REVIEW

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Translating genomic profiling to gastrointestinal cancer treatment

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Next-generation sequencing enables faster, cheaper and more accurate whole-genome sequencing, allowing genome profiling and discovery of molecular features. As molecular targeted drugs are developed, treatment can be tailored according to molecular subtype. Gastric and colorectal cancers have each been divided into four subtypes according to molecular features. Profiling of the esophageal cancer genome is underway and its classification is anticipated. To date, identification of *HER2* expression in gastric adenocarcinoma and *KRAS*, *NRAS* and *BRAF* mutations in colon cancer have proved essential for treatment decisions. However, to overcome therapy resistance and improve prognosis, further individualized therapy is required. Here, we summarize the treatment options for gastrointestinal cancer according to genomic profiling and discuss future directions.

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Background

The Human Genome Project was started in the 1990s and revealed final complete human genome sequence information in 2003, showing that 2.85 billion nucleotides encode only 20,000–25,000 protein-coding genes [1]. The Human Genome Project led not only to the discovery of the human genome sequence, but also to the development of improved sequencing technology. Today, next-generation sequencing enables faster, cheaper and more accurate whole-genome sequencing. Analysis of RNA sequences should be a useful tool for approaching the transcriptome, enabling the detection of gene expression, transcript isoforms, gene fusions and single nucleotide variants [2]. Methylation sequencing can investigate DNA methylation throughout the genome, providing the CpG island methylator phenotype and genome-wide methylation level. These technologies have been used for cancer identification and then applied in clinical practice, strongly suggesting that further understanding of cancer behavior could lead to personalized therapy.

As the cancer genome is better understood, it is expected that novel personalized therapy according to the cancer subtype will be more frequently developed for several kinds of cancer. For this review, keywords were searched in combination with 'genetic landscape' and 'gastric cancer', 'esophageal cancer' or 'colorectal cancer' (CRC) in PubMed, and then studies that evaluated genomic profile associated with gastrointestinal cancer were identified through reading, with several studies quoted at second hand. Liver and pancreatic cancer and gastrointestinal stromal tumor were not covered in this review due to the limited scope of the topic. Here, we summarize current treatments

KEYWORDS

- gastrointestinal cancer
- genome sequencing
- translational research

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for gastrointestinal cancer in accordance with its genomic profiling and their future potential.

• Targeting therapy & predictive value of genomic alterations

The developments of cancer biology knowledge can provide molecular alteration for targeting therapy. To date, there are several therapies with clinical utility in gastrointestinal tumors (Figures 1 & 2). Some targeted therapies are useful, but others are limited. Therefore, predictive biomarkers that might allow proper selection are needed. To date, HER2 overexpression ± amplification is key alteration anticipating the benefit of adding trastuzumab to systemic chemotherapy in esophageal adenocarcinoma (EAC) and gastric adenocarcinoma (GAC) [3]. CRC harboring RAS mutation tumor is resistant to EGFR inhibitors, thereby RAS status is evaluated before treatment [4–6]. Especially, BRAF mutation confers poor prognosis and therapy resistance, requiring combination of multimolecular strategy [7,8]. Interestingly, microsatellite instability (MSI) status is predictive marker for the benefit of immune checkpoint blockade therapy [9]. Similarly, EBV-associated GACs may be susceptible to immune modulation [10]. Thus, greater understanding of cancer genomic profiling and molecular biology should importantly lead to more personalizing cancer treatments.

Upper gastrointestinal cancer

• Esophageal cancer

Esophageal cancer is estimated to be the eighth most common cause of cancer in the world (456,000 cases) and the sixth most common cause of cancer death (400,000 deaths) [11]. Esophageal cancer has two common histologic types: esophageal squamous cell carcinoma (ESCC) and EAC.

• Genomic profiling in ESCC

Several reports regarding genomic sequencing analyses for ESCC show that mutations in cancer-associated genes facilitate cancer behavior (Table 1). As part of the International Cancer Genome Consortium, the Chinese group found eight highly mutated genes (*TP53*, *RBI*, *CDKN2A*, *PIK3CA*, *NOTCH1*, *NFE2L2*, *ADAM29* and *FAM135B*) from whole-genome sequencing or whole-exome sequencing of 88 ESCC samples [12]. Gao *et al.* performed exome sequencing on 113 ESCCs and identified mutations in genes involved in cell cycle and apoptosis

(*TP53*, *CCND1*, *CDKN2A*, *NFE2L2* and *RBI*) and histone modifiers (*MLL2*, *MLL3*, *KDM6A*, *EP300* and *CREBBP*) [13]. Regarding cell cycle and apoptosis pathways, *CCND1* showed copy number gain, whereas *CDKN2A* showed copy number loss. In the Hippo pathway, *FAT1*, *FAT2*, *FAT3* (27%) and *AJUBA* (7%) were mutated, and *YAP1* (4%) was amplified. In addition, they discovered that *EP300* mutation was associated with poor survival. Results of whole-exome or targeted deep sequencing of 139 paired ESCC cases and analysis of somatic copy number variations of more than 180 ESCCs were reported, identifying mutated genes such as *TP53*, *KMT2D*, *FAT1*, *FAT2*, *ZNF750*, *PIK3CA* and *NOTCH1*, and several focal somatic copy number variations, including *CCND1*, *EGFR*, *MYC*, *KRAS* and *CDKN2A* [14]. A large-scale genomic analysis of ESCCs from 144 patients in Japan identified mutated genes (*TP53*, *NOTCH1*, *KMT2D*, *NFE2L2*, *ZNF750*, *FAT1*, *PIK3CA* and *EP300*) and copy number variations (amplification of *CCND1*, *TERT*, *PCDH* cluster, *KLF5*, *FOXA1*, *EGFR*, *YAP1*; deletion of *CDKN2A*, *LRP1B* and *FAM190A*) [15].

