Current status and perspectives of genetic testing in gastrointestinal cancer (Review)

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Abstract. Genetic testing has become widespread in daily medical care for gastrointestinal (GI) cancers. However, unlike breast cancer and non-small cell lung cancer, in which personalized medicine targeting various driver genes is standardized, the incidence of targeted gene abnormalities in GI cancers is low. Nevertheless, such abnormalities may be linked to therapeutic agents and the further development of therapeutic agents for personalized medicine for GI cancers is desired. A liquid biopsy is of great benefit in offering clinical decision support, in applications such as GI cancer screening, surgical interventions, monitoring disease status and enhancing patient survival outcomes, all of which would contribute to personalized medicine. Germline genetic testing is required for several types of GI cancer, which shows clinical indications of hereditary predisposition. The increasing use of multigene panel testing has redefined gene-cancer associations, and consequently the estimate of cancer risk that vary from low to high penetrance. Comprehensive genetic testing can enable the detection of novel treatment targets and the discovery of undefined multiple diagnostic/predictive markers, which may enhance the molecular-level understanding of GI cancers. Genetic testing can also aid the design of more appropriate and adequate genomic-driven therapies for patients who may benefit from other standardized therapeutic methods.

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Abbreviations: GI, gastrointestinal; NGS, next-generation sequencing; FDA, U.S. Food and Drug Administration; MSI, microsatellite instability; TMB, tumor mutational burden; OS, overall survival; PARP, poly (ADP-ribose) polymerase; ICB, immune checkpoint blockade; PC, pancreatic cancer; DDR, DNA damage response; EGFR, epidermal growth factor receptor; MAPK, mitogen-activated protein kinases; CRC, colorectal cancer; mCRC, metastatic CRC; HR, hazard ratio; T-DXd, Trastuzumab-Deruxtecan; HER2, human epidermal growth factor receptor 2; WGS, whole genome sequencing; PDAC, pancreatic ductal adenocarcinoma; HRD, homologous recombination deficiency; ASCO, American Society of Clinical Oncology; PFS, progression-free survival; GC, gastric cancer; FGFR, fibroblast growth factor receptor; HCC, hepatocellular carcinoma; TERT, telomerase reverse transcriptase; ctDNR, circulating tumor DNA; cfDNA, cell-free DNA; MRD, minimal residual disease; LS, Lynch syndrome; FDR, first-degree-relative; HDGC, hereditary diffuse gastric cancer; DGC, diffuse type gastric cancer; WES, whole exome sequencing; EC, esophageal cancer

Key words: genetic testing, gastrointestinal cancer, multi-gene panel, hereditary tumor, comprehensive genetic analysis

Contents

1. Introduction

Gastrointestinal (GI) cancers include several malignancies of the gastrointestinal tract and organs such as the stomach, colon, liver, intrahepatic bile duct, gallbladder, and pancreas. GI cancers have epithelial cell or stromal origin, and GI cancers account for approx. 30% of all tumor cases in 2018. Together GI cancers have been responsible for over 3.5 million deaths which corresponds to 37% of the deaths from all human malignancies (1).

While clinical assessment of GI cancer has been performed with a physical examination, blood test, imaging, and endoscopy, recent advancements in genomics have led to the development of genetic analysis for diagnosis (2,3). In the modern era of precision medicine, genetic testing has been incorporated into routine clinical practice to assist decision-making regarding appropriate genetically matched treatments for patients with GI cancers (4). To acquire genetic information in a timely and cost-effective manner, a variety of

gene panel tests, which is based on next-generation sequencing (NGS), have been progressed and some have been approved by the U.S. Food and Drug Administration (FDA) as companion diagnostics for multiple molecular-targeted therapies (5). In contrast, the clinical value of genetic testing for GI cancer receptiveness is not well established. This review describes the current genomic landscape in GI cancers, and testing modalities that have prognostic, predictive, and therapeutic value. We provide an outline of the clinical use of multi-gene testing in GI cancers, and discuss the practical utility and potential of a liquid biopsy and whole genome sequencing.

2. Methods

Non-systematic review was conducted in a basis of an electronic search through the medical literature using PubMed and Google Scholar. The keywords 'genetic testing', 'Multi-gene panel testing', 'Whole genome sequencing', 'Next-generation sequencing', 'gastrointestinal cancer', 'colorectal cancer', 'pancreatic cancer', 'gastric cancer', 'hepatobiliary cancer', 'esophageal cancer', were searched. Guidelines and review articles from gastroenterology, oncology, endoscopy and genetics were included. When more than one guideline concerning the same subject was available, the latest one was picked up. Only full articles in the English language published in the last ten years were considered to be suitable for review. The exclusion criteria consisted duplicated articles, studies absent of diagnostic outcomes. Case reports, editorials, book chapters, correspondences, letters, and non-human research were not included. First, the titles were screened and appropriate studies were selected. Of these studies, the full text was acquired. A total of 258 articles were identified (Fig. 1).

3. Multi-gene panel testing

Comprehensive genomic profiling by NGS enables the detection of multiple genomic features in GI cancers. Multi-gene testing is conducted based on NGS platforms, and before sequencing, genomic regions of attention are chosen from the DNA sample (6). The sustainability and quality assurance of the molecular tumor board, named Expert Panel, examines the appropriate treatment methods such as drug treatment and participation in clinical trials, and the results were informed. Multi-gene panels are largely utilized in clinical settings for the identification of somatic and germline mutations in GI cancers, which lead to molecular classification, and prediction of therapeutic effect. It can also detect the gene which is involved in drug resistance. Additionally, microsatellite instability (MSI) and tumor mutational burden (TMB) are approved by the FDA as companion diagnostics for GI cancers.

Recently, the survival benefit of matched therapy using panel testing has been established. An encouraging impact on tumor response rates, patient outcome, and on detecting novel tools of molecularly-targeted therapy has been suggested by some clinical trials by utilizing multi-gene testing which may lead to personalized cancer treatments (7-9). The Know Your Tumor program testing matched therapies after multi-omics profiling provided suggestions for elucidated clinical trials and personalized therapy for patients with pancreatic cancer (PC). The outcomes of this trial revealed that the patients with *BRCA* mutations after Poly (ADP-Ribose) Polymerase (PARP) inhibitor treatment or those with mismatch repair deficiency after immune checkpoint blockade (ICB) treatment demonstrated 1-year survival benefit compared with patients who received unmatched therapies or those without an actionable molecular change (10). This study also showed that mutations in the DNA damage response (DDR) pathway were the most popular actionable alteration. These data would indicate a guarantee for this precision approach. Although the frequency of druggable genetic alterations in GI cancers is lower than that in breast cancer and non-small cell lung cancer, a variety of noble candidate genes have been identified over the past few years.

In this section, we summarize the current status of genetic tests and molecular-targeted therapies for GI cancer that are expected in the future (Table I).

