor registry platform that systematically collects representative RWD on molecular testing, treatment reality, and clinical outcomes of patients with metastatic (stage IV) gastroesophageal cancer receiving first-line systemic therapy in Germany. SAPHIR was approved by the responsible ethics committee and is registered at ClinicalTrials.gov (NCT04290806). The overall study design has already been described in detail.[29] In this study, patients aged $\geq$18 years with histologically confirmed stage IV (metastatic) GAC (C16.1-9) or GEJAC (C16.0) who were planned to undergo first-line systemic therapy and had signed an informed consent were included. For patients to be considered in the statistical analysis, the following variables were required to be documented: sex, date of birth, and at least one palliative systemic treatment including the date of its initial administration.

Eligible patients were recruited at 109 study sites in Germany, including office-based oncological practices, hospitals, and medical care centers. Patients were followed up for a maximum of 2 years. The data cut-off for the present analysis was 31 December 2023.

Central biobank

Within the SAPHIR registry, a central biobank was implemented with optional participation. All patients were asked to give written consent that their left-over tumor sample obtained as part of routine clinical care may be used for further research purposes. Upon patient agreement, tumor tissue samples were registered, key sample data (e.g. sample date, type, and origin) and storage location at the respective local pathologies were documented, and information was linked to the patient's clinical data collected in the SAPHIR registry via the electronic data capture (EDC) system. Automatic checks, manual central monitoring, and on-site monitoring guaranteed high-quality and comprehensive clinical data. Tumor samples were shipped from local pathologies and stored at the central pathology of the University Cancer Center Leipzig (Germany), which complies with best practice guidelines and the required standards for biobanks in terms of tissue collection, processing, and storage.

Biomarker profiling

Frequencies and results of clinically relevant biomarker testing carried out within the clinical routine at local pathologies were documented at the start of first-line treatment. Data on additional biomarker testing were updated at least every 3 months during follow-up.

For central biomarker profiling, tissue samples were characterized upfront with regard to the content and

quality of tumor cells. PD-L1, HER2, EBV, and CLDN18.2 were analyzed by immunohistochemistry (IHC) using the following antibodies: PD-L1 (22C3; Agilent, Santa Clara, CA), HER2 (4B5; Roche, Basel, Switzerland), and EBV (anti-EBV latent membrane protein, clones CS1-4; Dako, Agilent). In the case of an HER2 IHC score of 2+, *in situ* hybridization (ISH) was carried out using the ZytoDot 2C CISH Implementation Kit (ZytoVision, Bremerhaven, Germany). Scoring of PD-L1 was conducted using the CPS, the immune cell score (IC), and the tumor proportion score (TPS). PD-L1 positivity was defined as CPS $\geq$1 or TPS or IC $\geq$1%. CLDN18.2 testing was carried out with the VENTANA CLDN18 (43-14A) assay (Roche), assessing each staining intensity level.[30] Tumors were defined as CLDN18.2 positive, if $\geq$75% of the tumor cells demonstrated moderate-to-strong (2+/3+) CLDN18.2 membranous staining intensity. For determination of MMR/MSI status, IHC of the MMR proteins was carried out using the antibodies MLH1 (M1; Roche), PMS2 (A16-4; Roche), MSH2 (G219-1129; Roche), and MSH6 (SP93; Roche). dMMR was defined as the loss of expression of one or more of these proteins.

Data source and statistical analysis

The standardized EDC system (iOstudy office edc) provided by iOMEDICO, clinical variables collected during routine clinical practice, and quality control measures were previously described.[29] Overall survival (OS) was defined as the interval between the start of first-line treatment and the date of death from any cause. OS was estimated using the Kaplan–Meier method. Patients alive or lost to follow-up at data cut were censored at the last contact.

All statistical analyses were carried out using R software version 4.3.2 (2023-10-31; R Foundation, Vienna, Austria) 'Eye Holes' (Platform: x86_64-pc-linux-gnu (64-bit)).

RESULTS

Patient and tumor characteristics

Between December 2019 and March 2022, 509 patients with advanced GAC/GEJAC were enrolled in the SAPHIR registry, of which 473 patients were evaluable (Figure 1). A total of 418 patients gave their consent to participate in the biobank module for further translational research and samples were requested from the respective local pathologies. At database cut, samples of 161 patients were received by the central pathology and subsequently analyzed for the aforementioned predefined biomarkers. Most samples (76%) assessed by central pathology were derived from the primary tumor site, with 9% deriving from metastasis (15% unknown).