Interestingly, a high frequency of *TP53*, *CDKN2A*, *KMT2D*, *FAT1*, *PIK3CA* and *EP300* mutation, and copy number gain of *CCND1* and loss of *CDKN2A* seem to be common in all reports. In addition, a lower frequency of *RAS* mutation and *EGFR* amplification were shown. Moreover, genome sequencing revealed that the most common mutations in ESCC are C/G>T/A transitions, which were shown to be associated with an APOBEC cytidine deaminase [16,17]. Interestingly, Kosumi *et al.* reported that APOBEC expression is higher in ESCC than in normal tissue and is also associated with the rate of *PIK3CA* mutation, suggesting that APOBEC might have potential as a therapeutic target [18].

• Molecular targeting therapy for ESCC

Genomic sequencing analyses for ESCC show alteration of histone regulator genes, or genes involved in the cell cycle and the Wnt and Notch pathways, indicating that several targets and pathways could be considered as targets of therapy. Stimulation of the PI3K/AKT/MTOR pathway caused by PTEN loss has been shown to be a common contributor to ESCC development and progression [19]. Thus, PTEN loss or inhibitors of the PI3K/AKT/MTOR pathway might be therapeutic targets. However, in a study by Shigaki *et al.* *PIK3CA* mutation was

Figure 1. Altered pathway, genetic change and targeted therapy in gastric adenocarcinoma. Percentages of genetic change is described.

interestingly shown to be associated with favorable prognosis after esophagectomy in ESCC [20]. In addition, epigenetic regulator gene alteration is more common in ESCC than EAC, and might be another possible therapeutic target [19].

EGFR inhibitors for treatment of ESCC

To date, few molecular therapies have been effective in ESCC. EGFR is one of the most investigated molecular targets in ESCC because EGFR activation has been found in genome sequencing [15]. Cetuximab, a monoclonal antibody against EGFR, is effective in combination with radiotherapy for head and neck SCC [21]; however, a Phase III study of addition of cetuximab

to chemoradiation therapy did not lead to significantly longer survival for patients with ESCC [22]. In another Phase III study, gefitinib, a tyrosine kinase EGFR inhibitor, also did not show a significantly longer overall survival (OS) benefit [23]. Thus, EGFR-targeting therapies for ESCC appear to be ineffective.

Immunotherapy for ESCC

Immunotherapy has recently attracted attention, especially through the PD1–PDL1 signaling pathway [24]. PD1-positive expression (43.9%) is found to be associated with poor survival in ESCC patients [25]. A clinical trial that assesses the efficacy and safety of pembrolizumab for

Figure 2. Altered pathway, genetic change and targeted therapy colorectal cancer. Percentages of genetic change is described.

patients with resistance to current therapy is ongoing (NCT02054806).

• Genomic profiling in EAC

The incidence of EAC is rapidly increasing in western countries, and gradually increasing in Asian countries. EAC is one of the most mutated cancers along with bladder, colorectal, lung and melanoma [26]. Therefore, a better understanding of genomic features in EAC should lead to improved early detection and treatment outcomes.

Dulak *et al.* analyzed the mutations in 149 EACs using whole-exome sequencing and identified mutations in genes such as *TP53* (72%), *ELMO1* (25%), *DOCK2* (12%), *CDKN2A* (12%), *ARID1A* (9%), *SMAD4* (8%) and *PIK3CA* (6%), amplifications of *KRAS* (21%),

HER2 (19%), *EGFR* (16%), *CCND1* (10%) and *MET* (6%), and loss of *SMAD4* (34%), *CDKN2A* (32%) and *ARID1A* (10%) [27].

A>C transversions at AA sites are the most frequent mutations in EAC, which is quite different from the mutations in EACC [27,28]. Reflux of gastric and bile acids have been shown to be risk factors for Barrett's esophagus and EAC [29]. Importantly, exposure to bile acid cocktail and acid in esophageal cell leads to increased reactive oxygen species, which cause oxidative DNA damage [30]. Interestingly, an oxidatively damaged DNA has been shown to cause A>C transversions in *Escherichia coli* experiments [31]. Thus, It could be proposed that A>C mutations at AA sites in EAC might be induced through oxidative DNA damage caused by reflux of gastric and bile acid.

Wang *et al.* compared gene alterations between EAC and ESCC [19]. The ERBB pathway, especially *HER2* and *EGFR* amplification, TGF- β signaling and RAS/MEK/MAPK pathway were more altered in EAC than in ESCC. Conversely, the pathways altered at a lower frequency in EAC included PI3K/AKT/MTOR signaling, epigenetic regulation pathway, fibroblast growth factor signaling, the Keap/NRF2 pathway and the Notch signaling pathway. As *TP53* and *CDKN2A* were highly altered, the cell cycle pathway was equally altered in EAC and ESCC. Thus, according to genome sequencing, EGFR, *HER2* and RAS/MEK/MAPK pathway inhibitors might have potential for molecular targeted therapy.

Recently, Secrier *et al.* reported a whole-genome sequence analysis of 129 EAC samples and proposed a mutational classification with potential therapeutic relevance; C>A/T dominant (29%), DNA damage repair (DDR) impaired (18%) and mutagenic (53%) [32]. The novelty in this report was to identify simultaneous amplifications of *ERBB2* and *MET* and *in vitro* studies demonstrating the benefit from dual inhibition. Additionally, the DDR-impaired subgroup is associated with deficiency of homologous recombination pathway, such as *BRCA* mutation, and is sensitive for DDR-targeted treatment, such as PARP inhibitor in combination with DNA-damaging agent. The mutagenic subgroup had high mutation load (as expected) associated with high neoantigens and CD8 T-cell infiltration. Finally, *WEE1/CHK1*, G2/M-phase checkpoint regulators, were also identified as potential targets.

