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Review

Targeted therapy for gastric cancer: Molecular pathways and ongoing investigations☆

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ABSTRACT

Gastric cancer is currently the second leading cause of worldwide cancer mortality. Ongoing collaborative sequencing efforts have highlighted recurrent somatic genomic aberrations in gastric cancer, however, despite advances in characterizing the genomic landscape, there have been few advances in patient outcomes. Prognosis remains poor with a median overall survival of 12 months for advanced disease. The improved survival with trastuzumab, and more recently ramucirumab, underscore the promise of targeted and biologic therapies and the importance of molecular tumor characterization in gastric cancer. Here we review the most frequent actionable alterations in gastric cancer and highlight ongoing clinical investigations attempting to translate biologic understanding into improved clinical outcomes.

Contents

1. Introduction

Gastric cancer is the fourth most common cancer worldwide [1] and second leading cause of cancer mortality. There is significant geographic variability, with the highest incidence in east Asia [1], eastern Europe, and parts of south and central America. In the US, approximately 21,600 people were diagnosed in 2013, of whom 10,990 were expected to die [2]. Depending on tumor characteristics and stage [3], current treatment modalities include combinations of surgery, chemotherapy, and radiation therapy [4–7]. However even with maximal trimodality therapies, prognosis for gastric cancer remains poor, with a 25%–35% 5-year survival rate for loco-regional disease [4,8,9] and median survival ranging from 10 to 14 months in advanced disease [10,11]. Phase III

Abbreviations: EGFR, epidermal growth factor receptor (EGFR); ErbB, epidermal growth factor receptor family (ErbB); PI3K, phosphatidylinositol 3-kinase (PI3K); AKT, protein kinase B; mTOR, mammalian target of rapamycin; VEGF, vascular endothelial growth factor; ALK, anaplastic lymphoma kinase; MAPK-ERK, mitogen-activated protein kinase–extracellular signal-regulated kinase; HGFR, c-Met, hepatocyte growth factor receptor; FGFR, fibroblast growth factor receptor; OS, overall survival; PFS, progression free survival; GEJ, gastro-esophageal junction; RTK, receptor tyrosine kinase; TKI, tyrosine kinase receptor inhibitors; PFS, progression free survival; DFS, disease free survival; MTD, maximum tolerated dose; DLT, dose limiting toxicity; ORR, objective response rate; RR, response rate; AE, adverse events.

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trials in Asian populations have generally reported the best outcomes [12–14] and differences in surgical technique, pathologic evaluation, available chemotherapeutic agents, tumor location [15] and biology [16] have been suggested as rationale for these geographic variations in gastric cancer outcomes [17,18].

Improving molecular characterization has translated into better survival in select patients with advanced gastric and esophageal cancer. The trastuzumab for Gastric Cancer (ToGA) trial [19] showed that the use of the anti-*HER2* (epidermal growth factor receptor kinase 2) antibody trastuzumab in patients with gastric cancer overexpressing HER2 improves overall survival (OS) compared to standard platinum–fluoropyrimidine chemotherapy. While the findings of the ToGA trial suggest promising potential for targeted therapy, few identified actionable genomic alterations have met the same success. Recently, two phase III trials investigating the use of epidermal growth factor receptor (EGFR) directed therapy [11,20] in molecularly unselected patient populations failed to demonstrate an improvement in OS in patients with advanced gastric or esophageal cancer.

As advancing genomic technologies continue to refine the molecular characterization of gastric cancer how these findings will impact patient outcomes remains to be seen. In this review, we describe known potentially actionable genetic alterations and attempt to translate biologic understanding into improved patient outcomes.

2. Genomic landscape

Molecular profiling has transformed our understanding of tumor complexity and has led to the development of targeted treatments for multiple malignancies. Ongoing advances in genomic technologies are giving researchers the ability to profile genetic alteration at increasingly greater resolution and speeds [21]. Targeted "hot-spot" sequencing technologies interrogating known and common mutations in a given exon provided early information on mutational frequencies. Higher throughput next-generation sequencing technologies encompassing all exons in a given gene are supplanting hot-spot platforms [22], and provide improved granularity and allow discovery of novel mutations [23] in gastric cancer.

In gastric cancer the most commonly recurring genomic aberrations involve TP53, PIK3CA, ErbB2, ErbB3, ARID1A and KRAS [24] (Table 1). Other less frequent alterations include alterations in MET, FGFR1, MYC and CDH1 [25,26] (Table 1). Lee and colleagues [24] sequenced 237 gastric adenocarcinomas (47% diffuse type, 24% EBV positive) uncovering 474 mutations in 41 genes and identifying recurrent somatic mutations in PIK3CA, TP53, APC, STK11, CTNNB1 and CDKN2A, among others. Other groups have similarly reported on recurrent somatic alterations in gastric tumor sets [24,25,27,28]. Few studies have strictly investigated genomic differences between Asian and Western patients, although there is suggestion that East Asian patients have fewer PIK3CA mutations and PTEN loss, but higher frequencies of PTEN deletion compared to Caucasian populations [29]. Collaborative sequencing repositories including the cancer genome atlas (TCGA) and the catalog of somatic mutations in cancer (COSMIC) continue to refine mutational frequencies. Differences in the frequencies of genomic alterations occur between data sets, owing to differing sequencing approaches (hot-spot vs whole exome analysis) and possible biologic differences between the patient populations. High-throughput sequencing advances over the past 3 to 5 years have greatly increased our understanding of the genomic landscape of advanced gastric cancer.

Table 1
Recurrent somatic genetic alterations in gastric adenocarcinomas.