Colorectal cancer. For a decade, patients with KRAS/NRAS wild type are acceptable for therapy targeting the epidermal growth factor receptor (EGFR) signaling (11). *BRAFV600E* mutation is identified in approximately 8-10% of colorectal cancer (CRC) and generates a RAS-independent constitutional activation of the mitogen-activated protein kinases (MAPK) pathway, which leads to cell growth and survival and is a prognostic biomarker for patients with CRC (12). Even if some *BRAF* mutations are identified beyond the V600 hotspot in CRC, they do not present similar clinical, biological, and therapeutic results as the V600E mutation (13). These *BRAF non-V600E* mutated tumors tend to be well differentiated with left-sided tumor site and were correlated with improved prognosis and resistance to BRAF inhibitors, whereas some have a sensitivity to EGFR (14,15). Notably, the MSI phenotype, which can predict the efficiency of immune checkpoint blockade (ICB) therapies, was identified in approximately 20% of BRAFV600E CRC, regardless of the *BRAF* mutational status (12). BRAF inhibition has been said to cause a rapid feedback EGFR activation, which assists MAPK constitutive signaling. Continued proliferation and resistance of these tumors to BRAF inhibitor monotherapy may occur by EGFR-mediated stimulation of downstream signaling (16). In light of these, the combination strategy with the BRAF inhibitor, anti-EGFR agents, phosphatidylinositol-3-kinase (PI3K) inhibitors, or MEK inhibitors was investigated (17-20). These studies assisted the scheme of the BEACON CRC phase III study, which elucidated encorafenib, binimetinib, and cetuximab or encorafenib and cetuximab, or other treatment options, such as cetuximab and irinotecan or cetuximab and FOLFIRI (folinic acid, fluorouracil, and irinotecan). Metastatic CRC (mCRC) patients harboring a *BRAF* exon 15 p.V600E point mutation, with disease progression after one or two prior treatment approaches, were randomized. Conclusively, the median overall survival (OS) was prolonged over 3 months in the triplet and the doublet experimental regimens, compared to the control. Notably, median progression-free survival (PFS) was superior in the triple-combination group and in the association of the doublet group compared with the other group. These data indicated the clinical benefit of the molecular-targeting combination therapy in previously treated patients with mCRC harboring a *BRAF* exon 15 p.V600E point mutation (21). Although the two experimental regimens were

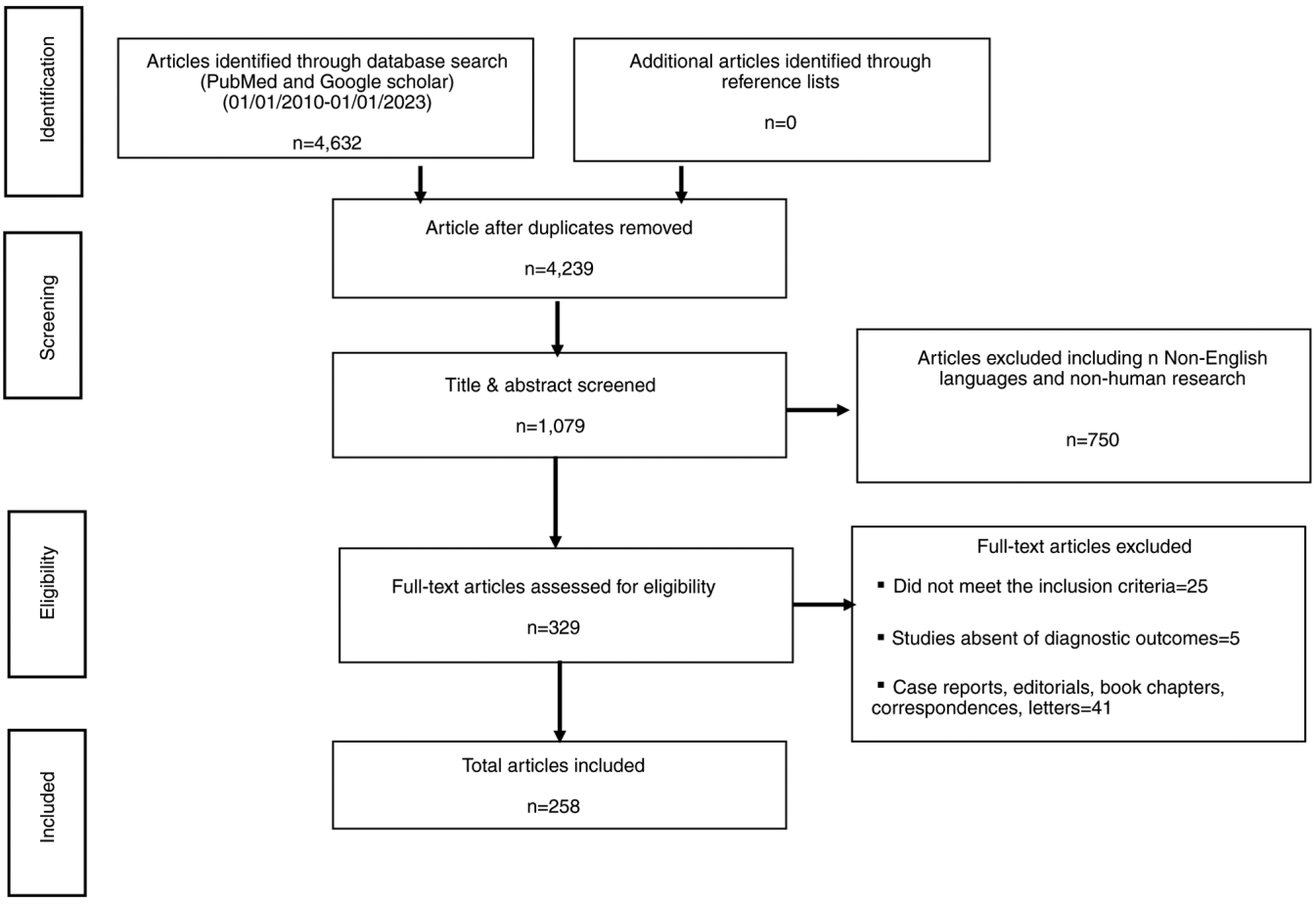

Figure 1. Flow diagram of the literature search modified from preferred reporting items for systematic reviews and meta-analyses.

not compared in the study the combination of encorafenib plus cetuximab, without the MEK inhibitor, is currently positioned as the standard for second- and third-line *BRAF* V600E-mutated mCRC (22). $KRAS^{G12C}$ (glycine 12 to aspartic acid) is one of the most popular *KRAS* mutations in CRC. A novel production of KRAS inhibitors may result in a revolutionary change in the treatment for patients with CRC (23). In a recent, convincing potencies of a direct $KRAS^{G12C}$ inhibitor were described. AMG 510 is a new small molecule that exclusively and irretrievably impaired $KRAS^{G12C}$ activity, by locking it in an inactive guanosine diphosphate-bound state. The initial study using AMG 510 in patients with advanced or metastatic $KRAS^{G12C}$ mutant solid tumors (CodeBreak-100; NCT03600883) demonstrated that ORR and DCR were 12.0 and 80.0%, respectively, in $KRAS^{G12C}$-mutated mCRC patients (24). Although the majority of CRCs initiate through the chromosomal instability pathway, 10-15% of CRCs occur based on the MSI pathway. MSI/dMMR CRCs are characterized by a high TMB with highly immunogenic neoantigens arising from frameshift mutations that induce high infiltration through activated cytotoxic T CD8+ lymphocytes (25,26). for the therapy of CRC patients with MSI/dMMR, who progressed after first or second chemotherapy (27). *HER2* gene amplification was found in approximately 1 to 8% of CRC patients (28). Although the prognostic implication of *HER2* amplifications is controversial, a negative predictive value of *HER2* amplifications for anti-EGFRs efficacy tends to be familiar (29). Phase II studies, named Heracles-A, and MyPathway, evaluated the combination therapy of trastuzumab plus lapatinib, and trastuzumab with pertuzumab. Convincing response rates of 30 and 32% and median PFS of 4.7 and, 2.9 months, respectively were demonstrated (30,31). Trastuzumab-Deruxtecan (T-DXd) is a new antibody drug conjugate consisting of a humanized anti-human epidermal growth factor receptor2 (HER2) antibody, a cleavable, peptide-based linker, and a potent topoisomerase I inhibitor. T-DXd showed a preliminary effect on HER2-positive mCRC refractory to standard therapy, which may lead to the increased advancement of precision treatment of HER2-positive CRC (32).

