



## Molecular profiling in gastroesophageal cancer – clinical routine and future perspective

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Received: 29 July 2019 / Accepted: 13 October 2019 / Published online: 8 November 2019  
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**Summary** Although several large clinical trials have been conducted in order to investigate targeted inhibition of several molecular pathways in gastric cancer, only a limited number of targeted therapies have been introduced in clinical routine. Besides scientific interest, international guidelines recommend investigation of some distinct molecular alterations, which are associated with therapeutic consequences. These are (i) human epidermal growth factor receptor 2 (HER2), (ii) programmed death receptor 1 (PD-L1) and (iii) microsatellite instability (MSI). There are some emerging markers, such as Epstein–Barr virus (EBV), which might also be associated with a favorable response to immunotherapy. These routine and potential markers will be further discussed in the scope of this short review.

**Keywords** Gastric cancer · Gastroesophageal junction cancer · Molecular markers

### Take home message

- Testing for HER2 is an international consensus in the diagnostic work-up of metastatic gastrointestinal tumors. After the introduction of very promising results with immunotherapy, identifying emerging biomarkers for the prediction of treatment response has become of particular interest. In this regard, analysis of PD-L1 staining with CPS score and investigation of MSI show a strong correlation with

treatment response; therefore, these investigations are already part of routine diagnostic biomarker investigations in the USA. Potential biomarkers for the prediction of successful treatment with immunotherapy such as EBV are promising; however, clinical and investigational data are not complete. There are several clinical trials, which test potential biomarkers for response prediction to immunotherapy such as tumor mutation burden, distinct labor alterations (neutrophil/lymphocyte ratio), or content of gut microbiome. These are investigational biomarkers and have no application for the routine assessments.

- In case of resectable settings, molecular profiling and biomarker investigations are still in early phases, where no routine recommendation for the diagnostic procedures is available.

### Introduction

Gastric cancer is a major contributor to global disease burden [1]. Even though survival has steadily increased during the past decades independent of the tumor stage, the prognosis remains poor [2]. Thus, molecular pathways, which drive tumor progression and metastasis, are of high clinical interest for the development of new targeted therapeutic approaches.

### HER2

Human epidermal growth factor receptor 2 (HER2) is overexpressed in up to 20% of all gastroesophageal (GE) tumors [3]. There exists varying information on the expression of HER2 and its association with the prognosis of this malignant disease. Although HER2 positivity is mainly associated with poorer survival [4], comparable survival times with HER2-negative patients were also shown [4]. Recently, Gu et al. demonstrated a meta-analysis of the prognosis of

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HER2-positive patients, where equal survival rates between HER2-negative and -positive patients were observed [5].

The pivotal ToGA (Trastuzumab for Gastric Cancer) trial was the first randomized, prospective, multicenter phase III trial to study the efficacy of first-line trastuzumab (a monoclonal antibody against HER2) in patients with HER2-positive advanced, metastasized or relapsed GE tumors [3]. Patients were randomly assigned to receive the standard chemotherapy combination of cisplatin plus fluorouracil/capecitabine with or without trastuzumab. Median overall survival (OS) was 13.8 months in the trastuzumab group, compared with 11.1 months in the control group (hazard ratio 0.74; 95% confidence interval [CI] 0.60–0.91;  $p=0.0046$ ). The longest survival (median 16 months) was seen in patients with the highest HER2 protein overexpression (defined by 3+ positive) and HER2 amplification. On the basis of this study, trastuzumab in combination with cisplatin and a fluoropyrimidine has been approved for the first-line treatment of advanced, metastasized or relapsed HER2-positive GE tumors. Consequently, an international consensus on the investigation of HER2 expression in advanced or metastatic settings was reached.

Two retrospective patient series investigating beyond progression trastuzumab continuation suggested a benefit in terms of an extension of overall survival and progression-free survival [6, 7]. However, two large clinical phase III trials investigating the anti-HER2 tyrosine kinase inhibitor lapatinib and chemotherapy/trastuzumab conjunction drug T-DM1 in second-line treatment of HER2-positive patients, who received trastuzumab previously, failed to show a survival benefit in further lines [8, 9]. A possible reason for this failure was potentially the conversion of HER2 positivity to a negative state. A phase II trial from Japan evaluated trastuzumab treatment beyond progression and showed that HER2 positivity is lost in up to 70% of patients, which might lead to an anti-HER2 treatment inefficiency [10]. There is lack of evidence whether patients with maintained HER2 positivity in second-line settings would benefit from an anti-HER2 treatment.

HER2 inhibition in combination with neoadjuvant treatment is currently under investigation in three large clinical trials. The HER-FLOT trial is a phase II trial, where HER2-positive resectable GE tumor patients receive a combination of trastuzumab and the chemotherapy regimen FLOT (5-fluorouracil, leucovorin, oxaliplatin, docetaxel) [11]. This trial published the interim results in ASCO 2014, where a pathological complete response rate of 22% could be achieved. In two further phase II trials, trastuzumab together with pertuzumab, another monoclonal antibody directed against HER2, and chemotherapy will be investigated in HER2-positive GE tumor patients [12, 13]. No data from these trials are available yet. Testing for HER2 expression in resectable settings might be use-

ful for further treatment decision, when the patients relapse. However, therapeutic consequences in the neoadjuvant setting do not exist.

### TCGA and molecular characterization

Recently, advances in technology and high-throughput analysis have improved our understanding of the genetic basis of gastric cancer. To provide a roadmap for patient stratification and trials of targeted therapies, the Cancer Genome Atlas (TCGA) Research Network has characterized 295 primary gastric adenocarcinomas and proposed a new classification of four different tumor subtypes consisting of Epstein–Barr virus positive, microsatellite instability (MSI), genomically stable and chromosomal instability subtypes [14].