Gene	Type of alteration	Frequency	Major cellular process	FDA-approved agent	Overall survival benefit
TP53	Mutation	43.80%	DNA damage response	None	Not tested
CDKN2B	Homozygous deletion	9.60%	DNA damage response	None	Not tested
BRCA2	Mutation	8.20%	DNA damage response	None	Not tested
BRCA1	Mutation	5.90%	DNA damage response	None	Not tested
MDC1	Mutation	7.30%	DNA damage response	None	Not tested
PIK3CA	Mutation	21.00%	Signal transduction, growth	None	Not tested
PIK3CA	Amplification	5.00%	Signal transduction, growth	None	Not tested
PTEN	Mutation	5.90%	Signal transduction, growth	None	Not tested
PIK3CG	Mutation	6.40%	Signal transduction, growth	None	Not tested
mTOR	Mutation	8.20%	Signal transduction, growth	Everolimus, temsirolimus	No (Ref. [83])
RPTOR	Mutation	5.50%	Signal transduction, growth	everolimus, temsirolimus	No (Ref. [83])
RICTOR	Amplification	7.70%	Signal transduction, growth	everolimus, temsirolimus	No (Ref. [83])
MYC	Amplification	11.90%	Cell cycle control	None	Not tested
CDKN2A	Homozygous Deletion	10.50%	Cell cycle control	None	Not tested
CDK6	Amplification	7.30%	Cell cycle control	None	Not tested
CCND1	Amplification	6.40%	Cell cycle control	None	Not tested
E2F7	Mutation	6.40%	Cell cycle control	None	Not tested
ERBB2	Amplification	10.50%	RTK, growth	Trastuzumab	Yes (Ref. [19])
ERBB2	Mutation	5%	RTK, qrowth	None	Not tested
ERBB3	Mutation	10.50%	RTK, growth	pertuzumab	Not tested
ERBB4	Mutation	9.40%	RTK, growth	None	Not tested
IGF1R	Mutation	5.90%	RTK, growth	None	Not tested
EGFR	Mutation	5%	RTK, growth	Erlotinib, gefitinib, afatanib	Not tested
EGFR	Amplification	5.40%	RTK, growth	Cetuxumab, panitumumab	No (Refs. [11,20])
KRAS	Mutation	11.40%	Ras–Raf–MEK–ERK	None	Not tested
KRAS	Amplification	5.40%	Ras–Raf–MEK–ERK	None	Not tested
DAB2	Amplification	8.20%	Ras–Raf–MEK–ERK	None	Not tested
PEG3	Mutation	9.60%	Survival-death regulation	None	Not tested
WWOX	Homozygous deletion	6.80%	Survival-death regulation	None	Not tested
CDH1	Mutation	7.80%	Invasion-metastasis	None	Not tested
AURKA	Amplification	7.30%	Growth	None	Not tested
ARID1A	Mutation	19.80%	Chromatin remodeling	None	Not Tested
FBXW7	Mutation	7.80%	Ubiquitin ligase	None	Not tested

Comprehensive gene lists are not presented due to space constraints, and can be found in the TCGA and COSMIC databases. Data are derived from the cancer genome atlas (TCGA) and catalog of somatic mutations in cancer (COSMIC) databases, accessed 2/2014.

3. ErbB family

The epidermal growth factor receptor family (ErbB) is composed of four structurally related receptor tyrosine kinases (RTK) (ErbB1, ErbB2, ErbB3, and ErbB4) [30], all of which play critical roles in regulating cell growth. Ligand binding, receptor overexpression, or structural alterations including truncations and mutations, have all been implicated in activating ErbB family receptors [31].

ErbB1 (EGFR) is overexpressed in 27%–64% of gastric cancers [27,32,33]. However, to date, no benefit in progression free or OS has been demonstrated in trials investigating EGFR inhibition. The phase III EXPAND trial [20] evaluated the addition of cetuximab, a monoclonal anti-EGFR antibody, to capecitabine–cisplatin in patients with advanced gastric or gastro-esophogeal junction (GEJ) tumors but failed to demonstrate a significant improvement in progression free survival (PFS). Similarly, the phase III REAL-3 trial [11] showed no OS benefit when the anti-EGFR antibody panitumumab was added to epirubicin, oxaliplatin, and capecitabine (EOX) in patients with advanced GEJ adenocarcinoma. In addition, limited retrospective biomarker analysis has failed to identify a clear patient subset who would derive benefit from EGFR-directed therapies [34]. To date, it remains unclear whether prospective selection for patients with EGFR pathway alterations would affect the patient outcomes.

Overexpression of ErbB2 (HER2) is found in approximately 6%–34% of gastric cancer samples [35,36] with some variability based on histologic subtype and location. The highest rates of expression have been observed in intestinal type tumors located proximally at the gastroesophageal junction [35]. Preclinical xenograft work suggested additional benefit by combining trastuzumab with platinum–fluoropyrimidine chemotherapy [37]. These findings were validated in the ToGA trial, which was the first phase III trial in gastric cancer to demonstrate enhanced survival with molecularly targeted therapy. In this trial, the addition of trastuzumab, a monoclonal anti-HER2 antibody, to standard cisplatin and 5-fluorouracil improved OS from 11.1 to 13.8 months in patients with HER2 amplified gastric adenocarcinomas [19]. This practice-changing trial highlights the power of molecular profiling and has spawned ongoing trials examining the role of trastuzumab and other HER2-directed therapies in both advanced and early stage disease (Table 2).

In addition to EGFR and HER2, ErbB3 (HER3) is another key member of the ErbB family that preferentially signals through the phosphatidylinositol 3-kinase (PI3K) pathway. The heterodimerization of HER3 and HER2 appears to be critical in driving tumor growth in breast cancers that overexpress HER2 [38]. A recent meta-analysis [39] found a correlation between overexpression of HER3 and shortened survival in solid tumors. Moreover, in gastric cancer, high expression of HER3 is

Table 2
Ongoing clinical investigations of molecularly targeted agents in gastric cancer.

