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Review Article

Gastric Cancer: Biomarkers and Therapeutic Approaches

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Abstract

Gastric cancer remains one of the most frequent malignant pathology worldwide.It is currently the fourth most common cancer found worldwide and the second leading cause of death by cancer. In 2020 over 1 milion new cases of GC were diagnosed, most of them were advanced which leads to a poor prognosis. Despite the great progress in the treatment of gastric cancer there are still critical needs to address. Due to the progress made in the molecular study of gastric cancer several targeted therapies have been developed to treat unresectable or metastatic gastric cancer. To date, there are three established biomarkers with good responses to targeted therapy: HER-2 (human epidermal growth factor receptor-2) positive for trastuzumab and trastuzumab deruxtecan, overexpression of PD-L1 for nivolumab, MSI-H (microsatellite instability high) for pembrolizumab. As new biomarkers shows up possibly there will be more treatment options for those patients with advanced/mGC. Promising results are seen in the most recent studied biomarkers Claudin18.2 and FGFR2b in this specific population. This review will sumarize the role of biomarkers, diagnostic methods and the advantage of molecular targeted therapy in advanced/mGC.

Keywords: Advantage; Biomarkers; Gastric Cancer; Recently; Targeted Therapies

Introduction

Gastric cancer is the second leading cause of death by cancer being an aggressive heterogeneous disease and still remains a serious problem worldwide [1]. The frequency of GC in SUA and Europe remains low, but it is considerably higher in Asia, especially in Japan and China. Among the important risk factors involved in the GC ethiopathogenesis we list Helicobacter Pylori infection,dietary factors, tobacco, obesity, and radiation. Approximately 95% of stomach cancers are adenocarcinomas. Based on Lauren clasification [2], there are two distinct types of adenocarcinoma: diffuse- associated with hereditary factors with poor prognosis and intestinal type, frequent endemic, associated with inflammation and infection due to Helicobacter Pylori, well differentiated and has a better prognosis. It is important to understand various molecular mechanism involved in proliferation and developement of the tumors to indentify the targets and underlying mechanism in order to find targeted-agents against them that brings efficiency in treatment and survival of those patients who missed the oportunity of a curative treatment and to offer new perspectives in therapeutic management. Based on genomic and epigenomic alterations The Cancer Genom Atlas (TCGA) uncovered four molecular subtypes of gastric cancer: Epstein-Barr virus (EBV), Microsatellite Instability (MSI), Genomically Stable (GS), and chromosomal instability (CIN) [3]. The best prognosis is associated with EBV subtypes and the worst prognosis with GS subtype. The MSI and CIN subtype have poorer overall survival than those with EBV subtype but better overall survival than those with GS subtype.

Various studies proved the efficacy of targeted-therapy alone or in combination with chemotherapy.

However, there is a lower frequency of biomarkers in GC therefore emphasis is placed on the precise detection of target molecules by appropriate diagnostic methods to select those patients who can benefit from targeted therapy. This review attempts

to expound the importance of understanding the mechanism and the relevance of established biomarkers (HER2, PD-L1 and MSI/MMR) and the promising new ones (fibroblast growth factor receptor 2 (FGFR2) ,Claudin 18.2 (CLDN18.2) along with their targeted-agents.

Key biomarkers with their targeted agents

There are well-established biomarkers and emerging one, but there where some studies with different molecular targets such as EGFR, PI3K/MTOR/AKT, MET which showed that targeted therapy against them didn't have any obvious advantage and not improve survivall.