### PD-L1

Programmed cell death ligand 1 (PD-L1) is a 40-kDA transmembrane protein that is activated among many cancer types and leads to an immunosuppressive tumor microenvironment. Thus, inhibition of PD-L1 and its receptor PD-1 have been intensively studied as novel treatment concepts in various cancer types [15]. A phase Ib clinical trial showed a promising overall response in gastric cancer when treated with the anti-PD-1 antibody pembrolizumab [16]. A further phase II trial emphasized this response in PD-L1 combined positive score (CPS)  $\geq 1\%$  patients; thus, pembrolizumab was approved in the USA for this indication [17]. Furthermore, a recent phase III trial in heavily pretreated patients with gastric cancer demonstrated an efficacy with another PD-1 inhibitor, nivolumab, in an Asian population, which led to its approval as salvage treatment in Japan [18]. Median OS was 5.26 months in the nivolumab group and 4.14 months in the placebo group (hazard ratio 0.63, 95% CI 0.51–0.78;  $p<0.0001$ ). Interestingly, pembrolizumab demonstrated different results in recent phase III trials in second line settings. In the Keynote-61 trial, pembrolizumab was not effective as second-line treatment option in PD-L1-positive preselected patients [19]. However, in the Keynote-181 trial, pembrolizumab was demonstrated to be effective in both adenocarcinoma and squamous cell carcinoma in second line, when CPS was  $\geq 10\%$  [20]. The Keynote-62 trial was presented within the ASCO 2019 [21]. The results of this study, which tested pembrolizumab as first-line treatment in advanced and metastasized gastroesophageal cancer, might indicate a survival benefit of patients with a CPS  $\geq 10\%$ . However, further investigation is necessary, since some subgroups developed a rapid progress despite having a CPS  $\geq 10\%$ .

According to National Comprehensive Cancer Network (NCCN) guidelines, CPS should be investigated in gastroesophageal carcinoma patients if metastatic disease is suspected. It is, however, important to men-

tion that neither pembrolizumab nor nivolumab has treatment approval by European authorities; thus, no evident recommendation for CPS testing can be made. Nevertheless, in many large European centers CPS is investigated routinely in metastatic settings.

### MSI

In gastric cancer, many studies have been conducted concerning the clinical and pathological characteristics of MSI. The majority of these studies show an association of a high MSI (MSI-H) with older age, female gender, distal third of the stomach, intestinal pathology, lower pTNM stage and lower number of infiltrated lymph nodes [22–26]. MSI-H gastric cancer is generally characterized by some distinct genetic features including increased number of tumor infiltrating lymphocytes and PD-L1 positivity [22, 26]. It is surmised that around 20% of all western gastric tumor cases are MSI-H [22, 26].

In 2017 immunotherapy with pembrolizumab was approved by the Food and Drug Administration (FDA) for the treatment of unresectable or metastatic, MSI-H solid tumors that have progressed following prior treatment and which have no satisfactory alternative treatment options [27]. Based on this “tissue agnostic approval”, investigation of MSI in tissues of all tumor types might have a therapeutic consequence. However, it is again important to mention that this kind of treatment has not been approved by European authorities.

The British MAGIC trial demonstrated a survival benefit for patients with resectable cancer when treated perioperatively with the chemotherapy combination epirubicin, oxaliplatin, and capecitabine [28]. However, a recent post hoc investigation of the tissue samples of the MAGIC trial suggested that those patients with MSI-H tumors benefited less from chemotherapy, indicating other treatment strategies should be offered for this selected patient group [29]. The post hoc investigation of the CLASSIC trial, where Asian patients were randomized to adjuvant chemotherapy versus surgery alone, did again show no survival benefit for patients with MSI-H, when postoperative treatment is given [30]. Randomized prospective trials with MSI-H patients, where chemotherapy in the resectable setting is omitted, are under investigation and will show us whether we could treat these patients solely with surgery or with an alternative therapy, such as immunotherapy. Therefore, it is too early now to say that MSI should be routinely tested in resectable settings.

### EBV

Tumor Epstein–Barr virus (EBV) positivity is an emerging marker, which might be introduced in personalized treatment for gastric cancer. A positivity rate of 8–10% in gastroesophageal cancers is

estimated [31]. Unlike other EBV-associated malignancies, the distribution of EBV-associated gastric carcinoma worldwide is approximately even. The prognostic value of EBV on the survival is not fully clarified; however, several studies indicate a better prognosis associated with EBV positivity [32–34]. Additional studies show an enhancement of PD-L1 expression in EBV-positive gastroesophageal tumors, which might suggest that immunotherapy targeting the PD-L1/PD-1 axis might be of benefit in this subgroup [35, 36]. Recently, a phase II biomarker trial demonstrated a 100% overall response rate in gastroesophageal cancer patients with EBV positivity, when treated with pembrolizumab in a second-line setting [37]. Due to the lack of evidence in large clinical trials, investigation of EBV is not routinely recommended in international guidelines. Nevertheless, testing for EBV is already part of routine diagnostics in many large academic hospitals.

**Funding** Open access funding provided by Medical University of Vienna.

**Conflict of interest** H.C. Pühr received travel support from Eli Lilly and Roche. A. Ilhan-Mutlu participated in advisory boards from Merck Sharp & Dohme and Servier, received lecture honoraria from Eli Lilly and Servier, is the local principle investigator for clinical trials sponsored by Bristol-Myers Squibb and Astellas.

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