Recently, NTRK gene fusions emerged as a greatly attractive target for the treatment of patients with cancer. A remarkable clinical significance is demonstrated by TRK inhibitors (larotrectinib, entrectinib). The *ALK* and *ROS1* genes, which encode for the homonym tyrosine kinase receptors, mediate various cellular biological activity via diverse signal transduction (33). *ALK*, *ROS1*, and *NTRK* fusions occur in 0.2 to 2.4% of CRCs (34). Hence, it needs to choose the population to be examined.

Pancreatic cancer. A recent study utilized whole genome sequencing (WGS) to map the genome of 100 pancreatic ductal adenocarcinoma (PDAC) specimens (35). Acknowledged common drivers of PDAC (*KRAS*, *TP53*, *CDKN2A,* and *SMAD4*) were emphasized in this study, and also various other

Table I. Key genetic alterations of gastrointestinal cancer and the corresponding targeted therapies.

Genomic profiling	Targeted agent
Gastric cancer	
HER2	Trastuzumab, Trastuzumab-Deruxtecan
FGFR	Bemarituzumab
VEGFR	N/A
MSI	Pembrolizumab
Colorectal cancer	
EGFR	Cetuximab, Panitumumab
BRAFV600E	Encorafenib
PIK3CA	N/A
MEK	Binimetinib
HER2	Trastuzumab, Trastuzumab-Deruxtecan
KRAS G12C	Sotorasib, Adagrasib
NTRK	Larotrectinib, Entrectinib
MSI	Pembrolizumab, ipilimumab
Pancreatic cancer	
KRAS	N/A
TP53	N/A
CDKN2A	N/A
SMAD4	N/A
EGFR	Erlotinib
NTRK	Larotrectinib, Entrectinib
ALK	N/A
BRAF	Encorafenib
PIK3CA	N/A
BRCA/HRD	Olaparib
MSI	Pembrolizumab
Hepatocellular carcinoma	
TERT	N/A
TP53	N/A
CTNNB1	N/A
VEGFR	Lenvatinib, Cabozantinib
ARID1A	N/A
CCND1	N/A
MET	N/A
PTEN	N/A
Biliary tract cancer	
FGFR	Pemigatinib, Infigratinib
IDH1/2	Ivosidenib
BRAF	N/A
TP53	N/A
HER2	Trastuzumab, Pertuzumab
PIK3CA	N/A
NTRK	Larotrectinib, Entrectinib

mutations at greatly lower frequencies were shown. Activating *KRAS* mutations are found in more than 90% of PDAC. Besides, *ALK* rearrangements, *NRG1* rearrangements, *NTRK* fusions, *ROS, BRAF*, *PIK3CA*, and a variety of cancer-associated genes identified as potential drivers have been detected (e.g., *STK11*, *RB1*, *GNAS*, *CHEK2*, and *ERBB2*), which may lead to potential targets. Inactivation of tumor suppressor genes, including *SMAD4*, *CDKN2A*, and, *TP53* is detected in advancing pancreatic intraepithelial neoplasia progression and arises in up to 50% (36). The frequency of persistently mutated genes then diminishes to less than 10%, which accumulates into central molecular pathways, such as KRAS, wingless and int, TGF-β signaling, DDR, NOTCH, RNA processing, cell cycle regulation (37). Associations of numerous pathways with survival have been detected by analyses of pathways in PDAC patients. DNA repair-related pathways were shown to contribute to a poor outcome (38). In preclinical vivo models and the clinical setting, several of these pathways can be actionable targets for treatment. *BRAF* mutation and *NTRK* gene fusions in KRASWT, MMR-D/MSI-H, and genetic alterations in homologous recombination deficiency (HRD) are considered to be prospective actionable mutations. The American Society of Clinical Oncology (ASCO) guidelines recommended early examination for actionable genetic alterations for PC patients who can be convincing candidates for subsequent therapy following first-line therapy (39). Patients with BRCA mutations, *NTRK* gene fusions, and MSI-H/MMR-D are likely to be provided personalized therapies, such as PARP inhibitors, *TRK* fusion inhibitors, and ICB therapy, respectively (10).

In ovarian and breast cancers, discriminate defects in Homologous Recombination DNA repair genes, such as germline mutations in *BRCA1*, *2*, and *PALB2*, somatic mutations in *BRCA1*, *2*, and promoter methylation of *BRCA1*, have been represented (40,41). BRCA mutations also contributed to promoting the risk for PC. *BRCA* genes encode for proteins involved in the HR repair of DNA double-stranded breaks. PC patients with deficient HR repair are predicted to be responsible for PARP inhibition. Hence, PARP inhibitors are efficient for selective patients with HRD owning to *BRCA1* or *BRCA2* mutations (42). According to recent experiments of genomic profiling in large populations of PDAC, the importance of HRD in predicting the efficacy on PARP inhibitors and platinum-based therapy was accumulated (10,43). ASCO guidelines recommended treatment with PARP inhibitor or platinum-based chemotherapy for patients with a germline *BRCA1*, *2* mutations. In a recent randomized phase III study (POLO), the efficacy of olaparib was demonstrated in germline *BRCA*-mutated metastatic PDAC (44), suggesting that HRD can effectively be targeted in pancreatic cancer. Due to Lynch syndrome or somatic MMR gene mutations, about 1% of PDAC patients have MMR-D/MSI-H (45,46). ASCO guidelines reported that pembrolizumab is advocated as a second-line therapy for PDAC patients with MMR-D/MSI-H (39).

Gastric cancer. The Phase III TOGA trial assessed the trastuzumab-containing regimen compared with standard first-line chemotherapy. Trastuzumab yielded a statistical improvement in terms of median OS, median PFS, and overall response rate (ORR) (47). According to these results, trastuzumab combined therapy come to be the standard treatment for advanced HER2-positive gastric cancer (GC). Although the continuous administration of trastuzumab after progression failed to improve PFS in patients with HER2-positive GC, a phase II

trial that assessed the efficacy and safety of T-DXd vs. a physician's choice of chemotherapy in patients with HER2-positive GC treated with two prior lines including trastuzumab (The DESTINY-Gastric01 trial) showed that OS, as a key secondary endpoint with T-DXd, was significantly improved (median OS 12.5 vs. 8.4 months, HR 0.59, P=0.01). According to these results of the DESTINY-Gastric01 trial, T-DXd was approved for the treatment of patients with HER2-positive unresectable GC in Japan (48,49).

Alterations in fibroblast growth factor receptor (FGFR) genes are found in gastric and gastro-esophageal junction cancers and frequency ranges between 3-7%. The most familiar alteration is amplifications, which are followed by rearrangements and mutations (50). The amplification level was revealed to be negatively associated with patients' prognosis (51). A first-in-class humanized fucosylated IgG1 monoclonal antibody directed against FGFR2b, bemarituzumab has demonstrated convincing results in a phase I study in solid tumors and FGFR2b-positive GC patients (52). The addition of bemarituzumab to chemotherapy was evaluated in first-line therapy in locally advanced, unresectable, metastatic HER2-negative and FGFR2b-positive GC patients. Notably, median PFS was prolonged to 9.5 months in the bemarituzumab group, compared to 7.4 months in the placebo group. Median OS was not reached in the bemarituzumab group vs. 12.9 months in the placebo arm (HR, 0.58, 95% confidence interval 0.35-0.95; P=0.03) and ORR was prolonged from 40 to 53% in the bemarituzumab group (53). Evaluation of Phase III trials in the near future is expected (NCT03694522).