EGFR and anti-EGFR-targeted therapy

The epidermal growth factor receptor belongs to the ErbB family receptors, a subfamily of four closely related receptor tyrosine kinases: EGFR (ErbB-1), HER2/neu (ErbB-2), Her 3 (ErbB-3) and Her 4 (ErbB-4). If there are mutations affecting EGFR expression or activity it becomes oncogenic. There were two trials who used anti-EGFR monoclonal antibodies (Cetuximab and Panitumumab) in first line. EXPAND study [4] explored the efficacy and safety of treatment with Cetuximab combined with XP regiments(capecitabine/cisplatin) in first line of advanced gastric cancer. There was 904 patients divided into two groups who received XP with Cetuximab and XP alone. Progression-free survival was 4.4 versus 5.6 months and overall survival was 9.4 versus 10.7 months with cetuximab combination vs. XP alone. No general benefit was seen from adding cetuximab to first-line capecitabine and cisplatin for treating patients with advanced gastric cancer (Table 1). REAL-3 study [5] tested the efficacy of panitumumab combined with EOX scheme (epirubicin/oxaliplatin/capecitabine) vs EOX. 553 patients were enrolled.One arm received EOX and Panitumumab and the others receieved EOX alone. The primary endpoint was OS . The secondary endpoint was PFS defined as the time from randomisation until documented disease progression or death from any cause . Median overall survival was reduced for EOX in combination with Panitumumab (8.8 months than 11.3 months for EOX) . Unfortunately, none of the EGFR inhibitors showed an improvement in the survival of pacients with advanced cancer.

Target	Trial	Number of patients	Regimen	Results(primary endpoint)	Reference
EGFR	EXPAND	904	Cetuximab+ XP vs. XP alone	Negative(PFS)	4
	REAL-3	553	Panitumumab+EOX vs. EOX	Negative (OS)	5

Abbreviations: EGFR: Epidermal Growth Factor Receptor, XP: Capecitabine/Cisplatin, EOX: Epirubicin/Oxaliplatin/Capecitabine, OS: Overall Survival, PFS: Progression-Free Survival

Table 1: Clinical trials with anti-EGFR monoclonal antibodies and their results.

PI3K/AKT/mTOR Pathway

The PI3K/AKT/mTOR pathway [6] has an important role because it is a promoter for cell growth, metabolism, survival, metastasis, and resistance to chemotherapy. It is demonstrated that in gastric cancer the PI3K/AKT/mTOR pathway has genetic alterations. Many molecular targeted therapy have gone through several clinical trials but none of them have been aproved for advanced gastric cancer treatment.

Target	Trial	Number of patients	Regimen	Results(primary endpoint)	Reference
AKT	JAGUAR	120	mFOLFOX+Ipatasertib vs. mFOLFOX+placebo	Negative (PFS)	7
mTOR	GRANITE-1	26	Everolimus vs. placebo	Negative (OS)	36
mTOR	RADPAC	300	Everolimus+Paclitaxel vs. Paclitaxel alone	Negative (OS)	

Abbreviations: PI3K: Phosphatidylinositol-3 Kinase, AKT: Phosphoinositide-3-Kinase–Protein Kinase B, mTOR:Mammalian Target Of Rapamycin, OS: Overall Survival, PFS: Progression-Free Survival

Table 2: Clinical trials with anti-PIK3/AKT/mTOR molecular targeted therapy and their results.

The JAGUAR trial [7] that used Ipatasertib-an AKT inhibitor- had no statistically significant PFS benefit. This trial is a phase II study with 120 patients (HER-2 negative or HER-2 unknown GC) with previously untreated locally advanced or metastatic GC randomized to receive Ipatasertib with FOLFOX and FOLFOX with placebo.The primary endpoint progression free survival was not reached. In second-line of treatment were two trials with anti-mTOR targeted therapy-everolimus. GRANITE-1 and RADPAC trials showed that there was no significant overall survival improvement for advanced gastric cancer that progressed after previous systemic chemotherapy (Table 2).