• Genomic profiling in GAC

Chronic infection with *Helicobacter pylori*, which can colonize the stomach, seems to be a risk factor for the development of distal GAC in particular, as well as gastric and duodenal ulcers [33,34]. In contrast, proximal GACs seem to be caused by gastroesophageal reflux disease and obesity [35]. Moreover, EBV has also been shown to be associated with GAC [36]. In east Asia, especially Japan and Korea, the incidence of distal GAC is high, whereas proximal GACs have a higher incidence in west Asia [37]. GAC has been divided into two histologic subtypes by Lauren classification: intestinal type, which typically correlates with *H. pylori* infection and intestinal metaplasia occurring in the antrum; and diffuse type, which seems to be poorly differentiated and tends to occur in younger patients [38]. This classification is clinically useful, but is not sufficient to lead to personalized management. Genome characterization should give further insight into specific molecular phenotypes and personalized treatment.

There are two major genomic sequencing analyses for GAC, both of which classified GAC into four subtypes (Table 2) [10,39]. First, The Cancer Genome Atlas (TCGA) Research Network has characterized 295 primary GAC into four molecularly distinct subtypes. The first subtype was EBV-associated cancer, which was characterized by an abundance of DNA promoter hypermethylation. Interestingly, *PIK3CA* mutations were found in 80% cases of the EBV subtype. In addition, mutation of *ARID1A* was detected in 55% of cases, and amplification of

Table 1. Genomic sequencing analyses for esophageal squamous cell carcinoma.

Sample number and method	Mutant gene (%)		Copy number variants (%)				Ref.
	>10%	<10%	Amplification		Loss		
			>20%	<20%	>20%	<20%	
WGS (n = 17) WES (n = 71) CGH (n = 123)	<i>TP53</i> (83)	<i>NOTCH1</i> (9), <i>RB1</i> (8), <i>FAM135B</i> (7), <i>CDKN2A</i> (4), <i>ADAM29</i> (4), <i>PIK3CA</i> (4)	<i>CCND1</i> (46), EGFR (24), <i>KRAS</i> (27)	<i>AKT1</i> (15)	<i>CDKN2A</i> (44)	<i>RB1</i> (11)	[12]
WES (n = 139) SNP array (n = 125) CGH array (n = 59)	<i>TP53</i> (60), <i>KMT2D</i> (26)	<i>EP300</i> (8), <i>PIK3CA</i> (7)	<i>CCND1</i> (46)	<i>EGFR</i> (11), <i>MYC</i> (9), <i>KRAS</i> (5)	<i>CDKN2A</i> (33)		[14]
WES (n = 113)	<i>TP53</i> (93), <i>FAT1–4</i> (27), <i>NOTCH1–3</i> (22)	<i>KMT2D</i> (19), <i>EP300</i> (10)	<i>CCND1</i> (33)	<i>EGFR</i> (6), <i>YAP1</i> (4)		<i>CDKN2A</i> (12)	[13]
WES (n = 144)	<i>TP53</i> (93)	<i>NOTCH1</i> (19), <i>KMT2D</i> (19), <i>NFE2L2</i> (16), <i>ZNF750</i> (16), <i>FAT1</i> (14), <i>PIK3CA</i> (10)	<i>CCND1</i> (46), <i>TERT</i> (22)	<i>KLF5</i> (9), <i>FOXA1</i> (7), <i>EGFR</i> (6), <i>YAP1</i> (5)	<i>CDKN2A</i> (47), <i>LRP1B</i> (20)	<i>CCSER1</i> (7)	[15]
CGH: Comparative genomic hybridization; SNP: Single nucleotide polymorphism; WES: Whole-exome sequencing; WGS: Whole-genome sequencing.							

CGH: Comparative genomic hybridization; SNP: Single nucleotide polymorphism; WES: Whole-exome sequencing; WGS: Whole-genome sequencing.

Table 2. Molecular subtype for gastric adenocarcinoma by The Cancer Genome Atlas and Asian Cancer Research Group.

Subtype	Mutation	Amplification	Features
TCGA			
MSI	<i>PI3K, HER2, HER3, EGFR</i>		<i>MLH1</i> silencing
GS	<i>CDH1, RHOA, ARID1, BCOR</i>		Adhesion and cell migration gene alterations, diffuse type
CIN	<i>TP53</i>	<i>EGFR, HER2, HER3, JAK2, FGFR2, MET, NRAS, KRAS, VEGFA</i>	RTKs/RAS activation
EBV	<i>PI3K, ARID1, BCOR</i>	<i>JAK2, HER2</i>	DNA promoter hypermethylation, PD-L1/2 expression, IL-12 signaling
ACRG			
MSI	<i>KRAS, PI3K, ALK, ARID1</i>		Intestinal type, good prognosis, less recurrence
MSS/EMT	lower number of mutations		Diffuse type, poor prognosis, loss of E-cadherin
MSS/TP53-	<i>TP53</i>	<i>HER2, EGFR, CCNE1, CCND1, MDM2, ROBO2, GATA6, MYC</i>	Intermediate prognosis and recurrence
MSS/TP53+	<i>APC, KRAS, ARID1, PI3K, SMAD4</i>		Intermediate prognosis and recurrence, EBV positive

ACRG: Asian Cancer Research Group; CIN: Chromosomal instability; EBV: Epstein–Barr virus; EMT: Epithelial–mesenchymal transition; GS: Genomically stable; MSI: Microsatellite instability; MSS: Microsatellite stable; TCGA: The Cancer Genome Atlas.