Hepatocellular carcinoma. Although the etiology of hepatocellular carcinoma (HCC) is unsatisfactorily evaluated, recent developments in genomic studies have provided a profound understanding of HCC advancement and may result in new approaches for prevention and treatment. *TP53, CTNNB1, ARID1A, ARID2, AXIN1, RB1,* and *NFE2L2* are the most common mutations in HCC. In a recent, catalytic telomerase reverse transcriptase (TERT) has been distinguished as a frequent driver mutation which is identified in 40-65% of HCC patients (54,55). *VEGFA, MYC, CCND1*, and *MET* are other oncogenes frequently amplified (56,57), *PTEN* is often suppressed (58) and *p16* is commonly deleted in HCCs (59). Present guidelines recommend chemotherapy, with sorafenib being the only first-line therapy for unresectable HCC because of its approval in 2007. A recent REFLECT trial demonstrated that lenvatinib revealed OS non-inferiority to sorafenib (60). The IMbrave 150 trial displayed a combination of atezolizumab plus bevacizumab showed better PFS and OS than that associated with sorafenib (61). Therefore, atezolizumab plus bevacizumab has been positioned as a first-line HCC therapy. According to the RESORCE trial, which showed the superiority of prognosis in patients with HCC whose disease progressed during sorafenib treatment, Regorafenib has been approved as a second-line therapy (62). Additionally, in a phase III trial (CELESTIAL), cabozantinib was presented to have met clinical endpoints, compared with control, as a second-line treatment (63). However, there are no molecular-targeted drugs that match these major genetic abnormalities, and personalized medicine is rarely conducted.

Biliary tract cancers. Biliary tract cancers have poor prognoses even when cytotoxic chemotherapy is applied. Based on the phase 3 ABC-02 and BT-22 trials, combined cisplatin with gemcitabine is the recent standard treatment in unresectable, metastatic biliary tract cancers (55,64). In the second-line setting, FOLFOX (folinic acid, 5-fluorouracil, and oxaliplatin) showed a prolonged OS compared with best supportive care in the phase 3 ABC-06 trial (65). The molecular analysis of biliary tract cancers has significantly improved understanding of the underlying pathological mechanism which may lead to novel targeted therapeutic approaches. *FGFR2* fusions and *IDH1/2* mutations are the most ordinary and clinically important genetic aberrations in intrahepatic cholangiocarcinoma, whereas *TP53* mutations, *KRAS* mutations, and *HER2* amplifications are the most meaningful genetic aberrations in extrahepatic cholangiocarcinoma (66).

4. Liquid biopsy

GI cancers account for a significant proportion of mortality worldwide (1). For these tumors, staging at diagnosis persists as the most principal prognostic factor. With the advancement of tumor biology, it has become important to search for basic knowledge such as pathology as well as for biomarkers that characterize tumors for a treatment approach. Although genome-based precision medicine is convincing, tissue-based genomic sequencing for first-line therapy decision-making in GI cancer remains obstacles owning to the long turnaround time between the receipt of tissue samples and reporting results. Liquid biopsy has the potential to detect circulating tumor DNA (ctDNR) from all tumors that shed into the circulation and can be used to assess intratumor genetic heterogeneity and overcome the limitations of tissue analysis. Circulating tumor cells, ctDNR, exosome, and microRNAs exist in the blood or other body fluids and exhibit the tumor condition in real-time. More recently, methods based on NGS have enabled ctDNA profiling as a replacement for tumor tissue sequencing (67,68). So far, assays applied for ctDNA can be categorized into two classes: those targeted for a single or small number of variants including CAPP-Seq, Safe-seq, Signatera, or ArcherDX, which have a limit of identification no more than 0.01% variant allele frequency, and those aimed at a broader coverage. These comprehensive panel-based sequencing assays which integrate genomic alterations as well as methylation status, are used for genotyping or early diagnosis and achieve a detection limit of approximately 0.2% in the Guardant Health Reveal test (69). Furthermore, cancer genomic testing using ctDNA has been commercialized and approved with an insurance. Meanwhile, although cell-free DNA (cfDNA)-based liquid biopsy test has been approved by the U.S. FDA to detect EGFR mutations in the ctDNA of patients with NSCLC who are candidates for targeted therapy with erlotinib and osimertinib (70), further studies are still required to confirm the clinical usefulness of ctDNA as prescribed by ASCO (71).

Several studies evaluated the possibility of ctDNA as a screening device for tumor progression. A recent study presented that in a high-risk population of 1493 enrolled patients in a prospective cohort study, a single ctDNA methylation marker, cg10673833, revealed distinguished diagnostic accuracy, with the sensitivity of 89.7%, and specificity of

86.8% for the finding of CRC and precancerous lesions (72). The promoter methylation of *APC* and *RASSF1A* in cfDNA was illustrated as frequent epigenetic results in patients with operable GC at an early stage (73). In HCC, when using NGS technology with a panel of regularly altered genes, in a prospective cohort of 30 patients, the ctDNA detection rate reached 63% with stage A based on the Barcelona Clinic Liver Cancer score (74). 81% of concordance rate was obtained between tissue and liquid biopsy. Distinguishment of HCC specimens from control cirrhotic and not cirrhotic tissue samples was reported with a specificity of 95% by a combination of five aberrant methylation biomarkers (75). Mutations of exons 9, 11, 13, and 17 of *KIT*, and exons 12, 14, and 18 of PDGFRA are important drivers of oncogenesis and exist in around 85-90% of gastrointestinal stromal tumors. Hence, the main part of the studies assessing the usefulness of ctDNA in gastrointestinal stromal tumors was focused on *KIT* and *PDGFRA* alterations (76).

In GI cancers, evaluation of minimal residual disease (MRD) through the study of ctDNA is not still defined, but has already been assessed in diverse analyses. In the TRACC study (NCT04050345) designed on stage II-III CRC, 6 out of 14 (43%) MRD-positive patients recurred whereas only 8 out of 93 (9%) MRD-negative patients did.

The TRACC study (NCT04050345) demonstrated that 6 out of 14 (43%) MRD-positive patients with stage II-III CRC recurred whereas only 8 out of 93 (9%) MRD-negative patients did. The most meaningful prognostic factor related with recurrence-free survival was shown to be ctDNA status. CRC patients at high risk of recurrence and who will really receive benefit from adjuvant therapy may be identified (77). ctDNA measurements provide the capability to guide surveillance while detecting latent candidates for escalated or de-escalated adjuvant therapy approaches in resected, stage I-III CRC. A report at the conference in ESMO 2021 evaluated somatic tissue mutations using MSK-IMPACT, and ctDNA utilizing Guardant360, FoundationOne, or MSK-ACCESS. ctDNA identification predicted the risk of recurrence in resected MSI-high patients and evaluated the effect of ICI (atezolizumab) on these MRD positive patients (NCT03832569). Meanwhile, the retrospective CORRECT trial, analysis of ctDNA, could predict the clinical utility of regorafenib and evaluate the survival in mCRC patients (78). Recently, the CIRCULATE-Japan trial, which involved a prospective nationwide patient-screening registry named GALAXY using the Signatera ctDNA assay, reported preliminary findings (79). The sample size of this observational study is 5,000 and 301 patients had clinical stages I, II, and III CRC with preoperative ctDNA identified in 50 (77%), 267 (95%), and 288 (96%) patients, respectively. Interestingly, ctDNA-positive status at 4 weeks showed a negative correlation with survival despite the association with *RAS*, *BRAF V600E*, and MSI status were not demonstrated. Notably, 99% of patients with ctDNA-negative clinical stage I-III survive for the postoperative 6-months.