Investigational compound	Target	Phase	Clinical trial identifier	Primary endpoint
HER family				
PF-00299804	HER1,2,4	II	NCT01152853	PFS
Pertuzumab/Trastuzumab	HER2	III	NCT01774786	OS
MM-111	HER2, HER3	II	NCT01774851	PFS
ASLAN001	pan-HER	II	NCT01614522	% HER2 phosphorylation
LJM716	HER3	I	NCT01602406	DLT
PF-00299804	HER1, 2, 4	II	NCT01152853	PFS
Poziotinib (HM781-36B)	Irreversible pan-HER	I–II	NCT01746771	DLT
Afatinib (BIBW 2992)	EGFR, HER2	II	NCT01522768	ORR
Lapatinib	EGFR, HER2	III	NCT00680901	OS
Nimotuzumab	EGFR	II	NCT01180166	PFS
Cetuximab	EGFR	II	NCT00275951	ORR
Nimotuzumab	EGFR	III	NCT01813253	OS
VEGF(R)/FGFR				
Dovitinib	FGFR, VEGFR, PDGFR	II	NCT01719549	RR, PFS
Lucitanib	FGFR, VEGFR	I-II	NCT01283945	MTD, ORR
Telatinib	VEGFR2, 3, c-kit	II	NCT00952497	PFS
Aflibercept	VEGFR1, 2	II	NCT01747551	PFS
Axitinib	VEGFR1, 2, 3, PDGF	I	NCT00842244	DLT
Cediranib (AZD2171)	VEGFR1, 2, 3	I	NCT00960349	AE, safety
ZD6474	EGFR, VEGFR, RET	I	NCT01183559	MTD
Brivanib	VEGFR2	I	NCT01046864	Safety, toxicity
Pazopanib	VEGFR2	II	NCT01130805	RR
Apatinib	VEGFR2	III	NCT01512745	OS, PFS
Ramucirumab	VEGFR2	III	NCT01170663	OS
MET signaling				
LY2875358	c-Met	II	NCT01874938	PFS
Foretinib (GSK1363089)	c-Met, VEGFR2	II	NCT00725712	ORR
Onartuzumab	c-Met	III	NCT01662869	OS
AMG 337	c-Met	II	NCT02016534	ORR
Rilotumumab (AMG 102)	HGF	I,II	NCT00719550	PFS
INC280	c-Met	I	NCT01324479	DLT, AE
PI3K-AKT-mTOR				
BYL-719	p110	I	NCT01613950	DLT, MTD
MK2206	AKT	I	NCT01705340	MTD
GDC-0068	AKT	II	NCT01896531	PFS
DS-7423	PI3K-mTOR	I	NCT01364844	AE
Histone acetylation				
Vorinostat	HDAC	I–II	NCT01045538	MTD
FR901228	HDAC	II	NCT00098527	RR
Other targets				
Saracatinib (AZD0530)	Src, Abl	II	NCT00607594	ORR
AMG 386	Ang1 and Ang2 (angiopoietin)	II	NCT00583674	PFS
Crizotinib	ALK, ROS1, c-Met	II	NCT02034981	ORR

Representative agents are shown, and a complete list can be found at clintrials.gov. Data are adapted from clintrials.gov, accessed 2/2014.

associated with tumor progression and significantly worse OS [40]. Interestingly, significantly higher levels of HER2 and HER3 have been detected in stage III–IV gastric adenocarcinoma compared to in stage I–II disease (22%–24.0% vs. 5.8%–7.7%, $p < 0.05$) [41]. Ongoing strategies to target ErbB family RTKs in gastroesophageal cancer include combinations of the dual EGFR-HER2 inhibitor lapatanib, the irreversible EGFR-HER2 inhibitor afatinib, and HER2 dimerization inhibitor pertuzumab (Table 2). Analogous to the CLEOPATRA study [42] in breast cancer, pertuzumab is currently being studied in a phase III trial in combination with trastuzumab, fluoropyrimidine and cisplatin as first-line therapy in patients with HER2-positive metastatic GEJ or gastric cancer (Table 2).

Phase I–III trials testing novel inhibitors of all four ErbB family receptors are ongoing in HER-2 positive gastric cancer, EGFR and HER2 co-expressing tumors, or HER2 amplified patients with recurrent or metastatic gastric cancer (Table 2).

4. Angiogenesis inhibition

Angiogenesis is a malignant hallmark [43] and has been a common therapeutic target using approaches including ligand inhibition [44], RTK inhibition, and inhibition of intracellular signaling kinases [45] or combinations of these strategies [46]. Elevated concentrations of both circulating [47] and tumoral vascular endothelial growth factor (VEGF) [48] have been described in patients with advanced gastric cancer and are correlated with decreased survival [49,50].

VEGF and the VEGF receptors (VEGFR1-4), the primary drivers for normal and pathologic angiogenesis, are overexpressed in 36%–40% of gastric cancers [51,52]. The anti-VEGF-A monoclonal antibody, bevacizumab, demonstrated a survival benefit in colorectal cancer, and early phase clinical trials suggested activity in gastric cancer [53,54]. However, the phase III AVAGAST [55] and AVATAR [56] trials have evaluated the addition of bevacizumab to first line platinum-fluoropyrimidine chemotherapy in advanced gastric cancer patients but failed to demonstrate improved survival. Pre-planned subgroup analysis from AVAGAST suggested an improved median survival in American as opposed to Asian cohorts [55], but specific implications of these regional differences remain to be determined.