Patient and tumor characteristics of all evaluable patients with available information on local biomarker testing (local testing group) and the subgroup of patients with additional available central test results (central testing subgroup) are presented in Table 1. The two patient groups were comparable regarding age, sex, Eastern Cooperative Oncology Group (ECOG) performance status, comorbidities, location of primary tumor, and metastatic sites. The median age at the start of the first-line treatment was similar (67 versus 68 years; local testing group versus central testing subgroup), with 73.6% and 74.5% male patients, respectively.

Real-world data on treatment and survival in clinical routine

Most patients in clinical routine received chemotherapy-doublet or chemotherapy-triplet (Table 1). Mono-chemotherapy was rarely administered (1.1%). A total of 15.9% of patients were treated with anti-HER2 targeted therapy, most frequently in combination with chemotherapy-doublet (11.4%) or chemotherapy-triplet (3.2%), and 77% of patients with HER2-positive tumors received anti-HER2 therapy.

The median OS for all patients with GAC/GEJAC included in this study was 11.6 months [95% confidence interval (CI) 10.4-12.7; Figure 2].

Frequencies of documented biomarker tests in clinical routine

For a total of 84.4% (n = 399) of patients with metastatic GAC/GEJAC, a biomarker test was documented prior to start of first-line treatment within clinical routine. The frequencies of documented tests for the individual biomarkers are presented in Figure 3. For most patients (78.6%, n = 372) a test for HER2 status was documented, followed by testing for PD-L1 expression in 31.3% (n = 148) of the patients, using different scoring methods such as CPS, IC, and TPS (Table 2). For 15.6% (n = 74) and 2.5% (n = 12) of the patients, a biomarker test for MSI and MMR was documented, respectively [MSI and/or MMR: 17.3% (n = 82)]. The most prevalent MSI detection method used in our real-world cohort within clinical routine was IHC (59.5%, n = 44/74), followed by sequencing (14.9%, n = 11/74), next-generation sequencing (2.7%, n = 2/74), and FISH (1.4%, n = 1/74). For 24.3% (n = 18/74) of the patients, the MSI testing method was unknown to the site. For two patients, more than one MSI testing method was documented. EBV testing was documented for 4.9% (n = 23) of patients. For 10.1% (n = 48) of patients, at least one other biomarker test was documented, such as neurotrophic tropomyosin receptor kinase (3.6%) and tumor mutational burden (0.4%). CLDN18.2 expression was not tested in clinical routine during the specific period of study conduct.

Biomarker test results of local and central testing

Biomarker test results determined at local pathologies within clinical routine (local testing) and for selected samples at the central pathology within SAPHIR (central testing) are detailed in Table 2.

In central testing, 11.8% of the patients were tested positive for HER2 and 75.2% (n = 121) for PD-L1, defining PD-L1 positivity as CPS $\geq$1 or TPS or IC $\geq$1%. dMMR/MSI-H (2.5%) was rarely identified. EBV positivity was detected only in one patient; 26.7% of patients were found to be positive for CLDN18.2.