Liquid biopsy is nearly ready to be approved not only for diagnosis but also for monitoring the acquisition of resistance to therapy in real-time due to its minimal invasiveness and easy collection. For example, in clinical and preclinical studies, *RAS* mutant clones have been elucidated as drivers of acquired resistance to anti-EGFR treatment (80). The appearance of acquired *RAS* mutations and alterations in other genes, such as *ERBB2*, *MET*, *FLT3*, *MEK*, and *EGFR* was suggested by an extensive observation of ctDNA using a ctDNA assay based on NGS during anti-EGFR treatment (81). In metastatic HER2-positive gastro-esophageal cancer, a longitudinal surveillance of serial plasma samples utilizing a ctDNA assay demonstrated that to be correlated to the resistance to trastuzumab in patients treated with trastuzumab in addition to chemotherapy (82). The CRICKET phase II study, the first prospective trial evaluating the efficacy of rechallenge approach with cetuximab and irinotecan, displayed the benefit in RAS/BRAF wild-type mCRC patients with acquired resistance to cetuximab. RAS mutation was not identified in patients who partially responded to treatment (83,84). Numerous clinical studies are currently assessing the role of liquid biopsy in anti-EGFR rechallenge (CHRONOS, NCT03227926; RASINTRO, NCT03259009) (83). We summarize the clinical relevance of liquid biopsy with ctDNA in GI cancer in Fig. 2.

In the near future, liquid biopsies with ctDNA will be essential for GI cancer treatment. A recent study showed that ctDNA analysis significantly reduced the screening period and improved the study enrollment rate compared with sequencing of tumor specimens in GI cancer. Collectively, ctDNA was found in 91.4% of patients (85). Besides, liquid biopsy permits the collection of repetitive samples during the course of the patient's therapy and the collection of clones that show resistance to ongoing treatment. ctDNA as circulating biomarkers can assess the response to ongoing treatments, thus rapidly guiding the medical choice for further chemotherapy regimen and the requirement to switch treatment strategy. Thus, the application of ctDNA-based analysis may provide great benefits in supporting clinical decision-making and improving patient prognosis, which may lead to personalized medicine.

5. Hereditary gastrointestinal cancer

In addition to the main purpose of predicting the drug effects, genetic testing may result in findings regarding germline variants (secondary findings). Multi-gene panel testing has been increasingly required and become broadly available in the research of hereditary cancer syndromes (86). Analysis of secondary findings has been discussed, and the results will be disclosed if the patient wishes to reveal them after discussing whether they should be disclosed in the expert panel. For patients and their families identified at risk by genetic testing, strategies for rigorous screening and risk-decreasing approaches for cancer prevention are important in their outcomes. They can accept genetic counseling consisting of medical geneticists and genetic counselors. Nowadays, guidelines state that patients with suspected hereditary CRC and PC should receive genetic counseling and be offered comprehensive genetic testing (87). The National Comprehensive Cancer Network guideline (88) plans a series of clinical outlines the way to approve multi-gene panel testing: i) when personal medical and/or family cancer history meets criteria for more than one hereditary cancer syndrome ii) when family cancer history does not meet established testing guidelines, but consideration of inherited cancer risk persists iii) in individuals concerned about cancer predisposition for whom family cancer history is limited or unknown. Hereditary breast and ovarian cancer

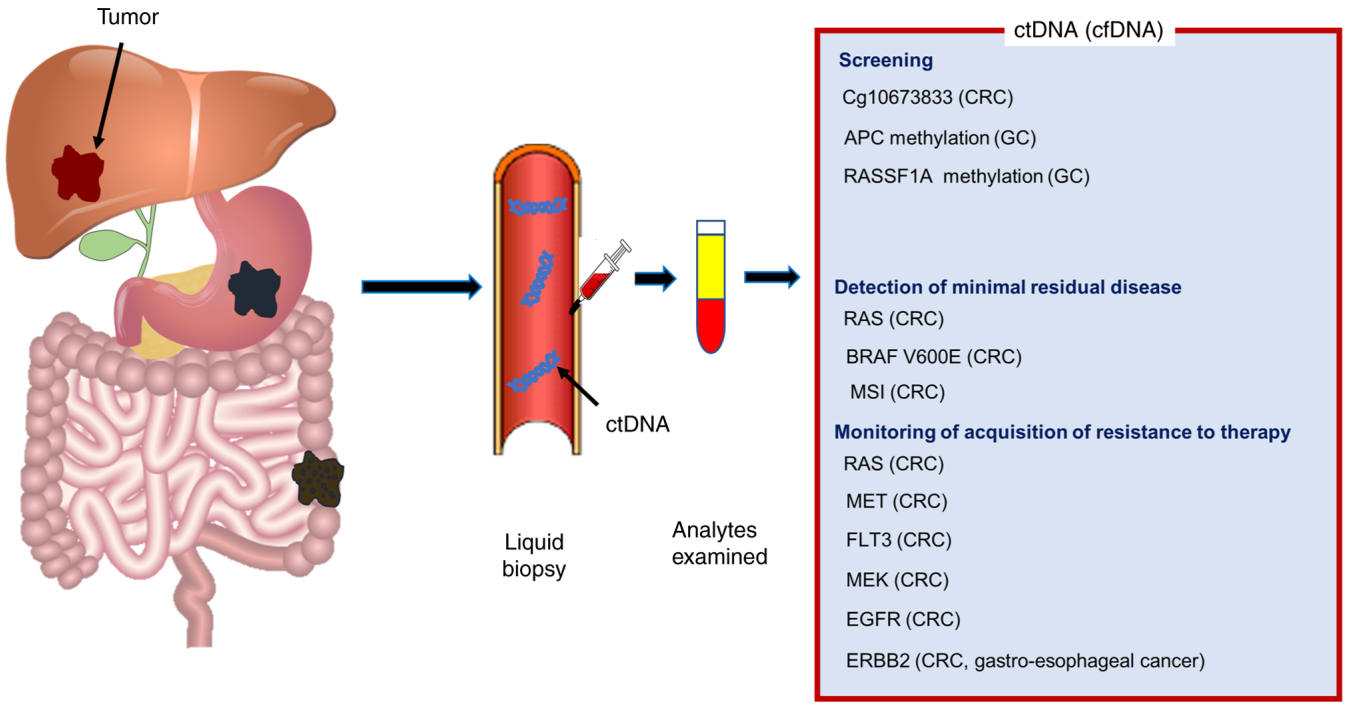

Figure 2. Clinical relevance of liquid biopsy with ctDNA in gastrointestinal cancer. Liquid biopsy with ctDNA and clinical relevance for screening, detection of residual disease and monitoring of acquisition of resistance to therapy. Liquid biopsies were obtained from peripheral blood and provide access to the genetic alteration by investigating ctDNA. ctDNA, circulating tumor DNA; CRC, colorectal cancer; GC, gastric cancer; cfDNA, cell-free DNA; HCC, hepatocellular carcinoma; FLT3, fms-like tyrosine kinase 3; MSI, microsatellite instability; ERBB2, Erb-B2 receptor tyrosine kinase 2.

have been investigated extensively by utilizing a multi-gene panel. It consists of genes as *BRCA1*, *BRCA2*, *PALB2*, *ATM*, *MLH1*, *MSH2*, *MSH6*, *TP53*, *CHEK2*, *STK11* and *PTEN* (89). A sensitivity to GI cancer was distributed among these genes, which led to personalized treatment and follow-up (88,89).

In GI cancers, the major organs involved with inherited cancer are the colon, pancreas, and stomach. Table II provides an overview of hereditary GI cancers, along with their genetic cause, cancer risks, and drug sensitivity.

Colorectal cancer. Lynch syndrome (LS) is one of the most familiar hereditary cancer syndromes that is caused by germline pathogenic variants in DNA MMR, including *EPCAM*, *MLH1*, *MSH2*, *MSH6*, and *PMS2* (90). Families with LS have a high risk of developing colorectal, small intestine, ureteral, urological, endometrial, ovarian, and hepatobiliary cancer, and are prone to progress cancer at a youthful age. The risk of advancing cancer in LS varies according to the causal gene (91). MSI testing is recommended by the American guidelines for all CRC patients with newly diagnosed CRC to find LS patients (92). When a pathogenic germline variant in MMR genes is detected by following genetic testing, LS is diagnosed. Microsatellite regions are involved in various genes contained in cancer initiation, and the accumulation of aberrations in these regions caused MSI-H. GI cancers with MSI-H are remarkably sensitive to ICI, suggesting that ICI should be efficient in LS (93).