MET and anti-HGF/MET-targeted therapy

The mesenchymal-epithelial transition receptor is stimulated by the hepatocyte growth factor (HGF). From there multiple signal pathways are activated and promote gastric cell proliferation [6]. There are various inhibitors antagonizing HGF/MET. We'll discuss about rilotumumab, a monoclonal antibody directed against HGF which was proposed for first line treatment. We extract data from a phase III study-RILOMET-1-[8] which shows that rilotumumab combined with epirubicin+cisplatin+capecitabine (ECX) vs placebo with ECX was not effective in improving clinical outcomes in patients with MET-positive gastric cancer. This study was stopped earlier due to a higher adverse events grade 3-4 in the rilotumumab group than in the placebo group. A phase III study of onartuzumab [9] plus mFOLFOX6 in patients with metastatic HER2-negative and MET-positive shows that addition of onartuzumab to mFOLFOX6 did not significantly improve the primary endpoint - OS (11.0 for combination vs 11.3 months for mFOLFOX6). PFS, ORR and safety-the secondary endpoints-were not improved. RILOMET-1 and METGastric results from two phase III were negative the reason why could be that MET gene is not the driver gene for gastric cancer (Table 3).

Target	Trial	Number of patients	Regimen	Results(primary endpoint)	Reference
HGF	RILOMET-1	609	Rilotumumab+ECX vs placebo+ECX	Negative (OS)	8
	METGastric	562	Onartuzumab+mFOLOFOX6 vs mFOLFOX+placebo	Negative(OS)	9

Abbreviations: HGF: Hepatocyte Growth Factor, ECX: Epirubicin+Cisplatin+Capecitabine, OS: Overall Survival

Table 3: Clinical trials with anti-HGF targeted therapy.

Currently Established Biomarkers in GC

HER-2

The receptor HER-2 (ERBB2) belongs to the human epidermal growth factor receptor family being related to EGFR receptor [10]. This biomarker is a receptor-tyrosine kinase that can be overexpressed or amplified in GC. It is a proto-oncogene involved in signaling pathways which leads to cell growth and differentiation. HER-2 was the first biomarker used to guied clinical decisions in GC. Many studies have demonstrated that HER-2 is present in several others malignancies including breast, ovarian, prostate, colorectal, lung and gastroesophageal tumors. HER-2 posivity has been identified in ~22% of advanced GC. The overexpression depens on the location (e.g gastro-esophageal jonction or cardia), the molecular subtype (Chromosomal Instability (CIN)), differentiation (well or moderately differentiated) and histologic subtype (generally, more associated with the intestinal type).

Nowadays , the detection of HER-2 may be done with IHC, ISH metods and NGS [11]. HER-2 immunostaining is generally assessed based on a score of the staining intensity (on a scale from 0 to 3+) and on the calculation of the proportion of stained tumor cells. HER-2 overexpression is defined as an IHC score of 3+. It is recommanded to start with IHC followed by ISH methods only when expression is 2+(equivocal). Positive (3+) or negative(0 or 1+) IHC results do not require further testing via ISH.

It is very important to know if there is HER-2 amplification/ overexpresion because the targeted therapy-trastuzumab-is well established for gastric cancer treatment.

Trastuzumab is a humanized monoclonal antibody that binds the extracellular domain of HER-2 receptor and inhibits the signaling pathway. There was a phase III trial called ToGA which included 594 patients with previously untreated advanced or metastatic gastric cancer that overexpress HER-2 which received chemotherapy in combination with Trastuzumab and chemotherapy alone in first line (Table 4) [12]. The data from this trial showed that there is 13.8 months OS in the arm with trastuzumab vs 11.1 months for chemotherapy alone(HR 0.74,p<0.01) (Table 4). Most common adverse events in both groups were nausea, vomiting and

neutropenia. Based on this study, adding trastuzumab to chemotherapy in first line for patients with HER-2 overexpression became the standard treatment.

There were several phase III studies which used another HER-2 targeting agents such as lapatinib (LOGiC study) [13], trastuzumab-emtansine (GATSBY study) [14], pertuzumab (JACOB study) [15] as first or second line treatment. Severe adverse reaction were seen in JACOB trial, almost 50% in the pertuzumab group. Diarrhoea was the most common serious adverse event in both groups. The primary endpoint in these three trials was overall survival that was not reached. All of these failed in bringing clinical outcomes (Table 4).