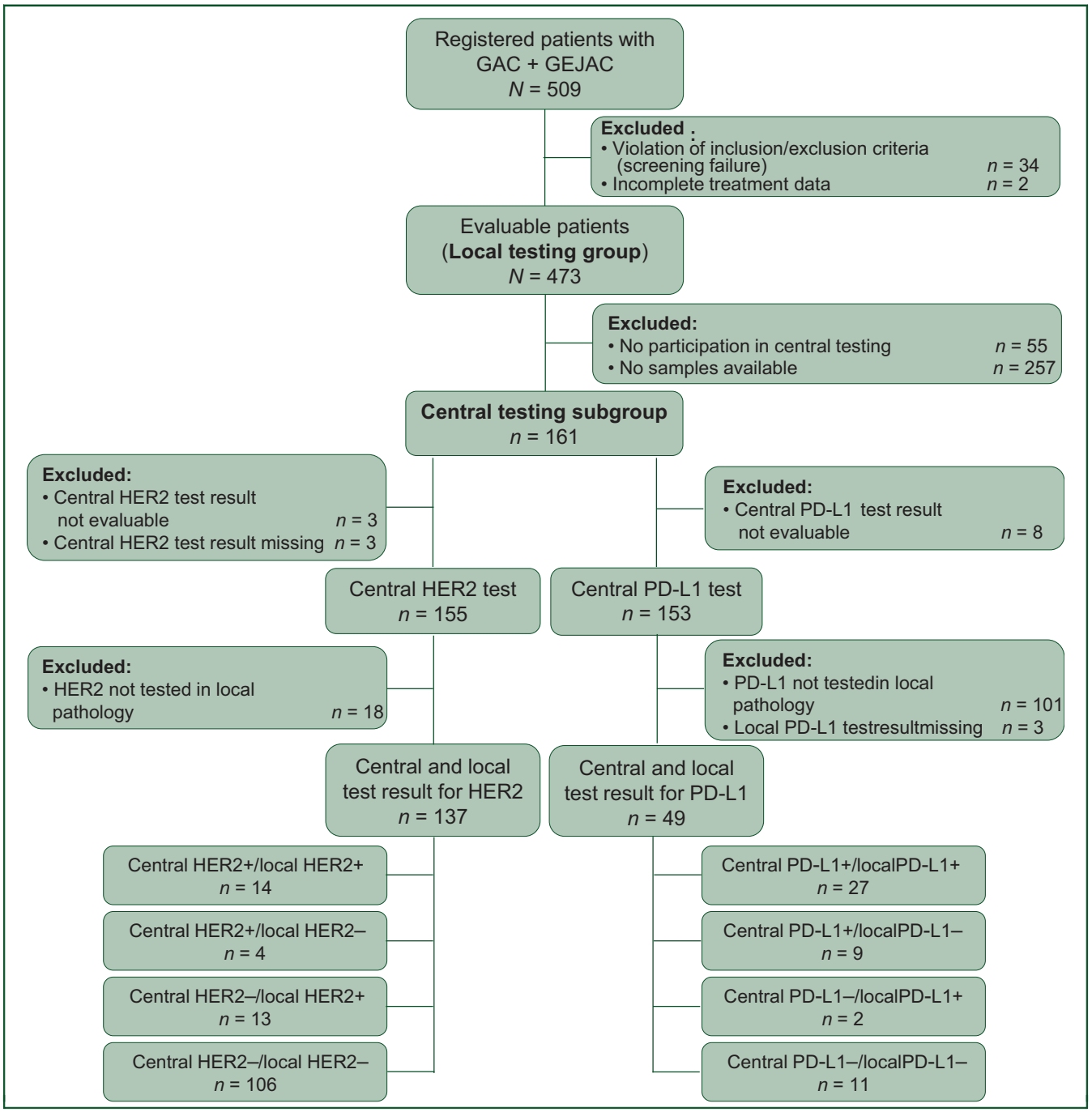

Figure 1. Cohort definition. Flowchart of all patients with advanced gastric and gastroesophageal junction adenocarcinoma (GAC/GEJAC) recruited into the SAPHIR registry from December 2019 until March 2022. All evaluable patients (local testing group) and patients with additionally available samples for biomarker testing at central pathology (central testing subgroup) were included in this analysis.
HER2, human epidermal growth factor receptor 2; PD-L1, programmed death ligand-1.

In local testing, 16.7% exhibited HER2 positivity, 17.8% PD-L1 positivity, and 2.5% the dMMR/MSI-H subtype, considering the number of all patients of the local testing group. None of the patients was tested positive for EBV. With regard to the number of documented tests within local testing, 21.2% of patients were tested positive for HER2, 56.8% ($n = 84$) positive for PD-L1, and 14.6% were dMMR/MSI-H.

To examine the deviations of HER2- and PD-L1 testing, biomarker test results of patients with both local and central test results available were compared (Table 3). Deviating test results were observed in 12.4% of the patients for HER2 and 22.4% for PD-L1.

DISCUSSION

Predictive biomarkers represent an essential component in the treatment of GAC/GEJAC, as targeted therapies and immunotherapies have been shown to improve clinical outcomes - but what about the corresponding biomarker tests in clinical routine? Are all patients tested to ensure that they can benefit from the associated therapies? Here we