VEGF receptor inhibition garnered greater clinical success in gastric cancer after *in vivo* studies suggested molecular inhibition of VEGFR-2 decreased tumor vascularity and increased endothelial cell apoptosis [57], and dual VEGFR-2 and EGFR inhibition led to inhibition of gastric tumor growth [58]. REGARD [59] was a phase III trial evaluating ramucirumab, a fully human IgG1 anti-VEGFR-2 monoclonal antibody, plus best supportive care, versus placebo in patients with advanced gastric cancer that have progressed after first line chemotherapy. In this trial, ramucirumab demonstrated a significant improvement in median OS compared to the placebo group (5.2 versus 3.8 months, $p = 0.047$) [59]. Early reporting of the phase III RAINBOW trial, testing ramucirumab in combination with paclitaxel for second line therapy after platinum–fluoropyrimidine failure, demonstrated an OS benefit of 9.6 versus 7.4 months versus paclitaxel alone [60]. In April of this year ramucirumab was approved in the US for advanced gastric or GEJ adenocarcinoma patients with progression on fluoropyrimidine or platinum-containing chemotherapy. With the recent success of ramucirumab, investigations with several other anti-angiogenic agents have begun. These include the VEGFR-2 inhibitor apatinib and the multi-targeted tyrosine kinase receptor inhibitors (TKIs) axitinib and pazopanib (Table 2).

5. Other RTK targets

Fibroblast growth factor receptor family members (FGFR1–4) are transmembrane RTKs whose downstream effectors include both the phosphoinositide 3-kinase (PI3K)–AKT and the mitogen-activated protein kinase–extracellular signal-regulated kinase (MAPK-ERK) pathways [61]. Amplification of FGFR2 is present in 5%–8% of gastric cancers [21] and is associated with lymphatic invasion and worse prognosis [62]. Prior preclinical work has suggested that FGFR2-amplified gastric cancers are sensitive to FGFR/VEGFR inhibition [63] and the FGFR inhibitor dovitinib is currently being tested in a phase II trial as monotherapy in patients with metastatic or unresectable gastric cancer with either FGFR2 amplification or polysomy (NCT01719549). The FGFR1-3 inhibitor AZD4547 is also being compared to paclitaxel in the SHINE trial, a phase II study again in patients with advanced gastric or GEJ cancer whose tumors exhibit FGFR2 amplification or polysomy (NCT01457846) (Table 2).

The hepatocyte growth factor receptor (HGFR, c-Met), encoded by the c-MET gene, is involved in epithelial–mesenchymal communication and is overexpressed in gastric cancer [64]. c-MET amplification is present in 2%–10% of gastric cancer patients [65], and is associated with higher tumor stage and grade and significantly shorter median survival [66]. Gastric cell lines with MET amplification appear to be susceptible to crizotinib, a small molecule inhibitor of anaplastic lymphoma kinase (ALK) and MET tyrosine kinase approved in NSCLC [67]. In a phase I trial, two out of four patients with MET-amplified (>5 copies) tumors treated with crizotinib experienced tumor reduction [65], with a delay in tumor progression, at 3 months. Rilotumumab, a monoclonal antibody against HGF, which binds c-Met, showed trends toward improved PFS and OS in combination with first line epirubicin, cisplatin and capecitabine (ECX) in patients with advanced gastric or GEJ cancers harboring high c-MET expression in a phase II trial [51]. Anecdotal evidence also supports activity of the c-MET inhibiting mAb Onartuzumab in MET-amplified gastric cancer [68], and further trials are ongoing. Unfortunately, a recent phase II trial evaluating foretinib, a dual c-Met/VEGFR2 inhibitor [69], showed minimal anti-tumor activity in molecularly unselected, previously treated patients with advanced or metastatic gastric cancer. Of the 5% of patients in this trial who exhibited MET amplification, foretinib was not associated with significant tumor regression despite evidence of inhibition from on-treatment biopsy samples [69]. It has been suggested that acquired resistance to MET inhibitors can develop through up-regulation of alternative signaling pathways [70,71] including EGFR, and downstream effectors in the PI3K and MAPK pathways. Ongoing trials are investigating additional mechanisms of targeting c-Met both as monotherapy and in combinatorial strategies, including combination with ramucirumab (Table 2).

6. PI3K/AKT/mTOR

The PI3K/protein kinase B (AKT)/mammalian target of rapamycin(mTOR) (PI3K/AKT/mTOR) pathway is a major effector of RTK signaling and one of the most frequently altered pathways in human malignancies. Exome sequencing has identified mutations of the PIK3CA gene, which encodes the catalytic subunit p110α, in over 5% of gastric cancers [24,72,73]. Preclinical work has demonstrated that hotspot PIK3CA mutations lead to constitutive PI3K pathway signaling in the absence of growth factors [74,75] or upstream activation *in vitro* and *in vivo* in gastric cancer samples [76]. In cases of advanced gastric cancer, amplification of PIK3CA is commonly detected and is associated with a poor prognosis [77,78]. Persistent PI3K signaling is a significant component of acquired resistance to upstream inhibitors [79,80] and has been demonstrated in multiple tumor types.

Several classes of PI3K pathway inhibitors are under evaluation in gastric cancer. The mTOR inhibitor everolimus, has been utilized successfully in treatment of neuroendocrine tumors and renal cell carcinoma [81,82]. However, despite documented pathway activity, the phase III GRANITE-1 study [83], which evaluated the efficacy of everolimus compared to best supportive care in molecularly unselected patients with advanced gastric cancer that progressed after previous chemotherapy, failed to show improved survival. Median survival was 5.39 months with everolimus versus 4.34 months with placebo (HR = 0.90; 95% CI, 0.75–1.08) [83]. Importantly, patients in this study were not pre-selected with respect to PI3K/AKT/mTOR pathway

alterations. The isoform specific p110-α inhibitor BYL719 and the heat shock protein 90 (Hsp90) inhibitor AUY922 are being evaluated in a phase I trial in patients with advanced gastric cancer with either a molecular alteration of PIK3CA, or an amplification of HER2 (NCT01613950). Dual PI3K/mTOR inhibitors have been shown to enhance 5-FU cytotoxicity *in vitro* and *in vivo* [84], especially in PIK3CA mutant gastric tumor cells, thought to be secondary to cellular heterogeneity in sensitivity to PI3K and mTOR inhibition [85]. Earlier dual PI3K/mTOR inhibitors have been plagued by difficulties with toxicities and ongoing trials including the phase I study of DS-7423 seeking to improve the therapeutic window (NCT01364844).