Janus kinase (*JAK2*) and *PDL1* genes and a high prevalence of *CDKN2A* promoter hypermethylation were also observed. Consequently, the PI3K pathway and inhibitory immune checkpoints might have a key role in targeted therapy. The second subtype was characterized by enrichment for MSI through silencing of mismatch repair genes, which involves mutations in several kinases including *PIK3CA* (42%), *HER3* (14%), *JAK2* (11%), *EGFR* (5%) and *HER2* (5%). Therefore, MSI tumors seem to be associated with activation of EGFR-MAPK and PI3K pathways. The third subtype was genomically stable tumors (20% of cases), which were characterized by the absence of extensive somatic copy number aberrations. This subtype likely contains the diffuse histological subtype and frequently shows alterations of genes associated with cell adhesion, including *RHOA* (15%), *CDH1* (26%) and *CLDN18/ARHGAP* (15%). The fourth subtype was chromosomal instability (CIN) (50% of cases), which involved the fewest mutations among the four subtypes but the highest frequency of mutation of p53. This subtype of tumor mostly occurs in the gastroesophageal junction/cardia. Gene amplifications were found in *HER2* (24%), *KRAS/NRAS* (18%), *EGFR* (10%), *PIK3CA* (10%), *HER3* (8%), *FGFR2* (8%) and *MET* (8%).

The Asian Cancer Research Group recently provided a new classification of GAC into four subtypes according to gene expression profiles [39]. First, loss of *MLH1* expression was defined as a MSI tumor. Second, microsatellite stable (MSS) tumors displaying loss

of E-cadherin were defined as MSS/EMT. Finally, MSS-epithelial tumors were divided into MSS/TP53+ and MSS/TP53 – according to TP53 activation status. This molecular subtype classification is associated with clinical prognosis. The MSS/EMT subtype tends to be diffuse type and shows the worst prognosis. The MSI subtype likely develops in the antrum (75%) and histologically correlates with an intestinal subtype (>60%), which shows the best prognosis of the four subtypes. MSS/TP53+ and MSS/TP53- are associated with an intermediate prognosis. With regard to the molecular status, the MSI subtype is associated with the presence of hypermutation in genes such as *ARID1A* (44.2%), *KRAS* (23%) and *ALK* (16.3%). Moreover, the PI3K pathway is stimulated in approximately 50% of cases. The MSS/EMT subtype shows a lower mutation rate than the other subtypes. The MSS/TP53-subtype has a high frequency of *TP53* mutations and CIN whereas MSS/TP53+ tumors exhibit frequent mutations in *APC*, *ARID1A*, *KRAS*, *PIK3CA* and *SMAD4*. Thus, there are both common features and differences between the TCGA and Asian Cancer Research Group classification (Table 2).

• Molecular target therapy for gastroesophageal adenocarcinoma

Several types of molecular targeted drugs have been tested in GAC and EAC; however, only HER2 inhibitor (trastuzumab) and VEGFR-2 inhibitor (ramucirumab) have a proven effect on long-term survival. Other molecular targeted

drugs, such as EGFR, VEGF, MET and mTOR inhibitors, were shown to have no advantage for prognosis. In many other cancers, genomic characterization has been useful for targeted therapy. For GAC and EAC, so far only HER2 is used as a predictive marker of trastuzumab efficacy [3]. However, many kinds of molecular targeted drugs have recently entered clinical trials (Table 3).

• HER2 inhibitor for gastroesophageal adenocarcinoma

A multicenter Phase III trial, ToGA randomly assigned patients with advanced or metastatic gastroesophageal cancer to chemotherapy with or without trastuzumab if their tumor samples were positive for overexpression/amplification of HER2 [3]. A total of 3665 patients were screened and 810 (22%) patients were HER2 positive. The patients who were assigned to the trastuzumab group experienced a higher response rate and better median OS (13.8 vs 11.1 months; $p = 0.0046$). Importantly, in subgroup analysis patients with strong HER2 positivity (tumors were 3p or 2p by immunohistochemistry and positive by FISH) showed a greater advantage of adding trastuzumab to chemotherapy (median OS: 16.0 vs 11.8 months). This trial indicated that trastuzumab with chemotherapy should be the first-line therapy for patients whose tumors are HER2-positive. However, a survival analysis by the US FDA after longer follow-up showed that initial benefits from trastuzumab reduced considerably [50]. Subsequently, lapatinib, an HER2 and EGFR inhibitor that already has been proven to have prognostic benefit for HER2-positive breast cancer, was evaluated in gastroesophageal adenocarcinoma; however, insufficient drug availability was a limitation of the study. Addition of lapatinib to capecitabine and oxaliplatin as first-line therapy did not show a significant advantage for OS in patients with HER2-amplified gastroesophageal adenocarcinoma [40]. In addition, the TyTAN study was not able to show an advantage of adding lapatinib to paclitaxel as a second-line therapy [41].

Further studies of HER2-targeted therapy are ongoing. The Phase III JACOB study is investigating the efficacy of adding pertuzumab to chemotherapy as a second-line therapy in patients with HER2-positive metastatic gastric or gastroesophageal junction cancer after trastuzumab as a first-line therapy (NCT01358877). Pertuzumab is an HER dimerization inhibitor that binds to a different location in HER2 from that bound

by trastuzumab. Moreover, an antibody–drug conjugate consisting of trastuzumab linked to the cytotoxic agent emtansine has been tested in the Phase II/III GATSBY study, in which trastuzumab emtansine was compared with taxane treatment as second-line therapy for advanced GAC. However, the T-DM1 group did not show a significant survival benefit compared with the taxane group [51]. Afatinib, which selectively and irreversibly binds to and inhibits both HER2 and EGFR, is being evaluated in a Phase II study in patients with HER2-positive esophagogastric cancer who have resistance to trastuzumab (NCT01522768).