Table 1. Patient and tumor characteristics

Characteristic	Local testing ($N = 473$)	Central testing ($n = 161$)
Age (years)[a]		
Median (range)	66.8 (37.3-90.6)	68.1 (38.8-90.6)
<65, *n* (%)	208 (44.0)	68 (42.2)
≥65, *n* (%)	265 (56.0)	93 (57.8)
Sex, *n* (%)		
Male	348 (73.6)	120 (74.5)
Female	125 (26.4)	41 (25.5)
ECOG PS[a], *n* (%)		
0	139 (29.4)	56 (34.8)
1	224 (47.4)	75 (46.6)
≥2	44 (9.3)	17 (10.6)
Missing/unknown	66 (14.0)	13 (8.1)
Comorbidities[a], *n* (%)		
Any comorbidity[b]	390 (82.5)	129 (80.1)
No comorbidity	83 (17.5)	32 (19.9)
CCI = 0[c]	345 (72.9)	114 (70.8)
CCI ≥ 1[c]	128 (27.1)	47 (29.2)
Location of primary tumor, *n* (%)		
GEJAC Siewert type I/II	161 (34.0)	57 (35.4)
GEJAC Siewert type III	74 (15.6)	25 (15.5)
GAC	238 (50.3)	79 (49.1)
Onset of metastases, *n* (%)		
Synchronous	354 (74.8)	126 (78.3)
Metachronous	86 (18.2)	26 (16.1)
Missing/unknown	33 (7.0)	9 (5.6)
Histological subtype[d], *n* (%)		
Intestinal type	95 (20.1)	36 (22.4)
Diffuse type	83 (17.5)	26 (16.1)
Mixed type	25 (5.3)	12 (7.5)
Missing/unknown	270 (57.1)	87 (54.0)
Grading, *n* (%)		
Grade 1/Grade 2	139 (29.4)	51 (31.7)
Grade 3/Grade 4	210 (44.4)	81 (50.3)
Missing/unknown	124 (26.2)	29 (18.0)
Number of distant metastatic sites[a], *n* (%)		
1	228 (48.2)	72 (44.7)
2	150 (31.7)	54 (33.5)
≥3	91 (19.2)	32 (19.9)
Missing/unknown	4 (0.8)	3 (1.9)
Selected sites of metastasis[a,e], *n* (%)		
Liver	229 (48.4)	80 (49.7)
Lymph node	171 (36.2)	64 (39.8)
Peritoneum	164 (34.7)	54 (33.5)
Lung	89 (18.8)	24 (14.9)
Bone	55 (11.6)	22 (13.7)
Brain	11 (2.3)	4 (2.5)
Prior gastrectomy, *n* (%)		
Yes	122 (25.8)	26 (16.1)
R0	79 (64.8)[f]	22 (84.6)[f]
No	350 (74.0)	126 (78.3)
Missing/unknown	1 (0.2)	9 (5.6)
First-line treatment strategy, *n* (%)		
Chemotherapy-doublet	204 (43.1)	67 (41.6)
Chemotherapy-triplet	145 (30.7)	45 (28.0)
Anti-HER2 therapy[g,h]	75 (15.9)	29 (18.0)
Immunotherapy[h,i,j]	18 (3.8)	7 (4.3)
Anti-angiogenic therapy[k]	24 (5.1)	8 (5.0)
Other	7 (1.5)	5 (3.1)

CCI, Charlson Comorbidity Index; ECOG PS, Eastern Cooperative Oncology Group performance status; GAC; gastric adenocarcinoma; GEJAC, gastroesophageal junction adenocarcinoma; HER2, human epidermal growth factor receptor 2; R0, Resection status 0 (complete resection); VEGF, vascular endothelial growth factor.

[a]At the start of the first-line treatment.

[b]Comorbidities according to CCI and other comorbidities combined.

[c]CCI according to Quan et al.[45]

[d]According to Laurén.[46]

[e]Relevant sites of metastases frequently documented in routine care (multiple answers possible).

[f]Proportion of patients with R0 from those with previous gastrectomy.

[g]Trastuzumab or trastuzumab—deruxtecan in combination with chemotherapy mono (1.1%), chemotherapy doublet (11.4%), chemotherapy triplet (3.2%), or anti-HER2 only (0.2%) for the local testing group and chemotherapy mono (3.1%), chemotherapy doublet (10.6%), or chemotherapy triplet (4.3%) for the central testing subgroup.

[h]Excluding the combination of anti-HER2 targeted therapy and immunotherapy in the local testing group (0.4%) and the central testing subgroup (0.6%).

[i]Approval of immunotherapeutic agents for GAC/GEJAC was at the end of the recruitment phase of SAPHIR and thus only a few patients with access to immunotherapeutic agents were enrolled.

[j]Nivolumab or pembrolizumab in combination with chemotherapy doublet (2.5%), chemotherapy triplet (1.1%), or immunotherapy only (0.2%) for the local testing group and chemotherapy doublet (2.5%), chemotherapy triplet (1.2%), or immunotherapy only (0.6%) for the central testing subgroup.

[k]Ramucirumab or bevacizumab in combination with chemotherapy mono (3.8%), chemotherapy doublet (0.6%), chemotherapy triplet (0.2%), or anti-VEGF only (0.4%) for the local testing group and chemotherapy mono (4.3%) or chemotherapy doublet (0.6%) for the central testing subgroup.

present a comprehensive analysis of biomarker testing, test results, and potential deviation between local and central testing in clinical routine care in Germany, based on data from the prospective, multicenter tumor registry SAPHIR.