Key-Note 811 study brings significantly higher objective response rate with the addition of pembrolizumab to trastuzumab and chemotherapy in patients with previously untreated unresectable or metastatic HER2-positive gastric or gastroesophageal junction adenocarcinoma [16]. The objective response observed was 74.4% for those with pembrolizumab vs 51.9% for those with placebo, with complete response in 11.3% vs 3.1%. The analysis from this trial accelerated approval of pembrolizumab in combination bases on the response rate and duration of response. This trial demonstrates that adding an immune checkpoint inhibitor to targeted therapy can be an efficent strategy to overcome HER2 resistance in GC.

Target	Trial	Number of patients	Regimen	Results(primary endpoint)	Reference
HER-2	ToGA	594	Trastuzumab+Capecitabine/FU+Cisplatin vs. Capecitabine/FU+Cisplatin alone	Positive (OS)	12
HER-2	LOGiC	545	Lapatinib+XELOX vs XELOX alone	Negative (OS)	13
HER-2	GATSBY	70	Trastuzumab-etamsine vs Paclitaxel or Docetaxel	Negative (OS)	14
HER-2	JACOB	780	Pertuzumab+trastuzumab+chemotherapy vs. Trastuzumab+chemotherapy	Negative (OS)	15

Abbreviations: HER-2: Human Epidermal Growth Factor Receptor, FU: Fluorouracil, XELOX: Capecitabine+Oxaliplatin, OS: Overall Survival

Table 4: Clinical trials with anti-HER-2 targeted therapy.

MSI/MMR

MSI can be defined as a form of genetic instability manifested by changes in the length of repeated single- to six-nucleotide sequences (known as DNA microsatellite sequences), which are caused by a functional defect in DNA MMR [17]. Most of MSI tumors are sporadic due to epigenetic inactivation of MLH1 gene while the others occurs in patients s with a mutation in a DNA MMR gene. MSI can be found in a wide range of solid tumor types such as colorectal cancer, gastric cancer, endometrial cancer. MSI can be detected by DNA polymerase chain reaction (PCR) or NGS; MMR protein expression can be detected via IHC. Tumors with over 30% expression of unstable microsatellites are called MSI-H(high) while tumors with 10-29% expression are considered MSI-L(low). MSI-H has been reported in ~4% of advanced gastric cancer. MSI-H is a favorable prognostic marker but is present in a lower percentage of gastric cancer. It predicts a poor response to 5-FU but adding oxaliplatin in regiments e.g FOLFOX can stop the adverse effect of MSI-H. But what if we add a targeted therapy?

It is known that programmed death 1 (PD-1) blockade has clinical benefit in microsatellite-instability–high (MSI-H) or mismatch-repair–deficient (dMMR) tumors after previous therapy. In 2015 the KEYNOTE-016 trial has identified MSI-H as a biomarker indicative of immunotherapy efficacy [18]. Two years later, FDA approved *pembrolizumab* (ICI) for the patients with advanced MSI-H/dMMR or metastatic tumors. There is data from three trials with pembrolizumab: KeyNote-062 (first-line treatment), KeyNote-061 (second-line treatment) and KeyNote-059 (third line treatment). KeyNote-062 trial shows that Pembrolizumab as monotherapy in first line treatment of advanced gastric cancer shows non-inferiority to standard chemotherapy in overall survival to patients with CPS>1%, with lower toxicity [19]. But adding Pembrolizumab to chemotherapy didn't improve results compared to chemotherapy alone. We