AKT, a key node in the PI3K cascade, has been effectively targeted by allosteric inhibitors in breast cancer cell lines with *PIK3CA* mutations and *HER2* amplifications [86]. MK2206, an allosteric AKT inhibitor, is being studied in early phase trials in mutationally selected and unselected patients with advanced gastric or GEJ cancers as well as other solid tumors (NCT01260701, NCT00963547). The AKT catalytic site inhibitor, GDC-0068, has also demonstrated anti-tumor activity in human cancer cell lines and xenograft models [87] and is currently being investigated in a multicenter phase II trial in gastric and GEJ cancer patients with locally advanced or metastatic disease (NCT01896531).

7. Proteomics and phosphoproteomics

Proteins are the ultimate effects of cellular function and genomic alterations that decrease or increase the relative abundance or activity of effector proteins are common across multiple malignancies. Functional proteomics allows for high throughput analysis of basal and phosphorylated proteins and has identified candidate proteins related to breast cancer outcomes [88] and pathopysiology [89,90] and is emerging as a useful adjunct in multiple malignancies. Early proteomic work in gastric cancer has identified candidate protein biomarkers for diagnosis [91,92] and possible predictive biomarkers for response to MET inhibition [93]. Additionally, phosphorylation patterns or ErbB1-3, cMET, PI3K, and IGF1R were shown to correlate with disease free survival in a large gastric cancer cohort [94]. Phosphoproteomic profiling has the ability to simultaneously assess the relative activity of multiple candidate target protein as well as monitor target activity during therapy. Small proteomic studies have identified candidate protein signatures associated with response to neoadjuvant chemoradiation [95] as well as resistance to 5-FU [96] in esophageal adenocarcinomas. The clinical applications of proteomic technologies remain largely investigational, but it is our opinion that proteomic interrogation is likely to identify multiple resistance mechanisms to targeted therapies and play an increasing role in developing molecularly directed therapies in gastric cancer.

8. Conclusions and future directions

The growing appreciation of the genomic landscape of gastric adenocarcinoma has led to the identification of several promising molecular targets including VEGR2, c-MET, FGFR1, 2, HER2, HER3, and members of the PI3K/AKT/mTOR pathway. While molecular characterization of gastric cancer continues to identify subsets of patients for genotype-directed therapeutic approaches, the response rates remain in the 25%–40% range across published trials, and novel molecularly directed approaches are needed. Breast cancer, non-small cell lung cancer, chronic myelogenous leukemia and melanoma have highlighted the inevitable development of acquired resistance to small molecule TKI monotherapy and the possibility of combination therapy to improve outcomes. Future biorepositories that include on-treatment and progression biopsies will help to identify acquired resistance mechanisms, further subdivide patients, and inform rational combinatorial approaches. Functional tumor assays including proteomics and phosphoproteomics may complement genomics and potentially narrow subsets of patients most likely to respond or be resistant to a given targeted therapy.

Here we have highlighted the recurrent actionable genomic alterations in gastric cancer and the ongoing attempts to translate these biologic insights into improved patient outcomes.