• VEGF inhibitor for gastroesophageal adenocarcinoma

The TCGA study showed that VEGF amplification is common in the CIN subgroup, and VEGF overexpression has been found in more than 50% of GAC [52]. The Phase III REGARD study showed that ramucirumab, a monoclonal antibody against VEGFR2, has benefit as second-line therapy in advanced GAC compared with best supportive care [45]. The Phase III RAINBOW study, which compared ramucirumab with paclitaxel versus paclitaxel alone as second-line therapy, also showed the advantage of adding ramucirumab [46]. However, the VEGF-directed monoclonal antibody bevacizumab was found to have no efficacy when added to chemotherapy in the AVAGAST study [44].

• EGFR inhibitor for gastroesophageal adenocarcinoma

EGFR is particularly amplified in CIN or MSS/TP53- subtypes [10,39]. Moreover, Kim *et al.* found that EGFR overexpression was present in 27.4% of GC patients and was associated with an unfavorable prognosis [53]. However, none of the EGFR targeted therapies showed a benefit in any trials. The EXPAND trial evaluated the benefit of adding cetuximab to first-line chemotherapy, which failed to show prognostic benefit [42]. The Phase III REAL3 study, which compared epirubicin, oxaliplatin and capecitabine with or without panitumumab, also did not show an OS benefit [43]. Importantly, populations of these two trials were not selected according to EGFR expression. Therefore, further evaluation of EGFR inhibitors in cancer with EGFR amplification or overexpression is needed. The results of the ENRICH study that evaluates the efficacy of adding nimotuzumab

Table 3. Randomized clinical trials of targeted therapies in esophageal and gastric adenocarcinoma.

Target	Study (NCT number)	Treatment	Median OS or PFS	HR (95% CI)	Ref.
HER2	ToGA (NCT01041404)	XP (n = 290) XP + trastuzumab (n = 294)	OS: 13.8 OS: 11.1	0.74 (0.60–0.91)	[3]
HER2	TRIO-013/LOGIC (NCT00680901)	CapeOx (n = 238) CapeOx + Lapatinib (n = 249)	OS: 10.5 OS: 12.2	0.91 (0.73–1.12)	[40]
HER2	TyTAN (NCT00486954)	Paclitaxel (n = 105) Paclitaxel + lapatinib (n = 98)	OS: 8.9 OS: 11.0	0.84 (0.64–1.11)	[41]
EGFR	EXPAND (EudraCT, number 2007-004219-75)	XP (n = 449) XP + cetuximab (n = 455)	PFS: 5.6 PFS: 4.4	1.09 (0.92–1.29)	[42]
EGFR	REAL-3 (NCT00824785)	EOC (n = 275) EOC + panitumumab (n = 278)	OS: 11.3 OS: 8.8	1.37 (1.07–1.76)	[43]
VEGF-A	AVAGAST (NCT00548548)	XP (n = 387) XP + bevacizumab (n = 387)	OS: 10.1 OS: 12.1	0.87 (0.73–1.03)	[44]
VEGFR-2	REGARD (NCT00917384)	Placebo (n = 238) Ramucirumab (n = 117)	OS: 5.2 OS: 3.8	0.77 (0.60–0.99)	[45]
VEGFR-2	RAINBOW (NCT01170663)	Paclitaxel (n = 335) Paclitaxel + ramucirumab (n = 330)	OS: 7.4 OS: 9.6	0.80 (0.68–0.96)	[46]
MET	RILOMET-1 (NCT01697072)	ECX (n = 300) ECX + rilotumumab (n = 300)	OS: 11.5 OS: 9.6	1.36 (1.05–1.75)	[47]
MET/HGF	METGastric (NCT01662869)	mFOLFOX6 mFOLFOX6 + onartuzumab	OS: 11.3 OS: 11.0	0.82 (1.05–1.75)	[48]
mTOR	GRANITE-1 (NCT00879333)	Placebo (n = 217) Everolimus (n = 439)	OS: 4.34 OS: 5.39	0.90 (0.75–1.08)	[49]

CapeOx: Capecitabine and L-OHP; ECX: Epirubicin, cisplatin and capecitabine; EOC: Epirubicin, oxaliplatin, and capecitabine; HR: Hazard ratio; mFOLFOX: Modified fluorouracil, folinic acid and L-OHP; OS: Overall survival; PFS: Progression-free survival; XP: Capecitabine and cisplatin.

in EGFR IHC 2+ or 3+ tumors are awaited (NCT01813253).

• Other agents

MET amplification is observed in the CIN subtype of GAC and a Phase II trial showed efficacy of *MET* inhibitor for GAC [54]. However, although a satisfactory result was expected in trials using *MET* inhibitor, they did not meet their end point. The Phase III RILOMET-1 trial compared ECX with and without rilotumumab as first-line therapy in *MET*-positive gastroesophageal cancer, showing that OS was significantly worse for the rilotumumab group [47]. The METGastric study showed that addition of onartuzumab to mFOLFOX6 was ineffective even in *MET*-positive patients [48].

The GRANITE-1 study, which evaluated everolimus versus placebo in patients with advanced GAC, did not show an OS benefit of adding mTOR inhibitor although there was an improvement in median progression-free survival [49].

FGFR2 amplification occurs especially in CIN or MSS/TP53– subtypes of GAC and is associated with a poor prognosis [55,56]. A clinical trial investigating the efficacy of *FGFR* inhibition for patients with *FGFR2* amplification

is ongoing. Results of the SHINE trial comparing the *FGFR2* inhibitor AZD4547 and paclitaxel in advanced GAC are anticipated (NCT01457846).