Molecular tumor characterization, including assessment of HER2 and PD-L1 expression and determination of the MMR/MSI subtype, is crucial for the selection of the appropriate treatment and therefore recommended before the start of systemic therapy.[5,7,15] In SAPHIR, especially for HER2, documented numbers of tests in clinical routine were high (78.6%) and comparable to frequencies reported in other real-world cohort studies (75%-88%).[31-33] Likewise, PD-L1 testing was relatively common in clinical routine, with 31.3% documented tests, considering that ICIs that target PD-1 or PD-L1 have only recently been approved for use in patients with advanced GAC/GEJAC for monotherapy or chemotherapy-immunotherapy combinations, i.e. during and at the end of the recruitment phase of SAPHIR. This also explains the use of different scoring methods for PD-L1 assessment in local testing (CPS, TPS, and IC), as current indications based on CPS and on the novel TAP scoring system only became relevant after these approvals. Similarly, testing for dMMR/MSI was relatively high considering the approval of pembrolizumab toward the end of the SAPHIR recruitment phase. By contrast, the frequency of EBV testing in our real-world cohort was found to be relatively low, suggesting that this test with no therapeutic consequence is not commonly used in routine clinical care in Germany.

The results of biomarker testing determined both within central and local testing, demonstrated a certain degree of variability (Table 2). The probability of a patient exhibiting a positive result for a specific biomarker, along with the selection of the appropriate therapy, is influenced by a multitude of factors. In clinical routine, this may guide the decision of the treating physician to perform biomarker testing on these patients. Hence selective testing may influence the results of biomarker distribution. This must be considered when evaluating and comparing data on the prevalence of biomarkers in a real-world setting. In this study, patients belonging to the central testing subgroup were not subject to selective biomarker testing and exhibited minimal missing data. In conclusion, it may be assumed that the values determined are in close alignment with the true prevalence for this subgroup.

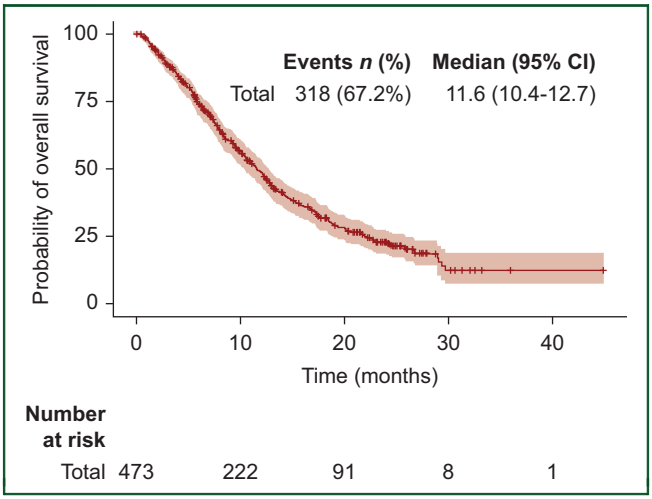

Figure 2. Overall survival of patients with advanced gastric and gastro-esophageal junction adenocarcinoma. All evaluable patients ($N = 473$) included in the SAPHIR registry were considered in this outcome analysis (recruitment: December 2019 to March 2022, follow-up of up to 2 years).
CI, confidence interval.

The prevalence of HER2 positivity in the literature varies considerably, mainly due to differences in tumor location (proximal versus distal), histological subtype according to Laurén (intestinal versus diffuse or mixed type), and grading,[3,5,34] with estimates ranging from 6% to 32% in patients with metastatic GAC/GEJAC.[34] In the present study, HER2 positivity rates fall within this range, with 11.8% for central testing. The precise assessment of HER2 for GAC/GEJAC has been repeatedly described as a challenging task and is further complicated by the fact that these cancers often exhibit heterogeneous intratumoral HER2 expression.[11] The deviation of test results for tumor specimens tested twice for HER2 expression (central and local) has already been observed in previous studies, most recently in the prospective multicenter VARIANZ study.[27,28] Test result deviation found in VARIANZ was 22.7%, whereby in most cases patients were tested locally positive for HER2, which was not confirmed by central testing.[28] In the present study, discordant HER2 results are also predominantly observed for cases in which the local HER2 test result was positive. Nevertheless, the HER2 test result deviation of 12.4% is considerably lower than that observed in VARIANZ, but comparable to the deviation presented in other studies involving European patient cohorts with 12% and 9%, respectively.[35,36] The proportion of deviating test results appears to be connected to intermediate HER2 expression levels and higher intratumoral HER2 heterogeneity.[27,28] In addition, the tumor localization and the histological subtype seem to impact HER2 test result deviations, with more deviations observed in distal GAC and tumors of the Laurén diffuse type.[27] It is of particular interest that trastuzumab-treated patients with HER2 test result deviations were found to have no benefit from targeted treatment with trastuzumab.[28,35] In the present study, the subgroup of patients with deviating HER2 test results is small ($n = 17$). Further investigation is therefore necessary to identify the underlying causes of HER2 test result deviations and their impact on the efficacy of targeted treatment, with the availability of additional empirical data.