Pancreatic cancer. Hereditary cancers caused by germline pathogenic variants are present in approximately 5-10% of PC. Individuals with at least one first-degree relative (FDR) with PC are at higher risk (OR 1.76). Patients with no less than one FDR with PC have elevated risk. The more FDR additionally increases this risk. If a family involves two concerned FDRs, the colleagues of this family are identified as FPC kindreds. Risk elevates promptly depending on the number of affected family members; 4.6-fold with one, 6.4-fold with two, and 32-fold with three affected FDRs (94). Well-defined genetic cancer sensitivity syndromes correlated with PC clarify a minority of this familial accumulation, as shown in Table II. In recent studies using gene-panel testing, some PCs harbor actionable *BRCA1/2* pathogenic or likely pathogenic variants (0-3% for *BRCA1* and 1-6% for *BRCA2*) were presented (95-97). While screening every person for PC is expensive due to the comparatively low occurrence of this disease and the deficiency of precise, and noninvasive screening methods, screening may have significance for patients who reveal elevated risk (98,99). Genetic testing seems to be worthwhile for patients with an increased risk of carrying a pancreas-related cancer susceptibility gene.

Gastric cancer. Hereditary Diffuse Gastric Cancer (HDGC is characterized by a high prevalence of diffuse-type GC in the family lineage. HDGC is an autosomal dominant inheritance caused by a germline *CDH1* mutation encoding the adhesion molecules E-cadherin. In Western countries, approximately 40% of HDGC families are shown to have germline mutations in *CHD1* (100). Genetic testing is recommended for HDGC candidates because it involves multiplex ligation-dependent probe amplification is recommended for HDGC candidates. Truncating mutations in *CDH1* and *CTNNA1* are thought to be responsible for this syndrome. Recently, exome sequencing

Table II. Characteristic feature of hereditary gastrointestinal cancers.

Disease	Causative genes	Inheritance trait	Gastrointestinal tumors (lifetime cancer risk)	Other malignancies	Drug sensitivity
Lynch syndrome	*MLH1, MSH2, MSH6, PMS2, EPCAM*	AD	CRC (22-74%) GC (11-19%) Small bowel cancer (1-4%) PC (3-4%) HCC and bile tract cancer (2-7%)	Endometrium, ovary, uterus, brain	ICI,
FAP	*APC*	AD	CRC (100 %) Duodenum and ampullary (4-12%) GC (<1%) PC (2%)	Thyroid, adrenal gland, brain	NSAID
JPS	*SMAD4*, *BMPR1A*	AD	CRC (39%) GC, PC, Small bowel cancer (21%)	None	N/A
PJS	*STK11*	AD	CRC (39%) GC (29%) PC (11-36%) Small bowel cancer (13%)	Breast, lung, ovary, uterus, testis, cervix	N/A
LFS	*TP53*	AD	CRC (12.5%) GC (4.8%)	Sarcoma, breast, adrenal gland brain, lung	N/A
MUTYH-associated polyposis	*MUTYH*	AR	CRC (40-100%) Duodenum (4%)	Thyroid,	N/A
Cowden syndrome	*PTEN*	AD	CRC (9-16%)	Breast, thyroid, endometrium, brain, kidney	N/A
HDGC	*CDH1*	AD	GC (70-80%)	Breast	N/A
HBOS	*BRCA1, BRCA2, PALB2*	AD	PC (1-7%)	Breast, ovary, prostate, skin	PPAP inhibitor
FAMMM	*CDKM2A*	AD	PC (17%)	Skin (melanoma), lung, larynx, breast	N/A

CRC, colorectal cancer; GC, gastric cancer; PC, pancreatic cancer; FAP, familial adenomatous polyposis; JPS, Juvenile polyposis syndrome; PJS, Peutz-Jeghers syndrome; LFS, Li-Fraumeni syndrome; HDGC, Hereditary diffuse gastric cancer; HBOS, hereditary breast ovarian cancer; FAMMM, familial atypical multiple mole melanoma syndrome; AD, autosomal dominant inheritance; AR, autosomal recessive inheritance; NSAIDs, non-steroidal anti-Inflammatory drugs; PARP, poly ADP ribose polymerase; N/A, not applicable.

identified germline mutations of some related genes, such as *INSR*, *FBXO24*, *MAP3K6*, *PALB2*, *RAD51C*, *MET*, and *DOT1L* as other latent candidate genes for HDGC (101).

The increasing use of multigene panel testing has redefined gene-cancer associations, and consecutively, cancer risk assesses that penetrance values range from low to high. Cancer screening approvals and preventive strategies adapted by germline mutation will enable us to improve clinical prognosis for patients at greatest risk of cancer and their kindreds.

6. Comprehensive genetic analysis

As mentioned above, standards of cancer molecular diagnostics, including multi-gene panels have been launched and developed in clinical settings. In contrast, these tests cover only a certain number of associated genomic alterations in coding regions of the genome. Because cancer genomes evolve in a while, it is recommended to utilize comprehensive NGS techniques over restricted-gene tests. Recent advances in NGS as large-scale sequencing technology allow one to investigate the entire genome (WGS), the exons within all known genes (whole exome sequencing, WES), or total RNA (whole transcriptome sequencing) (102). WGS is theoretically straightforward. DNA is randomly fragmented by physical shearing, and 30-50x sequence depth (90-150 Gb) of the individual human whole genome is ordinarily sequenced for both cancer and normal genomes, which result in comprise 99% of the total human genome (103). WGS strategies can identify unexplored mutations, such as untranslated regions, introns,

Table III. Ongoing clinical trials of gastrointestinal cancer classified on comprehensive entire genetic testing.

Sequencing/NCT number	Type of trial	Clinical purpose	Results	Detection method	Comments
WGS					
NCT02759657 (COMPASS)	Cohort	Diagnostic	Active, not recruiting	Tissue	Comprehensive molecular characterization of PDAC for better treatment selection
NCT03254121 (HEPCASUS)	Cohort	Diagnostic	Completed	Tissue	Genome studies of HCC developed in hepatitis C patients with sustained virological response
NCT03718897	Cohort/prospective	Prognostic	Recruiting	Tissue	Identification of prognostic gene Mutations in biliary tract cancer Using WGS
NCT04597710	Cohort/prospective	Diagnostic/ predictive	Recruiting	Tissue	Utility of WGS to aid clinical decision making in patients referred for liver resection
NCT05242237	Cohort/prospective	Prognostic	Recruiting	Blood	The prognostic value of CTC isolated by a novel microfluidic platform in liver cancer patients
WES					
NCT04694391	Case-Control/ prospective	Diagnostic	Recruiting	Tissue/blood	Genomic study of relapse EC after radiotherapy
NCT02127359	Cohort/prospective	Diagnostic	Completed	Tissue	The study is to perform WES on cancer cells and normal tissues to develop better ways to treat and prevent cancers
NCT03486574	Family-based/ prospective	Diagnostic	Enrolled by invitation	Blood	Research for associated genes for developing GC in family member with first-degree relatives
NCT03982173 (MATILDA)	Single Group	Therapeutic	Active, not recruiting	Tissue	A phase II WES-based basket trial for combination therapy with durvalumab and tremelimumab in patients with metastatic solid tumors
NCT03108885	Case-Only/ prospective	Predictive	Enrolled by invitation	Tissue/blood	Measuring cfDNA during the course of treatment for EC as a marker of response and recurrence
NCT04955808	Case-Only/ prospective	Diagnostic	Recruiting	Tissue/blood	The utility of biospecimen collection in identifying genetic changes in patients with solid tumors or multiple myeloma undergoing surgery
NCT02851004	Single Group	Therapeutic	Terminated	Tissue/blood	The efficacy and safety of BBI608 in combination with pembrolizumab in mCRC
NCT05048524	Single Group	Diagnostic	Recruiting	Tissue/blood	The feasibility of SLOG regimen in patients with localized PC.
NCT03832621 (MAYA study)	Single Group, open label	Diagnostic	Active, not recruiting	Tissue/blood	The efficacy and safety of nivolumab, ipilimumab and temozolomide combination in patients with MSS, MGMT-silenced mCRC.