emphasize that there is a little bit differences in the results of KeyNote-590 trial that shows that adding Pembrolizumab to chemotherapy vs chemotherapy alone in gastroesophageal junction improved overall survival [20] . KeyNote-061 (pembrolizumab vs paclitaxel) shows that pembrolizumab did not significantly improve OS but was associated with higher 24-month OS rates than paclitaxel. For third line treatment was KeyNote-059 trial which demonstrated that adding Pembrolizumab in monotherapy after at least two lines of treatment brings a promising activity [21]. Pembrolizumab has demonstrated promising antitumor activity in patients with advanced gastric cancer with PD-L1 CPS ≥1 and CPS ≥10 irrespective of MSI-H status. In these three trials were identified the patients with MSI-H (the status was determinated by polymerase chain reaction testing): 7.3% in KeyNote-062,5.3% in KeyNote-061 and 4% in KeyNote-059.In those patients with MSI-H tumors, OS and PFS were prolonged with pembrolizumab vs chemotherapy. The findings suggest that MSI-H status may be a biomarker for pembrolizumab benefit in these malignancies.

PD-L1

PD-1 (programmed cell death-1) is an immune check-point receptor expressed on the activated T-cells [22]. Its ligand PD-L1 (programmed cell death ligand-1) is found on the tumor cells but also on the imune cells that penetrate the tumor (dendritic cells, macrophage, T and B lymphocytes). PD-1 and PD-L1 act like co-inhibitory factors that can limit the development of the T cell response. The PD-1/PD-L1 interaction has a inhibitor role to the immune response to minimize the possibility of chronic autoimmune inflammation. When PD-1 binds to PD-L1 basically tells the T-cell to leave the other cell alone. This happens in a malignant proliferation where can be found the PD-L1 expression on the tumor cells that helps them to hide from an immune atack. The point is to make a immune checkpoint blockade with monoclonal antibodies. Either PD-1 or PD-L1 are therapeutics targets, both of them have been helpful in treating different types of cancers. PD-L1 expression has been detected in various tumors, including lung, colon, ovarian and gastric cancers. PD-L1 expression is detected using IHC. Tumors are considered PD-L1 positive if the CPS score is one or higher. CPS score (combined positive score) is defined as the number of positive tumor cells, lymphocytes and macrophages, divided by the total number of viable tumor cells multiplied by 100. Prevalence of PD-L1 has been reported for several positivity thresholds throughout various studies: 67-73% CPS ≥1, 29-31% CPS ≥5, and 16-18% CPS ≥10 . Various studies have shown discordant levels of PD-L1 in the primary tumor vs metastatic sites, probably due to the intratumoral heterogenity of PD-L1 expression. For an accurate diagnosis of PD-L1 expression there should be obtained multiple biopsy samples(at least five).

Monoclonal antibodies against PD-1 (*nivolumab and pembrolizumab*) have demonstrated efficacy in patients with advanced gastric cancer in clinical trials. ATTRACTION-2, a phase III trial, showed that giving nivolumab in monotherapy to advanced gastric cancer patients previously treated with two or more previous chemotherapy regiments has improved the primary endpoint- OS (5,26 vs 4,14) and prolonged the progression-free survival (Table 5) [23]. 12-month overall survival rates were 26,2% vs10,9%.This trial indicate that nivolumab might be a new treatment option. A phase III CheckMate-649 trial recently showed that adding nivolumab in combination with chemotherapy (capecitabine and oxaliplatin every 3 weeks or leucovorin, fluorouracil, and oxaliplatin every 2 weeks) vs chemotherapy alone in first line improves OS (13.1 vs 11.1; hazard ratio [HR] 0·71 [98·4% CI 0·59-0·86]; p<0·0001) and PFS in patients with CPS>5 (Table 5) [24]. Most common adverse events (≥25%) were nausea, diarrhoea, and peripheral neuropathy across both groups. Nivolumab plus chemotherapy represents a new standard first-line treatment for these patients.

A reliable biomarker for anti-PD-1 ICI is the molecular subtype. EBV+ and MSI-H tumors signaling responses to pembrolizumab (overall response rates of 100% in EBV+ and 85.7% in MSI-H). It is very important to understand the molecular and immunological characteristics of GC to know what is the best option ICI treatment for patients with advanced gastric cancer.