The estimated prevalence of PD-L1 positivity for GAC with CPS $\geq$1 is between 50% and 60%; however, values >70% have also been reported.[7,37] This is in concordance with the PD-L1 positive rate observed in our real-world study in central testing if PD-L1 test results with TPS or IC $\geq$1% were included. To the best of our knowledge, central and local test results for PD-L1 have not yet been compared in another real-world study. Using the lowest threshold for PD-L1 positivity (CPS $\geq$1 or TPS or IC $\geq$1%) in both patient groups, we found discordant PD-L1 test results in 22.4% of the patients. These were mostly tumors found to be PD-L1 negative locally, but positive in central testing. It should be noted, however, that these data are based on a limited number of patients, as both local and central test results were only available for a small

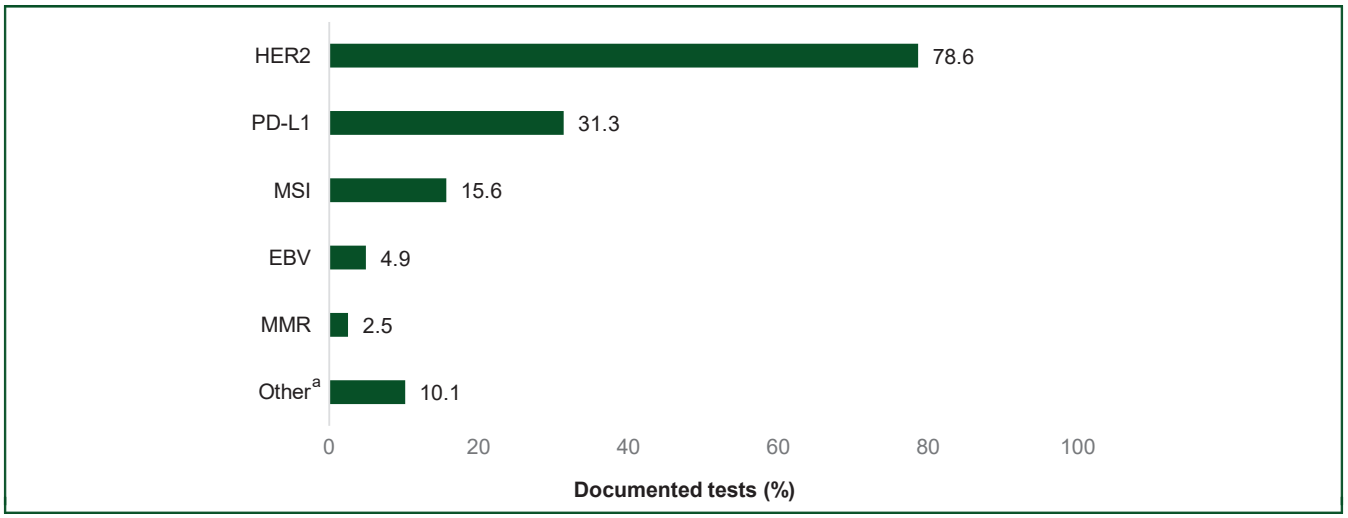

Figure 3. Documented biomarker tests in clinical routine. Documented biomarker tests prior to the start of first-line treatment for patients with advanced gastric and gastroesophageal junction adenocarcinoma (GAC/GEJAC) in SAPHIR ($N = 473$). Multiple answers for tested biomarkers per patient were possible.
EBV, Epstein—Barr virus; HER2, human epidermal growth factor receptor 2; MMR, mismatch repair; MSI, microsatellite instability; PD-L1, programmed death ligand-1.
[a]At least one other biomarker test was documented.