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References

[1] A. Jemal, et al., Global cancer statistics, CA Cancer J. Clin. 61 (2) (2011) 69–90.
[2] R. Siegel, D. Naishadham, A. Jemal, Cancer statistics, 2013, CA Cancer J. Clin. 63 (1) (2013) 11–30.
[3] P. Lauren, The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification, Acta Pathol. Microbiol. Scand. 64 (1965) 31–49.
[4] D. Cunningham, et al., Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer, N. Engl. J. Med. 355 (1) (2006) 11–20.
[5] C.C. Earle, et al., Neoadjuvant or adjuvant therapy for resectable gastric cancer? A practice guideline, Can. J. Surg. 45 (6) (2002) 438–446.
[6] G. Group, et al., Benefit of adjuvant chemotherapy for resectable gastric cancer: a meta-analysis, JAMA 303 (17) (2010) 1729–1737.
[7] J.S. Macdonald, et al., Chemoradiotherapy after surgery compared with surgery alone for adenocarcinoma of the stomach or gastroesophageal junction, N. Engl. J. Med. 345 (10) (2001) 725–730.
[8] S.R. Smalley, et al., Updated analysis of SWOG-directed intergroup study 0116: a phase III trial of adjuvant radiochemotherapy versus observation after curative gastric cancer resection, J. Clin. Oncol. 30 (19) (2012) 2327–2333.
[9] P. van Hagen, et al., Preoperative chemoradiotherapy for esophageal or junctional cancer, N. Engl. J. Med. 366 (22) (2012) 2074–2084.
[10] D. Cunningham, et al., Capecitabine and oxaliplatin for advanced esophagogastric cancer, N. Engl. J. Med. 358 (1) (2008) 36–46.
[11] T. Waddell, et al., Epirubicin, oxaliplatin, and capecitabine with or without panitumumab for patients with previously untreated advanced oesophagogastric cancer (REAL3): a randomised, open-label phase 3 trial, Lancet Oncol. 14 (6) (2013) 481–489.
[12] W. Koizumi, et al., S-1 plus cisplatin versus S-1 alone for first-line treatment of advanced gastric cancer (SPIRITS trial): a phase III trial, Lancet Oncol. 9 (3) (2008) 215–221.
[13] W. Koizumi, et al., Addition of docetaxel to S- without platinum prolongs survival of patients with advanced gastric cancer: a randomized study (START), J. Cancer Res. Clin. Oncol. 140 (2) (2014) 319–328.
[14] S. Sakuramoto, et al., Adjuvant chemotherapy for gastric cancer with S-1, an oral fluoropyrimidine, N. Engl. J. Med. 357 (18) (2007) 1810–1820.
[15] R.E. Schwarz, K. Zagala-Nevarez, Ethnic survival differences after gastrectomy for gastric cancer are better explained by factors specific for disease location and individual patient comorbidity, Eur. J. Surg. Oncol. 28 (3) (2002) 214–219.
[16] V.E. Strong, et al., Comparison of gastric cancer survival following R0 resection in the United States and Korea using an internationally validated nomogram, Ann. Surg. 251 (4) (2010) 640–646.
[17] M. Sasako, et al., Five-year outcomes of a randomized phase III trial comparing adjuvant chemotherapy with S-1 versus surgery alone in stage II or III gastric cancer, J. Clin. Oncol. 29 (33) (2011) 4387–4393.
[18] K. Bickenbach, V.E. Strong, Comparisons of gastric cancer treatments: east vs. west, J. Gastric Cancer 12 (2) (2012) 55–62.
[19] Y.J. Bang, et al., Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial, Lancet 376 (9742) (2010) 687–697.
[20] F. Lordick, et al., Capecitabine and cisplatin with or without cetuximab for patients with previously untreated advanced gastric cancer (EXPAND): a randomised, open-label phase 3 trial, Lancet Oncol. 14 (6) (2013) 490–499.
[21] N. Deng, et al., A comprehensive survey of genomic alterations in gastric cancer reveals systematic patterns of molecular exclusivity and co-occurrence among distinct therapeutic targets, Gut 61 (5) (2012) 673–684.
[22] J. Zhang, et al., The impact of next-generation sequencing on genomics, J. Genet. Genomics 38 (3) (2011) 95–109.
[23] A. Grada, K. Weinbrecht, Next-generation sequencing: methodology and application, J. Invest. Dermatol. 133 (8) (2013) e11.
[24] J. Lee, et al., High-throughput mutation profiling identifies frequent somatic mutations in advanced gastric adenocarcinoma, PLoS One 7 (6) (2012) e38892.
[25] A.M. Dulak, et al., Exome and whole-genome sequencing of esophageal adenocarcinoma identifies recurrent driver events and mutational complexity, Nat. Genet. 45 (5) (2013) 478–486.
[26] Z.J. Zang, et al., Exome sequencing of gastric adenocarcinoma identifies recurrent somatic mutations in cell adhesion and chromatin remodeling genes, Nat. Genet. 44 (5) (2012) 570–574.
[27] A.M. Dulak, et al., Gastrointestinal adenocarcinomas of the esophagus, stomach, and colon exhibit distinct patterns of genome instability and oncogenesis, Cancer Res. 72 (17) (2012) 4383–4393.