Target	Trial	Number of patients	Regimen	Results(primary endpoint)	Reference
PD-1	Attraction-2	493	Nivolumab vs placebo	Positive(OS)	23
PD-1	CheckMate-649	1581	Nivolumab+chemotherapy vs chemotherapy alone	Positive(OS, PFS)	24

Abbreviations: OS: Overall Survival, PFS: Progression-Free Survival

Table 5: Clinical trials with anti-PD-1 targeted therapy.

VEGF/VEGFR-2

The tumoral angiogenesis represents proliferation of new blood vessels which supplies a tumor with oxygen and nutrients to sustain optimal growth. Different studies have shown that a higher tumoral angiogenesis is bound to a poor prognosis.There are two strategies

for tageting VEGF : (1) we can use a monoclonal antibody anti-VEGF (e.g bevacizumab), to inhibit the proangiogenenic effect of VEGF or (2) we can use a VEGF receptor monoclonal antibody (e.g ramucirumab) or a small tyrosine kinase inhibitor molecules (e.g Apatinib).

The most used test for angiogenesis quantification is microvascular density based on labeling with an anti-CD31 monoclonal antibody [25].

The AVAGAST trial evaluated the efficacy of adding *bevacizumab* to capecitabine-cisplatin in the first line of advanced gastric cancer treatment [26]. The primary objective,overall survival, was not reached (Table 6). The median OS was 12.1 months with bevacizumab and chemotherapy vs 10.1 with placebo and chemotherapy. Rainfall trial used ramucirumab, a VEGF receptor monoclonal antibody, in first line of treatment in combination with chemotherapy(cisplatin and capecitabine) [27]. By adding ramucirumab to chemotherapy the OS (secondary endpoint) was not improved, even if the primary endpoint-PFS-was significantly prolonged and it is not recommended as first-line treatment (Table 6). So, in first line of treatment we cannot add targeted therapies for VEGF/VEGFR because none of them showed significant benefits regarding the survival.

Ramucirumab is a humanized monoclonal antibody that binds to VEGFR-2 and block the binds of VEGF-A,VEGF-C,VEGF-D preventing the proliferation, permeability, and migration of human endothelial cells. We have positive data from two studies which proved that adding Ramucirumab in second line in monotherapy or in combination with chemotherapy (paclitaxel) improved overall survival to advanced gastric cancer patients. A phase III RAINBOW trial showed the benefit from adding ramucirumab to chemotherapy (paclitaxel) vs chemotherapy alone (OS : 9,6 months for combination vs 7,4 ,p=0,047). The REGARD trial also proved that ramucirumab in monotherapy can increase the survivall (OS 5,2 vs 3,8 months,p=0,047) (Table 6) [28].

Apatinib an oral small molecular of VEGFR-2 tyrosine kinase inhibitor can be used as third line of treatment to those who failure to second-line chemotherapy. From the phase III trial of Apatinib: median overall survival (mOS) was significantly prolonger in the apatinib group compared with in the placebo group (195 days versus 140 days; HR= 0.71; 95% CI (0.54 □ 0.94); P < 0.016). Median progression-free survival (mPFS) was also prolonged in the apatinib group compared with the placebo group (78 days versus 53 days, HR = 0.44, 95% CI (0.33 □ 0.61), P < 0.0001) [29]. This study confirmed the efficacy and safety of apatinib in patients with advanced gastric cancer (Table 6).

Regorafenib an oral multi-kinase inhibitor, has positive data from phase II trial called INTEGRATE.The patients received Regorafenib vs placebo, the primary endpoint being PFS.The PFS was improved for regorafenib vs placebo (2,6 months vs 0,9) (Table 6) [30].