Colorectal cancer

CRC is the third most common cancer in men (746,000 cases) and the second most common in women (614,000 cases). Although more than half of CRC cases occur in advanced nations, mortality is lower in this population (694,000 deaths) than that in less developed regions [11]. Almost 80% of CRCs occur sporadically. Some hereditary diseases, such as Lynch syndrome and familial adenomatous polyposis, increase susceptibility to CRC [57] and approximately 2% of cases of CRC are associated with inflammatory bowel diseases [58].

The TCGA network project collected CRC samples and corresponding germline DNA samples from 276 patients [59]. After genome analysis, CRC was classified into two major groups: nonhypermuted (84%) and hypermuted (16%). Three-quarters of hypermuted cancers had high MSI due to MLH1 silencing. One-quarter had somatic mutation in mismatch repair genes and polymerase E resulting in an extremely frequent mutation rate; this type was

termed ultramutated cancers. *ACVR2A*, *APC*, *TGFBR2*, *BRAF*, *MSH3*, *MSH6*, *SLC9A9* and *TCF7L2* were highly mutated in hypermutated cancers; in particular *BRAF* (V600E) mutations were more frequent compared with nonhypermutated cancers. However, the mutation rate of *TP53* and *APC* was significantly lower in hypermutated cancer. In nonhypermutated cancer, *APC*, *TP53*, *KRAS*, *PIK3CA*, *FBXW7*, *SMAD4*, *TCF7L2* and *NRAS* were frequently mutated. Somatic copy number alterations were more common in nonhypermutated than hypermutated cancers. *FHIT*, *RBFOX1*, *WWOX*, *SMAD4*, *APC*, *PTEN*, *SMAD3* and *CF7L2* were found to be deleted, whereas *MYC*, *ERBB2*, *IGF2*, *USP12*, *CDK8*, *KLF5*, *HNF4A*, *WHSC1L1/FGFR1* and *IRS2* were found to be amplified. The TCGA group study reported that CRC shows activation of the WNT signaling pathway, inactivation of the TGF- β signaling pathway, enhanced activity of MYC and frequent genomic aberrations of the MAPK and PI3K pathways, suggesting that WNT-signaling inhibitors, small-molecule β -catenin inhibitors, and RTK-RAS and PI3K pathway inhibitors might have clinical relevance.

The CRC classification of the TCGA group is one of the largest cohort studies, but other studies indicating gene expression-based CRC classifications also exist [60–66]. To develop gene subtype CRC classifications, the CRC Subtyping Consortium (CRCSC) evaluated a total of 18 CRC datasets ($n = 4151$ patients) and identified four subtypes: CMS1, MSI immune (14%); CMS2, canonical (37%); CMS3: metabolic (13%); and CMS4, mesenchymal (23%) (Table 4) [67]. CMS1 was characterized by MSI, high CpG island methylator phenotype (CIMP), hypermutation including *BRAF* mutations, immune infiltration and worse survival after relapse. CMS2 was characterized by high somatic copy number alterations and activation of the WNT pathway and MYC. CMS3 was characterized by mixed MSI status, low somatic copy number alterations, low CIMP, *KRAS* mutations and metabolic deregulation. CMS4 was characterized by high somatic copy number alterations, stromal infiltration, TGF- β activation, angiogenesis and worse relapse-free and OS. This classification suggests the potential of subtype-based therapy; for instance, immune checkpoint therapy might be effective for the MSI status [9]. Moreover, metabolic activation might be a therapeutic target for CMS3 and

TGF- β signaling inhibition might be effective for CMS4.

• Molecular-targeted therapy for CRC

KRAS has currently a key role for CRC molecular targeted therapy. *KRAS* mutation tumor is associated with resistance to EGFR inhibitor [4–6]. Nowadays, standard first-line therapy for advanced CRC is chemotherapy in combination with EGFR inhibitor or VEGFR inhibitor, if RAS mutation exists, EGFR inhibitor should not be used [68]. As after second-line therapy, several molecular targeted drugs, such as BRAF inhibitors, MEK and PI3K pathway inhibitors, are used or are under trial (Table 5).

• EGFR inhibitor for CRC

The addition of EGFR inhibitor to chemotherapy significantly prolongs the OS of patients with advanced CRC; however, this benefit is limited to *KRAS* wild-type tumors [4–6]. Importantly, it was recently shown that not only mutation in *KRAS* exon 2 but all *RAS* mutations, including *KRAS* exon 3 or 4, *NRAS* exon 2, 3 or 4 or *BRAF* exon 15, should predict inefficacy of EGFR inhibitor [8]. Moreover, mutations in *ERBB2*, *EGFR*, *FGFR1*, *PDGFRA* and *MAP2K1* were identified as mechanisms of resistance to EGFR blockade, suggesting that combination therapies with EGFR inhibitor and a drug targeting resistance-related genes might overcome primary and secondary resistance to EGFR targeting therapy [76]. The HERACLES trial indicated efficacy of dual inhibition of HER2 and EGFR for patients with HER2-positive CRC [77]. Further studies of combination therapy with EGFR inhibitors should be performed.

• BRAF inhibitors for CRC

KRAS and *BRAF* mutation analysis in the CRYSTAL and OPUS randomized clinical trials showed that *BRAF* mutation, which activates the MEK/ERK pathway through its downstream effectors, was present in 8.8% of CRCs, and that OS for patients with *BRAF*-mutant CRC was worse than that for *BRAF* wild-type cases [7]. A Phase II pilot study of vemurafenib in 21 patients with metastatic CRC harboring the identical *BRAF* V600E mutation concluded that vemurafenib monotherapy did not show meaningful benefit [78]. BRAF (V600E) inhibition leads to rapid feedback activation of several pathways. The EGFR pathway was activated by *BRAF* blockage in colon cancers through feedback activation [79].