[28] J. Lee, S.H. Ou, Towards the goal of personalized medicine in gastric cancer—time to move beyond HER2 inhibition. Part I: targeting receptor tyrosine kinase gene amplification, Discov. Med. 15 (85) (2013) 333–341.
[29] M.L. Chong, et al., Phosphatidylinositol-3-kinase pathway aberrations in gastric and colorectal cancer: meta-analysis, co-occurrence and ethnic variation, Int. J. Cancer 134 (5) (2014) 1232–1238.
[30] Y. Yarden, M.X. Sliwkowski, Untangling the ErbB signalling network, Nat. Rev. Mol. Cell Biol. 2 (2) (2001) 127–137.
[31] N.E. Hynes, H.A. Lane, ERBB receptors and cancer: the complexity of targeted inhibitors, Nat. Rev. Cancer 5 (5) (2005) 341–354.
[32] M.A. Kim, et al., EGFR in gastric carcinomas: prognostic significance of protein overexpression and high gene copy number, Histopathology 52 (6) (2008) 738–746.
[33] R. Langer, et al., Prognostic significance of expression patterns of c-erbB-2, p53, p16INK4A, p27KIP1, cyclin D1 and epidermal growth factor receptor in oesophageal adenocarcinoma: a tissue microarray study, J. Clin. Pathol. 59 (6) (2006) 631–634.
[34] A.F. Okines, et al., Biomarker analysis in oesophagogastric cancer: results from the REAL3 and TransMAGIC trials, Eur. J. Cancer 49 (9) (2013) 2116–2125.
[35] C. Gravalos, A. Jimeno, HER2 in gastric cancer: a new prognostic factor and a novel therapeutic target, Ann. Oncol. 19 (9) (2008) 1523–1529.
[36] T. Yano, et al., Comparison of HER2 gene amplification assessed by fluorescence in situ hybridization and HER2 protein expression assessed by immunohistochemistry in gastric cancer, Oncol. Rep. 15 (1) (2006) 65–71.
[37] K. Fujimoto-Ouchi, et al., Antitumor activity of trastuzumab in combination with chemotherapy in human gastric cancer xenograft models, Cancer Chemother. Pharmacol. 59 (6) (2007) 795–805.
[38] T. Holbro, et al., The ErbB2/ErbB3 heterodimer functions as an oncogenic unit: ErbB2 requires ErbB3 to drive breast tumor cell proliferation, Proc. Natl. Acad. Sci. U. S. A. 100 (15) (2003) 8933–8938.
[39] A. Ocana, et al., HER3 overexpression and survival in solid tumors: a meta-analysis, J. Natl. Cancer Inst. 105 (4) (2013) 266–273.
[40] M. Hayashi, et al., High expression of HER3 is associated with a decreased survival in gastric cancer, Clin. Cancer Res. 14 (23) (2008) 7843–7849.
[41] X.L. Zhang, et al., Comparative study on overexpression of HER2/neu and HER3 in gastric cancer, World J. Surg. 33 (10) (2009) 2112–2118.
[42] J. Baselga, et al., Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer, N. Engl. J. Med. 366 (2) (2012) 109–119.
[43] D. Hanahan, R.A. Weinberg, Hallmarks of cancer: the next generation, Cell 144 (5) (2011) 646–674.
[44] L.M. Ellis, D.J. Hicklin, VEGF-targeted therapy: mechanisms of anti-tumour activity, Nat. Rev. Cancer 8 (8) (2008) 579–591.
[45] N. Ferrara, VEGF and the quest for tumour angiogenesis factors, Nat. Rev. Cancer 2 (10) (2002) 795–803.
[46] G. Bergers, D. Hanahan, Modes of resistance to anti-angiogenic therapy, Nat. Rev. Cancer 8 (8) (2008) 592–603.
[47] S.Y. Oh, et al., Clinicopathologic significance of HIF-1alpha, p53, and VEGF expression and preoperative serum VEGF level in gastric cancer, BMC Cancer 8 (2008) 123.
[48] S.E. Kim, et al., The clinicopathological significance of tissue levels of hypoxia-inducible factor-1alpha and vascular endothelial growth factor in gastric cancer, Gut Liver 3 (2) (2009) 88–94.
[49] D. Cabuk, et al., Vascular endothelial growth factor, hypoxia-inducible factor 1 alpha and CD34 expressions in early-stage gastric tumors: relationship with pathological factors and prognostic impact on survival, Oncology 72 (1–2) (2007) 111–117.
[50] S. Juttner, et al., Vascular endothelial growth factor-D and its receptor VEGFR-3: two novel independent prognostic markers in gastric adenocarcinoma, J. Clin. Oncol. 24 (2) (2006) 228–240.
[51] R. Wadhwa, et al., Gastric cancer—molecular and clinical dimensions, Nat. Rev. Clin. Oncol. 10 (11) (2013) 643–655.
[52] Y. Zhou, et al., Clinicopathological significance of E-cadherin, VEGF, and MMPs in gastric cancer, Tumour Biol. 31 (6) (2010) 549–558.
[53] H.I. Hurwitz, et al., Efficacy and safety of bevacizumab in metastatic colorectal cancer: pooled analysis from seven randomized controlled trials, Oncologist 18 (9) (2013) 1004–1012.
[54] M.A. Shah, et al., Multicenter phase II study of irinotecan, cisplatin, and bevacizumab in patients with metastatic gastric or gastroesophageal junction adenocarcinoma, J. Clin. Oncol. 24 (33) (2006) 5201–5206.
[55] A. Ohtsu, et al., Bevacizumab in combination with chemotherapy as first-line therapy in advanced gastric cancer: a randomized, double-blind, placebo-controlled phase III study, J. Clin. Oncol. 29 (30) (2011) 3968–3976.
[56] L. Shen, et al., Bevacizumab plus capecitabine and cisplatin in Chinese patients with inoperable locally advanced or metastatic gastric or gastroesophageal junction cancer: randomized, double-blind, phase III study (AVATAR study), Gastric Cancer (2014) (in press).
[57] M.F. McCarty, et al., ZD6474, a vascular endothelial growth factor receptor tyrosine kinase inhibitor with additional activity against epidermal growth factor receptor tyrosine kinase, inhibits orthotopic growth and angiogenesis of gastric cancer, Mol. Cancer Ther. 3 (9) (2004) 1041–1048.
[58] Y.D. Jung, et al., Effects of combination anti-vascular endothelial growth factor receptor and anti-epidermal growth factor receptor therapies on the growth of astric cancer in a nude mouse model, Eur. J. Cancer 38 (8) (2002) 1133–1140.
[59] C.S. Fuchs, et al., Ramucirumab monotherapy for previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (REGARD): an international, randomised, multicentre, placebo-controlled, phase 3 trial, Lancet 383 (9911) (2014) 31–39.
[60] J. Clin. Oncol. 32 (Suppl. 3) (2014) (abstr LBA7).
[61] K. Matsumoto, et al., FGFR2 gene amplification and clinicopathological features in gastric cancer, Br. J. Cancer 106 (4) (2012) 727–732.