Target	Trial	Number of patients	Regimen	Results(primary endpoint)	Reference
VEGF	AVAGAST	774	Bevacizumab+XP vs XP alone	Negative (OS)	26
VEGFR-2	RAINFALL	645	Ramucirumab+XP	Negative(did not improve overall survival,even if PFS-primary endpoint) was significantly prolonged)	27
VEGFR-2	RAINBOW	39	Ramucirumab+paclitaxel vs paclitaxel alone	Positive (OS)	28
VEGFR-2	REGARD	355	Ramucirumab vs placebo	Positive (OS)	28
VEGFR-2	APATINIB TRIAL	267	Apatinib vs placebo	Positive (OS and PFS)	29
VEGFR-2	INTEGRATE	152	Regorafenib vs placebo	Positive (PFS)	30

Abbreviatons: VEGF: Vascular Endothelial Growth Factor, VEGFR-2: Vascular Endothelial Growth Factor Receptor-2, XP: Capecitabine/Fluorouracil+Cisplatin, OS: Overall Survival, PFS: Progression-Free Survival

Table 6: Clinical trials with anti-VEGF/VEGFR-2 targeted therapy.

Emerging Biomarkers in GC

Claudin18.2

Claudins are a family of transmembrane proteins. Claudins are present throughout the body, but there are two specific isoform of Claudin18, localized in certains tissue: Claudin18.1 is the dominant isoform in normal,healthy lung tissue and Claudin18.2 the dominant isoform in normal, healthy gastric epithelial cells [31]. So, Claudin18.2 is a tight-junction protein which is expressed in the gastric epithelia. This biomarker is involved in controlling the flow of molecules between cells.It has proprieties as a selective barier and contributes to cell-to-cell epithelial adhesion. When there is a malignat transformation the normal structure of the cells is loss and the cell-cell adhesion may be disrupted. As a result, Claudin18.2 can be more exposed and more accesible to antibodies. The presence of Claudin18.2 is found throughout malignant transformation both in the primary tumor site and metastatic disease. Detecting the presence of CLDN18.2 identifies a previously undefined patient population. Recent studies have shown approximately 36% of advanced cancer patients are CLDN18.2 positive (high expression). Among mGC biomarkers CLDN18.2+ is highly prevalent. The presence of CLDN18.2 can be detected by standars IHC staining methods being expressed in both diffuse- type tumors and intestinal-type tumors (tumors with diffuse histology are asociated with poorer prognosis). CLDN18.2 may be expressed when tumors develop in esophageal, pancreatic, lung, and ovarian tissues as well.

Zolbetuximab- an experimental monoclonal antibody-that targets CLDN18.2 has positive results in two trials. A global phase III trial –SPOTLIGHT- included patients with Claudin 18.2-positive, HER2-negative, locally advanced unresectable or metastatic gastric cancer [32]. One arm received Zolbetuximab plus mFOLFOX6 and the other arm received placebo plus mFOLFOX6. The initial results from this study were presented in January at the 2023 American Society of Clinical Oncology (ASCO) Gastrointestinal (GI) Cancers Symposium. On 14 april 2023 *The Lancet* published online detailed data from the phase III SPOTLIGHT trial evaluating first-line treatment with zolbetuximab [33].The primary endpoint is progression-free survival of participants treated with combination of zolbetuximab plus mFOLFOX6. The secondary endpoint include overall survival, objective response rate, duration of response, safety , tolerability and quality-of-life parameters. Zolbetuximab was shown to significantly increase both progression-free and overall survival compared to chemotherapy. The median progression-free survival was 10,61 months in the zolbetuximab group versus 8,67 months in the placebo group (Table 7). The study also showed that zolbetuximab plus mFOLFOX6 significantly prolonged OS, reducing the risk of death by 25.0% (HR=0.750; 95% CI: 0.601-0.936; P=0.0053). Median OS was 18.23 months for zolbetuximab and chemotherapy vs 15.54 months for chemotherapy and placebo (95% CI: 13.47-16.53).

At the ASCO Plenary Series from March 2023 Session first-line treatment with zolbetuximab in combination with capecitabine and oxaliplatin (CAPOX) extended overall survival in patients with claudin-18.2 (CLDN18.2)-positive/HER2-negative locally advanced or metastatic gastric adenocarcinoma according to results of the GLOW trial reported by Rui-Hua Xu (Table 7) [34]. For the zolbetuximab and placebo arms progression-free survival rates were 35% vs 19% at 12 months and 14% vs 7% at 24 months (HR = 0.687, P = .0007). Similarly, overall survival rates were 58% vs 61% at 12 months and 29% vs 17% at 24 months (HR = 0.771, P = .0118). This novel CLDN18.2 targeted therapy-Zolbetuximab- is more closer to be approved in gastric cancer.