Table III. Continued.

Sequencing/NCT number	Type of trial	Clinical purpose	Results	Detection method	Comments
NCT03023436	Single Group, open label	Diagnostic/ therapeutic	Recruiting	Tissue/blood	The survival benefit and safety of cytoreductive surgery combined with Hyperthermic Intraperitoneal Chemotherapy and chemotherapy in gastric cancer with peritoneal metastasis.
WTS					
NCT03886571	Cohort/prospective	Diagnostic	Recruiting	Tissue/blood	An observational, biospecimen collection protocol to develop a bank of pancreatic cancer tissue and normal tissue.
NCT03573791	Case-control/ prospective	Diagnostic	Recruiting	Tissue	The purpose of this trail is to identify the biomarkers to predict resistance to neoadjuvant therapy.
NCT03840460	Cohort/prospective	Diagnostic	Recruiting	Tissue/blood	A study in PDAC to enable further disease characterization and the development of predictive and prognostic biomarkers
NCT04249739	Non-Randomized	Therapeutic	Recruiting	Tissue	Pembrolizumab + capecitabin/ oxaliplatin or pembrolizumab + trastuzumab + capecitabine/ cisplatin in GC
NCT02015169	Single Group	Therapeutic	Completed	Tissue	Phase II study of neoadjuvant XELOX + Lapatinib in HER2-positive GC patients with liver metastasis
NCT03841799 (COLON-IM)	Cohort/prospective	Diagnostic	Recruiting	Tissue	Assessment of colorectal tissue microenvironment (neutrophils infiltrate) of patients with benign or malignant colorectal lesion
NCT03260712	Single Group	Predictive	Active, not recruiting	Tissue	Evaluation of pathological predictive factors for response and toxicity which are responsible for chemotherapy and pembrolizumab.
NCT04554771 (BASALT)	Randomized	Treatment	Recruiting	Blood	Blood-borne assessment of stromal activation in EC to guide tocilizumab therapy

WGS, whole genome analysis; WES, whole exome analysis; WTS, whole transcriptome analysis; CRC, colorectal cancer; GC, gastric cancer; PC, pancreatic cancer; EC, esophageal cancer; CTC, circulating tumor cell; N/A, not applicable.

promoters, non-coding functional RNA, and mitochondrial genomes, as well as coding mutations and somatic copy number alterations. WGS also provides a range of diagnostic significance, including new detection in rare cancer mutations (104). WGS analysis will enable us to clarify the functions of these unknown genomic regions and further understand the whole landscape of cancer genomes (105). The comprehensive genetic testing for GI cancer now being examined in clinical studies is reviewed in Table III.

A recent genomic-based study of glioblastoma patients examined the usefulness of WGS/RNA-seq vs. targeted panels (106). WGS/RNA-seq detected more conceivably criminal clinical findings than targeted panels in 90% of cases, with an average of 16-fold more unique conceivably criminal variants identified for each patient. In PC, WES of germline DNA from whole blood of Japanese familial pancreatic cancer patients revealed novel germline susceptibility genes, *FAT1* and *FAT4*, which encode the large transmembrane proteins

Table IV. Selective ongoing clinical trials of multi-omics study for gastrointestinal cancer.

Multi-omics study	Type of trial	Clinical purpose	Phase	Results	Detection method	Comments
NCT02342158 (PERMED-01 trial)	Single Group	Diagnostic	N/A	Active, not recruiting	Tissue/blood	Identification molecular alterations to guide individualized treatment in advanced solid tumor
NCT03546127 (MULTIPLI-0)	Cohort/ prospective	Diagnostic	N/A	Completed	Tissue/blood	A feasibility study in France to assess sample circuit and to perform analyzes within a limited time in CRC.
NCT03951792	Case-control/ prospective	Diagnostic	N/A	Enrolled by invitation	Tissue/stool	Time longitudinal study of the microbiome in CRC
NCT04318834 (COMPASS-B-MUHC)	Single Group, open label	Diagnostic		Recruiting	Tissue	Identification of actionable molecular alterations of biliary tract cancer through WTS.
NCT04622423	Cohort/ prospective	Diagnostic	N/A	Recruiting	Tissue	Advanced therapies for liver metastasis in CRC and PC.
NCT04871321	Cohort/ prospective	Diagnostic	N/A	Recruiting	Tissue/blood	Biomarker discovery in patients within patients with advanced biliary tract cancer who received nab-paclitaxel plus gemcitabine-cisplatin
NCT05234450	Case-only/ prospective	Diagnostic	N/A	Recruiting	Tissue	Identification of different tumor subgroups in pancreatic neuroendocrine tumors and carcinomas regardless of their grade and stage.
NCT03429816 (OPPOSITE)	Single Group, open label	Therapeutic	N/A	Active, not recruiting	Tissue	Correlation of molecular subtypes with histological response after neoadjuvant therapy in patients with EC and GC.

WGS, whole genome analysis; WTS, whole transcriptome analysis; CRC, colorectal cancer; GC, gastric cancer; PC, pancreatic cancer; EC, esophageal cancer; N/A, not applicable.

protocadherins (107). Thus, WES for PC patients would offer significant information about high-risk pathogenic germline variants in hereditary cancer syndromes. A study focusing on rare genetic variants using WGS through analysis of heterozygous premature truncating variants showed that 20 significant genes, including *PALD1*, *LRP1B*, *COL4A2*, *CYLC2*, *ZFYVE9*, *BRD3*, *AHDC1* were identified, which would play an important role in risk prediction of high-risk patients in families identified at risk (108).

In CRC, a novel tumor suppressor, *ARID2* was detected based on WES analysis of younger patients (109). Substantial augmentation for mutations in 4 out of 23 coding and 12 out of 15 noncoding driver genes was shown in the mCRC cohort compared with primary CRC by using WES. Mutations in *PIK3CA* were significantly reduced in mCRC among detected putative drivers (110). Six of the newly found coding driver genes, *ZFP36L2*, *BCL*, *BCL9L*, *ELF3*, *LMTK3*, and *TGIF1* are not detected in the CRC-specific MSK-IMPACT panel. Similarly, WGS analysis of metastatic vs. matched primary colorectal lesions, 65% of somatic mutations originate from a common progenitor, with 15% being tumor- and 19% metastasis-specific (111). Both primary- and metastasis-specific mutations maintain high levels of BRCAness. Recurrently mutated non-coding elements such as ncRNAs RP11-594N15.3, AC010091, SNHG14, 3' UTRs of *FOXP2*, *DACH2*, *TRPM3*, *XKR4*, *ANO5*, *CBL*, *CBLB* and efferocytosis-/PD-L1were identified. Numerous metastasis-specific mutations were detected, including non-silent mutations of *FAT1*, *FGF1*, *BRCA2*, *TP53*, and *KDR*, splice site mutations of *JAK2* and 3'-UTR mutations in *KDR*, *PDGFRA*, and *AKT2* genes, suggesting the existence of a high degree of mutational discordance between metastatic and primary tumor (111). An original dataset containing

whole genomes analysis from 60 single-cell collecting samples before therapy and after metastatic relapse resection demonstrated that three non-synonymous and one stop codon mutations specific to the recurrent lineage in four different genes, PKHD1, PCDHB15, CSF1R, and CC2D1B, were detected in CRC patients. Moreover, a distinctive mutagenic prototype distinguishing the cancer cells from the recurrent lesion illustrated by a substantial contribution of COSMIC signatures SBS35 and SBS17b was identified (112).