[62] X. Su, et al., FGFR2 amplification has prognostic significance in gastric cancer: results from a large international multicentre study, Br. J. Cancer 110 (4) (2014) 967–975.
[63] L. Xie, et al., FGFR2 gene amplification in gastric cancer predicts sensitivity to the selective FGFR inhibitor AZD4547, Clin. Cancer Res. 19 (9) (2013) 2572–2583.
[64] L.J. Appleman, MET signaling pathway: a rational target for cancer therapy, J. Clin. Oncol. 29 (36) (2011) 4837–4838.
[65] J.K. Lennerz, et al., MET amplification identifies a small and aggressive subgroup of esophagogastric adenocarcinoma with evidence of responsiveness to crizotinib, J. Clin. Oncol. 29 (36) (2011) 4803–4810.
[66] F. Graziano, et al., Genetic activation of the MET pathway and prognosis of patients with high-risk, radically resected gastric cancer, J. Clin. Oncol. 29 (36) (2011) 4789–4795.
[67] G.A. Smolen, et al., Amplification of MET may identify a subset of cancers with extreme sensitivity to the selective tyrosine kinase inhibitor PHA-665752, Proc. Natl. Acad. Sci. U. S. A. 103 (7) (2006) 2316–2321.
[68] D.V. Catenacci, et al., Durable complete response of metastatic gastric cancer with anti-Met therapy followed by resistance at recurrence, Cancer Discov. 1 (7) (2011) 573–579.
[69] M.A. Shah, et al., Phase II study evaluating 2 dosing schedules of oral foretinib (GSK1363089), cMET/VEGFR2 inhibitor, in patients with metastatic gastric cancer, PLoS One 8 (3) (2013) e54014.
[70] J. Qi, et al., Multiple mutations and bypass mechanisms can contribute to development of acquired resistance to MET inhibitors, Cancer Res. 71 (3) (2011) 1081–1091.
[71] A. Danilkovitch-Miagkova, B. Zbar, Dysregulation of Met receptor tyrosine kinase activity in invasive tumors, J. Clin. Invest. 109 (7) (2002) 863–867.
[72] I. Vivanco, C.L. Sawyers, The phosphatidylinositol 3-kinase AKT pathway in human cancer, Nat. Rev. Cancer 2 (7) (2002) 489–501.
[73] S. Volinia, et al., Molecular cloning, cDNA sequence, and chromosomal localization of the human phosphatidylinositol 3-kinase p110 alpha (PIK3CA) gene, Genomics 24 (3) (1994) 472–477.
[74] J.F. Liu, et al., Up-regulation of PIK3CA promotes metastasis in gastric carcinoma, World J. Gastroenterol. 16 (39) (2010) 4986–4991.
[75] A.G. Bader, S. Kang, P.K. Vogt, Cancer-specific mutations in PIK3CA are oncogenic in vivo, Proc. Natl. Acad. Sci. U. S. A. 103 (5) (2006) 1475–1479.
[76] S. Velho, et al., The prevalence of PIK3CA mutations in gastric and colon cancer, Eur. J. Cancer 41 (11) (2005) 1649–1654.
[77] J. Shi, et al., Highly frequent PIK3CA amplification is associated with poor prognosis in gastric cancer, BMC Cancer 12 (2012) 50.
[78] J. Shi, et al., Frequent gene amplification predicts poor prognosis in gastric cancer, Int. J. Mol. Sci. 13 (4) (2012) 4714–4726.
[79] J.A. Engelman, J. Settleman, Acquired resistance to tyrosine kinase inhibitors during cancer therapy, Curr. Opin. Genet. Dev. 18 (1) (2008) 73–79.
[80] S.J. Klempner, A.P. Myers, L.C. Cantley, What a tangled web we weave: emerging resistance mechanisms to inhibition of the phosphoinositide 3-kinase pathway, Cancer Discov. 3 (12) (2013) 1345–1354.
[81] W.W. Ma, A.A. Adjei, Novel agents on the horizon for cancer therapy, CA Cancer J. Clin. 59 (2) (2009) 111–137.
[82] M. Dong, A.T. Phan, J.C. Yao, New strategies for advanced neuroendocrine tumors in the era of targeted therapy, Clin. Cancer Res. 18 (7) (2012) 1830–1836.
[83] A. Ohtsu, et al., Everolimus for previously treated advanced gastric cancer: results of the randomized, double-blind, phase III GRANITE-1 study, J. Clin. Oncol. 31 (31) (2013) 3935–3943.
[84] B. Bhattacharya, et al., Pharmacologic synergy between dual phosphoinositide-3-kinase and mammalian target of rapamycin inhibition and 5-fluorouracil in PIK3CA mutant gastric cancer cells, Cancer Biol. Ther. 13 (1) (2012) 34–42.
[85] M. Mazzoletti, et al., Combination of PI3K/mTOR inhibitors: antitumor activity and molecular correlates, Cancer Res. 71 (13) (2011) 4573–4584.
[86] Q.B. She, et al., Breast tumor cells with PI3K mutation or HER2 amplification are selectively addicted to Akt signaling, PLoS One 3 (8) (2008) e3065.
[87] J. Lin, et al., Targeting activated Akt with GDC-0068, a novel selective Akt inhibitor that is efficacious in multiple tumor models, Clin. Cancer Res. 19 (7) (2013) 1760–1772.
[88] W.S. Yang, et al., Proteomic approach reveals FKBP4 and S100A9 as potential prediction markers of therapeutic response to neoadjuvant chemotherapy in patients with breast cancer, J. Proteome Res. 11 (2) (2012) 1078–1088.
[89] S.W. Lam, C.R. Jimenez, E. Boven, Breast cancer classification by proteomic technologies: current state of knowledge, Cancer Treat. Rev. 40 (1) (2014) 129–138.
[90] Y.K. Chae, A.M. Gonzalez-Angulo, Implications of functional proteomics in breast cancer, Oncologist 19 (4) (2014) 328–335.
[91] L.L. Lin, H.C. Huang, H.F. Juan, Discovery of biomarkers for gastric cancer: a proteomics approach, J. Proteomics 75 (11) (2012) 3081–3097.
[92] J.Y. Wu, et al., Discovery of tumor markers for gastric cancer by proteomics, PLoS One 9 (1) (2014) e84158.
[93] D. Torti, et al., A preclinical algorithm of soluble surrogate biomarkers that correlate with therapeutic inhibition of the MET oncogene in gastric tumors, Int. J. Cancer 130 (6) (2012) 1357–1366.
[94] J. Lee, et al., A novel proteomics-based clinical diagnostics technology identifies heterogeneity in activated signaling pathways in gastric cancers, PLoS One 8 (1) (2013) e54644.
[95] J. Slotta-Huspenina, et al., A specific expression profile of heat-shock proteins and glucose-regulated proteins is associated with response to neoadjuvant chemotherapy in oesophageal adenocarcinomas, Br. J. Cancer 109 (2) (2013) 370–378.
[96] W. Shan, et al., A proprietary multianalyte test for predicting extreme resistance to neoadjuvant 5-FU based chemoradiation (CTRT) in esophageal adenocarcinoma (EC), J. Clin. Oncol. 32 (suppl 3; abstr 51) (2014).