Table 2. Biomarker test results determined by local and central testing

Details on testing results	Central testing (n = 161)	Local testing (N = 473)	
	% of all patients	% of all patients	% of documented tests
HER2 test documented	N = 161	N = 372	
Positive	19 (11.8)	79 (16.7)	79 (21.2)
Negative	136 (84.5)	293 (61.9)	293 (78.8)
Not evaluable/no test documented[a]	3 (1.9)	101 (21.4)	101 (N/A)
Missing	3 (1.9)	—	—
IHC score			
IHC 0-1	129 (80.1)	151 (31.9)	151 (40.6)
IHC 2/ISH positive	—	4 (0.8)	4 (1.1)
IHC 2/ISH negative	7 (4.3)	7 (1.5)	7 (1.9)
IHC 3	19 (11.8)	40 (8.5)	40 (10.8)
IHC 2/ISH missing	—	7 (1.5)	7 (1.9)
Not evaluable/no test documented[a]	3 (1.9)	163 (34.5)	163 (N/A)
Missing	3 (1.9)	—	—
PD-L1 test documented	N = 161	N = 148	
CPS ≥10	46 (28.6)	28 (5.9)	28 (18.9)
10> CPS ≥5	24 (14.9)	11 (2.3)	11 (7.4)
5> CPS ≥1	50 (31.1)	16 (3.4)	16 (10.8)
CPS <1 or missing but TPS or IC ≥1%	1 (0.6)	29 (6.1)	29 (19.6)
Negative	32 (19.9)	58 (12.3)	58 (39.2)
Not evaluable/no test documented[a]	8 (5.0)	325 (68.7)	325 (N/A)
Missing	—	6 (1.3)	6 (4.1)
MMR/MSI test documented	N = 161	N = 82	
dMMR/MSI-H[b]	4 (2.5)	12 (2.5)	12 (14.6)
Potentially dMMR/MSI-H[c]	—	67 (14.2)	67 (81.7)
Neither dMMR nor MSI-H[d]	152 (94.4)	3 (0.6)	3 (3.7)
Not evaluable[e]/no test documented[a]	5 (3.1)[e]	391 (82.7)	391 (N/A)
Missing	—	—	—
EBV test documented	N = 161	N = 23	
Positive	1 (0.6)	—	—
Negative	157 (97.5)	23 (4.9)	23 (100)
Not evaluable/no test documented[a]	3 (1.9)	450 (95.1)	450 (N/A)
Missing	—	—	—
CLDN18.2 test documented	N = 161	—	
Positive[f]	43 (26.7)	—	—
Negative	115 (71.4)	—	—
Missing	3 (1.9)	—	—
Staining intensity			
Negative	71 (44.1)	—	—
Weak (1+)	23 (14.3)	—	—
Moderate (2+)	27 (16.8)	—	—
Strong (3+)	37 (23.0)	—	—
Not evaluable	2 (1.2)	—	—
Missing	1 (0.6)	—	—
pTC			
pTC ≥75%	47 (29.2)	—	—
75% > pTC ≥50%	9 (5.6)	—	—
pTC <50%	101 (62.7)	—	—
Not evaluable	2 (1.2)	—	—
Missing	2 (1.2)	—	—

Data are number of documented tests (%), unless otherwise indicated.
CLDN18.2, Claudin 18 isoform 2; CPS, combined positive score; dMMR, mismatch repair deficiency; EBV, Epstein—Barr virus; HER2, human epidermal growth factor receptor 2; IC, immune cell score; IHC, immunohistochemistry; ISH, *in situ* hybridization; MSI, microsatellite instability; MSI-H, high microsatellite instability; N/A, not applicable; PD-L1, programmed death ligand-1; pTC, positive tumor cells; TPS, tumor proportion score.
[a]Number of patients with nonevaluable tests for central testing and patients with no documented tests for local testing.
[b]dMMR and/or MSI-H.
[c]Patients with potentially dMMR or MSI-H status, as only MMR or MSI test results were documented.
[d]MMR and MSI test result documented, but neither dMMR nor MSI-H.
[e]Patients with nonevaluable test results may have potentially dMMR/MSI-H status.
[f]Defined as ≥75% of tumor cells showing moderate-to-strong (2+/3+) CLDN18.2 staining intensity.

number of patients (n = 49). Although PD-L1 expression is one of the best-studied treatment-relevant biomarkers in immunotherapy, accurate assessment remains challenging.[26] PD-L1 expression exhibits high inter- and intratumoral heterogeneity and PD-L1 scoring is influenced by various methodological factors, including antibody selection and staining protocols.[10] Upon the availability of additional data, it would be of interest to investigate the potential causes of PD-L1 test deviations and to ascertain whether an influence on treatment efficacy can be identified.

Table 3. Details on test result deviations for central and local testing of HER2 and PD-L1

Test results	HER2	PD-L1[a]
Evaluable patients, *N*	473	473
Consent to central testing, *n*	418	418
Local test results documented, *n/N* (%)	372/473 (78.6)	148/473 (31.3)
Central and local test results available, *n*	137	49
Concordant test results, *n/n* (%)	120/137 (87.6)[b]	38/49 (77.6)[b]
Central +/local +, *n*	14	27
Central −/local −, *n*	106	11
Deviating test results, *n/n* (%)	17/137 (12.4)[b]	11/49 (22.4)[b]
Central +/local −, *n*	4	9
Central −/local +, *n*	13	2

Please refer also to Figure 1 for a detailed depiction of the patient subgroups analyzed.
CPS, combined positive score; HER2, human epidermal growth factor receptor 2; IC, immune cell score; PD-L1, programmed death ligand-1; TPS, tumor proportion score; +, positive; −, negative.
[a]PD-L1 positivity is defined as CPS ≥1 or TPS or IC ≥1%.
[b]Proportion of test results from the total number of available local and central tests.