Target	Trial	Number of patients	Regimen	Results(primary endpoint)	Reference
Claudin18.2	SPOTLIGHT	565	Zolbetuximab + mFOLFOX6 vs mFOLFOX6 alone	Positive (PFS)	32,33
Claudin18.2	GLOW	507	Zolbetuximab+CAPOX vs CAPOX alone	Positive(PFS)	34

Abbreviations: PFS: Progression-Free Survivall

Table 7: Clinical trial with anti-CLDN18.2 targeted therapy.

FGFR2b

Fibroblast growth factor receptor 2 (FGFR2) is one of four FGFR family members that encode transmembrane receptor tyrosine kinases being involved in normal cell development. FGFR2b is the IIIb splice isoform of FGFR2. The FGFR signaling axis contributes to tumor progression by enhancing angiogenesis and proliferation. The splice variant is expressed in various types of epithelial cells where tumors may begin to grow (pancreatic, breast, endometrial, cervical, lung, and colorectal cancers). FGFR2b can be associated with higher T stage and higher N stage. Its overexpression can be detected using IHC or gene amplification by ctDNA. FGFR2b positivity has been observed in 30% of mGC suggesting that targeting FGFR2b may be an important therapeutic strategy. In present, there is no approved FGFR inhibitor for FGFR2 positive gastric cancer but there is a selective FGFR2b monoclonal antibody-*bemarituzumab*-investigated in the first phase III randomized trial for patients with first line advanced cancer. The FIGHT trial selected patients with HER-2 negative, unresectable locally advanced or metastatic GC and overexpression of FGFR2b [35,36]. In phase II trial of bemarituzumab combined with mFOLFOX6 in first line of treatment vs mFOLOFOX, at the time of the primary analysis and at a median follow-up of 10.9 months (IQR 6.3–14.2), median progression-free survival was 9.5 months (95% CI 7.3–12.9) in the bemarituzumab group and 7.4 months (5.8–8.4) in the placebo group (Hazard Ratio [HR] 0.68 [95% CI 0.44–1.04; p=0.073). In this exploratory phase II trial no significant improvement in PFS was seen but treatment with bemarituzumab showed promising clinical efficacy. The results from this trial may bring major changes in the treatment for FGFR2 positive mGC.

Conclusions

We have seen that a lot of studies tried or are trying to find effective biomarkers that may bring an important role in diagnosis, classification, and molecular characterization of GC. NCCN Guidelines support using biomarkers to help guide the path forward for patients and recommend testing for all established biomarkers (HER2, MSI, PD-L1) at diagnosis if metastatic cancer is documented. As we have seen in various clinical trial there is a higher prevalence of emerging biomarkers (36% were CLDN18.2 positive and 30% were FGFR2b+). Their targeted drugs showed promising results which means that they are new targets of interest in advanced GC. While zolbetuximab is expected to see a first approval soon, we are waiting for data from phase III studies for bemarituzumab that can bring major changes in GC treatment.

Targeted therapy for established biomarkers (HER-2, PD-L1,MSI) had improved outcomes and showed significal survival benefit in advanced GC patients.

Unfortunately, there were negative results from EGFR, VEGF or mTOR – targeted therapy, but the anti-VEGFR-2 monoclonal antibody-rambucirumab- and a VEGFR2-tyrosine kinase inhibitor-apatinib- has demonstrated their efficacy in unselected patients but there needs to be more biomarkers explored for treatment response.

With the development of biotechnology, the molecular classification of GC will be more precise. The future treatment for advanced GC will be a clinical-pathological-molecular combined classification and guided individualized approach.

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