Table 4. Molecular subtype for Colorectal Cancer Subtyping Consortium.

Subtype	Mutation	Copy number alteration	Activated pathway	Protein expression
CMS1 MSI immune (14%)	Hypermutation <i>BRAF</i> mutation	Low	Immune pathway	DNA repair, cell cycle, apoptosis, inflammation
CMS2 Canonical (37%)	Low mutated <i>TP53</i> mutation	High <i>EGFR</i> amplification	WNT, MYC and epithelial pathway	β -catenin
CMS3 Metabolic (13%)	Intermediate mutated <i>KRAS</i> , <i>PIK3CA</i> mutation	Intermediate	Epithelial pathway	β -catenin, receptors, IGFBP2
CMS4 Mesenchymal (23%)	Low mutated	High	Mesenchymal, TGF- β , stromal, VEGF	NOTCH3, VEGFR2, fibronectin,

MSI: Microsatellite instability.

Moreover, activation of the PI3K/AKT pathway was found to be a mechanism of resistance to BRAF inhibitors [80]. Another study showed that MAPK pathway reactivation was a mechanism of resistance to vemurafenib [81]. These results suggested the potential of combination therapy with BRAF inhibitor and other targeting drugs, such as EGFR, MEK and PI3K inhibitors. Several trials investigating the efficacy of combination therapy with BRAF inhibitor are in progress and promising results are expected (NCT01086267, NCT01524978, NCT01719380, NCT01750918, NCT01791309 and NCT02164916).

• MEK & PI3K pathway inhibitor for CRC

Single MEK inhibitors were not found to have clinical benefit [82–84]. This result is most likely due to feedback upregulation of other pathways, especially PI3K and Wnt pathways [85–87]. Therefore, combination therapy with MEK and PI3K pathway inhibitor is required. Importantly, this combination therapy is effective even for tumors harboring *KRAS* or *BRAF* mutations, suggesting the potential to prolong survival in those patients [88,89]. Several clinical trials are underway to investigate the benefit of different inhibitor combinations of PI3K and MAPK pathways. Final results of ongoing trials are still awaited.

• Trials based on molecularly defined subgroups for CRC

Following the discovery of molecular alterations in CRC, novel therapeutic strategies based on molecular subtype have been proposed. The FOCUS4 trial, which opened for enrollment in the UK, was designed to perform genotype determination from biopsy samples. After standard

first-line chemotherapy, patients were enrolled into a specific randomized trial arm based on the results of the molecular tests (ISRCTN 90061546). The MODUL trial is a study of biomarker-driven maintenance therapy for first-line treatment of mCRC. After 16 weeks of induction therapy with FOLFOX and bevacizumab, patients were divided into several arms of maintenance treatment according to their tumor subtype (NCT02291289). Further studies in which patients receive a treatment regimen based on molecular abnormalities are expected.

Future perspective

Genomic profiling of gastrointestinal cancer has become possible because of technological developments. It is hoped that understanding the cancer genomic landscape will lead to personalized therapy and better prognosis.

High-throughput experimental methods, ‘omics’ including genomics, transcriptomics, proteomics, metabolomics and epigenomics, have become more routine, potentially providing more knowledge of protein interaction, gene expression and metabolic profile data. However, it is difficult to analyze and get something useful from huge data. The recently report showed method how to Integrate multiple omics data [90]. Integration of multiple omics data are revolutionizing our understanding of cancer biology targeted in cancer therapy. Further clinical use of ‘omics’ are expected and should be carefully performed [91].

Patient-derived xenograft (PDX) models could have the potential to help drug screening, biomarker discovery and preclinical testing of personalized medicine approaches [92]. Many studies have indeed shown that tumors in PDX mice

recapitulate the histologic, molecular and biological features of the original tumors [93]. Choi *et al.* showed that genetic and histologic characteristics of GAC were highly consistent between primary and PDX tumors [94]. Investigating the relationship between PDX tumor and drug sensitivity revealed genomic features and mechanism correlated with drug resistance, therefore facilitating the development of effective personalized therapeutics. PDX model should be key tool connecting the tumor genetic feature and clinical application.

Although genetic screening of the primary tumor has been established, characterization of metastatic lesions remains unclear. Typically, the personalized therapy strategy is decided based on primary tumor tissue at the time of diagnosis. During progression of the metastatic process, the molecular differences between primary tumors and metastatic disease may increase [95]. Interestingly, one study of metastatic CRC reported that *KRAS* and *PIK3CA* mutation rates are similar between primary tumors and metastases, whereas metastatic lesions have more frequent *TP53* mutations and less frequent *BRAF* mutations compared with primary tumors [96]. Murata *et al.* showed that the LINE-1 methylation level, which is a surrogate marker of genome-wide methylation level, was similar between primary tumor and metastases. In addition, the mutation rate of *KRAS*, *BRAF* and *PIK3CA*, and the MSI status were concordant [97]. These studies

indicate that some features of metastatic lesions are similar to the primary tumor, while others are different. Thus, to aim to cure metastatic disease it is important to identify the genomic landscape of metastatic lesions and targeted therapy should be selected according to both metastatic and primary lesions.