In esophageal cancer (EC), somatic mutations and copy number alterations in multiple chromosome segments, encoding *MYC* on 8q24.21, *PIK3CA* and *SOX2* on 3q26, *CCND1*, *SHANK2*, *CTTN* on 11q13.3, and *KRAS* on 12p12 were detected using WES. Amplifications of *EGFRvIII* and *EGFRvIVa* mutants were identified, representing a novel finding in African-American EC that may lead to clinical practice (113). WGS can lead to the detection of novel treatment targets and the discovery of new genomic biomarkers, which may eventually develop the treatment modalities for patients with GI tumors.

7. Discussion and future perspective

In this review, we focused on the latest advances in genetic testing for the diagnosis and management of GI cancer. With the introduction of Sanger sequencing and polymerase chain reaction, laboratory genetic testing became an important instrument for the genomic profiling of cancers in clinical settings. However, although a variety of genes that are mutated in GI malignancies are known, none of the mutations has had clear actionability, and DNA analyses of GI cancers were not a part of clinical oncology until relatively recently. The concept of massively parallel sequencing led to the development of multi-gene panels, that cover the entire spectrum of all acknowledged targeted genes and assist in selecting a useful therapy (5). Studies of germline variants that contribute to cancer predisposition now help detect individuals who have a high-risk for some heritable cancers. Multi-gene panel testing has the capacity to provide significant advances in daily oncology practice. However, there are still several obstacles to be addressed before multigene panel testing can be effectively applied to patients with GI cancer.

One of the major issues regarding the use of multi-gene panel for precision medicine is the lack of appropriate treatment. The proportion of GI cancers that have clearly actionable genetic alterations is comparatively low, and there is no gene-tailored therapy for the majority of patients with GI cancer. From a translational viewpoint, the persistent success of comprehensive genetic testing will depend principally on the testing's clinical utility and ability to identify the treatable targets. To increase the number of patients whose tumors can be successfully treated, it would be indispensable to use strategies such as large-scale analyses in preclinical settings to increase our understanding of the biological processes driving cancer and to identify biomarkers for cancer diagnostics and new drugs. Comprehensive genome profiling might represent one of these strategies, and the continued progress in such profiling may lead to genetic testing as a first option in the treatment of GI cancers.

Liquid biopsies are an ideal sample source that reflects individual characteristics and the heterogeneity of GI cancers. The widespread clinical applications of ctDNA-based assays for therapy decision-making and monitoring of tumors are based on promising preliminary findings, but many challenges remain. The ctDNA levels in plasma are prone to be inconsistent and low, causing a variety of detection thresholds. Additionally, a negative ctDNA finding may be attributed to low copy number identification instead of the absence of ctDNA. Hence, the restricted sensitivity of a ctDNA examination is an essential issue in patients who have early-stage cancer as well as a lower level of plasma ctDNA. The low level of ctDNA in the plasma may require the usage of ultrahigh-depth sequencing and sophisticated statistical models for the purpose of decreasing background error rates for very low variant allele frequencies (114). False-positive ctDNA results can also be caused by DNA fragments from the clonal hematopoiesis of indeterminate potential or non-neoplastic hematopoietic stem cells can be reduced by conducting an advanced bioinformatics analysis or by comparing the results of ctDNA sequencing with the findings obtained from leukocytes and/or matched tumor tissues (115). A high-intensity cfDNA sequencing analysis based on the combination of cfDNA and white blood cell DNA analysis provides both the *de novo* detection of tumor-derived changes and the clarification of MSI, the TMB, mutational profiles, and the sources of somatic mutations identified in cfDNA (115). A quantitative ctDNA evaluation and methylation uncovering may increase the specificity of ctDNA identification and consequently allow to distinguish benign from cancerous GI disease, even at early tumor stages. Further explorations by a large number of clinical trials are necessary for the standardization of the detection process as well as the clinical application of liquid biopsies.

Among the multiple technical platforms that are now available, the WGS strategy is now the most effective way to construct a comprehensive image of the genomic variation in a tumor. The widespread use of WGS technologies in clinical settings seems no longer a distant dream, but the application of WGS strategy possesses tremendous challenges in light of the sequencing costs, computational processing, long-term storage, and meaningful biological interpretation. Moreover, WGS needs particular ethical and regulatory frameworks to handle accidental and secondary genomic detections in the germline. However, in light of the estimation that the costs of sequencing will result in the historic descending tendency, a more gradual approval of WGS approaches for a more improved stratification and subtyping of rare tumors may be attainable in the short period. Algorithms that can dependably support the latent significance of new genetic issues and then associate these issues to theoretical or assumed clinical activity with limited manual interference are required. The advancement of those algorithms will be essential for decreasing analysis and explanation costs and reducing the turnaround time for clinical strategy The most modern WGS platforms such as Illumina NovaSeq 6000 system can handle a great volume of specimens in comparatively short turnaround times, which makes WGS more practical (116).

Recent advances in cancer research have revealed intratumor heterogeneity at the cell levels, epigenetic profiles, and interferences with the tumor microenvironment. Hence, the

incorporation of multiple layers of information for individual cancer cells is crucial for a comprehensive knowledge of the mechanisms of cancer initiation (117). The addition of 'omics' to a molecular word suggests a comprehensive, or worldwide evaluation of a set of molecules. A multi-omics study is a data-driven biological analysis in which the data sets are diverse individual omic analyses, such as genomics, epigenomics, transcriptomics, proteomics, metagenomics, and microbiomics that are used to investigate physiological or pathological phenomena and characterize biomolecular systems at different levels. The recent advances in high-throughput technologies for genomics and transcriptomics have resulted in a paradigm shift toward multi-omics investigations, large-scale research collaborations, and the design of computational algorithms (118). Multi-omics studies for GI cancer currently being evaluated in clinical trials are summarized in Table IV. Multi-omics investigations have been applied in a variety of clinical studies for a better detection of clinical subtypes or drug resistance, the prediction of efficient combined therapies, and the exploration of novel biomarkers. For instance, integrated proteogenomic data together with genomic and transcriptomic data of CRCs, which were illustrated by The Cancer Genome Atlas, demonstrated that a chromosome 20q amplicon was correlated with the great inclusive alterations at both messenger RNA and protein levels. In addition, the incorporation of proteomics data provides the detection of important 20q candidates, including *HNF4A* (hepatocyte nuclear factor 4, alpha), *TOMM34* (translocase of outer mitochondrial membrane 34), and *SRC* (SRC proto-oncogene, nonreceptor tyrosine kinase), suggesting that incorporated proteogenomic analyses will enable novel developments in cancer diagnosis and treatment (119). A study that performed a multi-omics characterization of molecular features of GC, using WGS, WES, and RNA-seq for 35 GC patients before and after their neoadjuvant chemotherapy, showed that *C10orf71* was associated with treatment resistance, whereas *MYC* and *MDM2* amplification mutations were associated with treatment sensitivity (120).

8. Conclusion

Multi-gene testing should be widely applied in clinical settings, not only for greater insights into tumor biology but also to drive cancer treatment. New clinical studies should apply multigene testing toward the goal of finding novel targeted therapies. The rapid analysis of genetic alterations with real-time monitoring of therapy responses by ctDNA can optimize new therapeutic strategies. The comprehensive characterization of GI cancers by genetic testing will contribute to a better molecular-level understanding of cancer, and it will contribute to more appropriate and effective genomic-driven therapies for patients who might not benefit from standardized therapy or experimental interferences in the context of clinical studies. Due to several challenges to be resolved such as the costs, restricted sensitivity, and time consumption to carry out, genetic testing should be used when standard therapeutic approaches have been completed at present.

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Competing interests

The authors declare that they have no competing interests.

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