The prevalence of dMMR or MSI-H in patients with advanced GAC/GEJAC is relatively low (<5%).[20] In the present study, the frequency of dMMR/MSI-H determined in the centrally tested subgroup was even slightly lower, with 2.5%. Likewise, EBV positivity was low in this study (0.6% of centrally tested patients), while it is estimated that EBV-associated GAC accounts for ∼7.5% of global cases.[38] A recent real-world cohort study reported a comparably low prevalence, with EBV positivity of 1%.[32] One potential explanation for the observed differences, both in dMMR/MSI-H status and in EBV frequency, is the diversity of the respective patient cohorts examined, as both biomarkers correlate with sex, age, histological subtype, and tumor location,[10,32,38,39] which may be different in our study and other published cohorts.

For CLDN18.2, a prevalence of 30%-33% in patients with GAC/GEJAC is estimated, whereby CLDN18.2-positive tumors are defined as moderate to strong (2+/3+) membrane staining in ≥75% of tumor cells, following the results of relevant clinical trials.[22-24,40,41] As CLDN18.2 represents a novel therapeutic target, comprehensive data on the prevalence of this biomarker are of particular interest. The CLDN18.2 positivity rate determined in our centrally tested real-world cohort was 26.7%. The expression of CLDN18.2 appears to be influenced by a number of different factors.[41] Moreover, the use of different antibodies may potentially impact the results obtained.[41] As for other biomarkers, the precise assessment of CLDN18.2 presents certain challenges. A high intratumoral heterogeneity was observed and the evaluation process is described to require a considerable degree of expertise.[41,42]

The median OS of the SAPHIR study cohort was in accordance with the literature and comparable to that reported recently in a large real-world cohort study in Germany, considering the population receiving first-line treatment.[7,43] The treatment strategies applied indicate that patients are treated according to the current recommendations (start of first-line between 2019 and 2022).[7] As many as 77% of patients with HER2-positive tumors received anti-HER2 targeted therapy. Immunotherapy was administered to 3.8% of patients. It must be taken into consideration that the approval of nivolumab and pembrolizumab occurred at the end of the enrollment period in SAPHIR and is dependent on both PD-L1 expression level and HER2 status.[7,12-15,17,44] At the time of the database cut, the PD-L1 expression level was documented in 31.3% of patients. The analysis of treatment strategies applied depending on HER2 and PD-L1 will be of interest once additional data are available.

Strengths and methodological limitations

The prospective data collection, the participation of a large number of sites (office-based oncological practices, hospitals, and medical care centers) all over Germany, and the recruitment of a study cohort selected by almost no inclusion/exclusion criteria are the most notable strengths of this study. A limitation is that the availability of samples for central testing was limited, despite a high rate of consent to participation in the biobank module and good cooperation by local pathologists. As a result of the data collection time (paused shortly after the approval of ICIs), local testing rates will likely not reflect the current standard of testing in the clinical routine.

CONCLUSION

This study provides valuable insights into molecular testing in patients with advanced GAC/GEJAC who started treatment in clinical routine in Germany from 2019 to 2022 and presents positivity rates of relevant biomarkers in a real-world cohort in Europe at this time. Patients were frequently tested for actionable biomarkers associated with approved targeted therapies. Nevertheless, there is still potential for further improvement. Discrepancies in HER2 and PD-L1 test results were found between local and central testing, which could be due to multiple reasons as discussed earlier. This is of particular interest, as the precise, yet challenging, assessment of biomarkers is crucial for the selection of the appropriate treatment and subsequently for the outcome of patients with GAC/GEJAC in routine care because patients with tumors not tested accurately for actionable biomarkers might not receive the most effective treatment.

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DISCLOSURE

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DATA SHARING

The data supporting the findings of the study are not openly available due to data privacy protection regulations. Inquiries regarding the data used for this study can be directed to the corresponding author.

ETHICS STATEMENT AND CONSENT TO PARTICIPATE

The study complies with the current local legal and regulatory requirements to ensure the protection of patients' personal data, including collection of patient informed consent. All procedures carried out in studies involving human participants were in accordance with the ethical standards of the National Research Committee and with the 1964 Helsinki Declaration and its later amendments. The study protocol was reviewed and approved by the ethics committees of the State Medical Association at Baden Württemberg (F-2019-114, date of initial ethical approval: 17 December 2019; amendment F-2019-114#A1: 11 November 2021), and the local institutional review board for each study site in Germany. SAPHIR is registered at ClinicalTrials.gov (NCT04290806). Written informed consent was obtained from all patients before enrollment.

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