Recently, liquid biopsy has attracted a lot of attention and has been actively studied and developed. Liquid biopsy is a minimally invasive technique to investigate tumor behavior using circulating tumor cells (CTCs), cell-free DNA (ctDNA) and exosomes. Analysis of CTC and ctDNA enables evaluation of whether systemic therapy would be successful and determination of clonal evolution during targeted therapies [98,99]. For example, in breast and prostate cancers a change in CTC counts predicts the prognosis after treatment [100,101]. In CRC, acquisition of resistance to EGFR inhibitor occurs during its use due to changes in a heterogeneous pattern of mutations in *KRAS*, *NRAS*, *BRAF* and *EGFR* [102]. Interestingly, a change in the proportion of *KRAS*-mutated alleles or copy number can be dynamically detected during EGFR inhibitor therapy using ctDNA [103,104]. Also, in ESCC, ctDNA is considered to be a valuable biomarker for tracking and evaluating tumor status [105]. Taken together, these findings indicate that liquid biopsy has the potential to detect mutations associated with secondary acquired drug resistance and heterogeneity of the tumor beyond the

Table 5. Randomized clinical trials of targeted therapies in colorectal cancer.

Target	Study	Treatment	Median OS or PFS	HR (95% CI)	Ref.
VEGF-A	AVF2107g trial (NCT00109070)	IFL (n = 411) IFL + bevacizumab (n = 402)	OS: 20.3 OS: 15.6	0.66	[69]
VEGF-A	NO16966 trial (NCT00069095)	Oxaliplatin-based (n = 700) Oxaliplatin-based + bevacizumab (n = 701)	OS: 21.3 OS: 19.9	0.89 (0.76–1.03)	[70]
EGFR (<i>KRAS</i> wild-type)	CRYSTAL (NCT00154102)	FOLFIRI (n = 176) FOLFIRI + cetuximab (n = 172)	PFS: 8.7 PFS: 9.9	0.68 (0.50–0.94)	[4]
VEGF-A/EGFR (<i>KRAS</i> wild-type)	PEAK (NCT00819780)	FOLFOX + bevacizumab (n = 143) FOLFOX + panitumumab (n = 142)	OS: 24.3 OS: 34.2	0.62 (0.44–0.89)	[71]
VEGF-A/EGFR (<i>KRAS</i> wild-type)	FIRE-3 (NCT00433927)	FOLFIRI + bevacizumab (n = 171) FOLFIRI + cetuximab (n = 171)	OS: 25.6 OS: 33.1	0.70 (0.53–0.92)	[72]
VEGF-A/EGFR (<i>KRAS</i> wild-type)	CALGB/SWOG 80405 (NCT00265850)	Chemotherapy + bevacizumab (n = 559) Chemotherapy + cetuximab (n = 578)	OS: 29.0 OS: 29.9	0.92 (0.78–1.09)	[73]
VEGF-A and EGFR	PACCE (NCT00115765)	CT + bevacizumab (n = 525) CT + bevacizumab + panitumumab (n = 528)	PFS: 11.4 PFS: 10.0	1.27 (1.06–1.52)	[74]
Multikinase	CORRECT (NCT01103323)	Placebo (n = 255) Regorafenib (n = 505)	OS: 5.0 OS: 6.4	0.77 (0.64–0.94)	[75]

CT: Chemotherapy; FOLFIRI: Fluorouracil, folinic acid and irinotecan; FOLFOX: Fluorouracil, folinic acid and L-OHP; HR: Hazard ratio; IFL: Irinotecan, folinic acid and fluorouracil; OS: Overall survival; PFS: Progression-free survival.

primary mutation. Further study and clinical applications of this field should be expected.

Conclusion

This review describes the genomic profiling for select gastrointestinal cancers and therapy according to genomic features. GACs and CRCs have each been divided into four subtypes according to molecular features. Profiling of the EAC cancer genome is currently ongoing; however, it may turn out to more homogenous than GAC. To date, identification of *HER2* expression in GAC and *KRAS*, *NRAS* and *BRAF* mutations in CRC has proved essential for treatment decisions. Further targeting therapy has developed according to new genomic landscape. In addition, individualized therapy according to molecular subtype has been proposed and applied into clinical trial. Not only primary tumor genomic features, specific alterations in circulating DNA, RNA and tumor cells are useful to understand the heterogeneity of cancer and monitor during therapy response and surveillance. Furthermore, PDX might facilitate development of more detailed personalized therapy regimens. In summary, more understanding

the cancer genomic landscape will lead to better personalized therapy and better prognosis.

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EXECUTIVE SUMMARY

Background

- Next-generation sequencing enables faster, cheaper and more accurate whole-genome sequencing.
- The Cancer Genome Atlas has characterized the landscape of common cancer types using genome sequencing, exome sequencing, methylome DNA sequencing, RNA sequencing and protein arrays.
- Many molecularly targeted drugs targeting the cancer hallmarks have been developed, leading to personalized therapy according to molecular subtype.

Upper gastrointestinal cancer

- Gastric adenocarcinoma was classified into four subgroups by The Cancer Genome Atlas and Asian Cancer Research Group according to molecular features.
- Esophageal adenocarcinoma should be treated according to *HER2* expression.
- Molecular targeting therapy for esophageal squamous cell carcinoma is not yet established but these tumors may be quite susceptible to immune modulations.

Colorectal cancer

- Colorectal cancer (CRC) Subtyping Consortium divided CRC into four subtypes (CMS1: microsatellite instability immune [14%], CMS2: canonical [37%], CMS3: Mmetabolic [13%], CMS4: mesenchymal [23%]).
- CRC should be treated according to *KRAS*, *NRAS* and *BRAF* mutation status. CRCs with high microsatellite instability seem very susceptible to immune modulations.

Future perspective

- Cancer should be treated according to molecular features.
- Patient-derived xenograft and liquid biopsy (circulating tumor cells, ctDNA, exosome) should lead to further molecular profiling and improved individual therapy.

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