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The evolving role of OMICS in gastrointestinal tumor biology and clinical practice

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Abstract

Due to late diagnosis, high molecular diversity, and limited response to therapeutic intervention, gastrointestinal (GI) cancers result in a considerable number of cancer-related deaths. Classic clinicopathological classification and single omics analysis fail to adequately convey the extensive biological complexity related to progression of disease, developed therapy resistance and recurrence of the disease. As a result, the recent introduction of integrated multi-omics including genomics, epigenomics, transcriptomics, proteomics, metabolomics and spatial profiling as well as the emerging use of liquid biopsy is substantially reshaping our understanding of and clinical approach to GI cancers. This review summarizes developments to illustrate how the incorporation of multi-omics across GI tumors offers a better understanding of GI cancers and providing more precise and less costly ways to detect disease earlier, develop molecular subtypes of the tumors with greater accuracy for the purpose of developing an individualized risk stratification system for patients. Furthermore, the article will discuss the growing use of minimal residual disease monitoring and the use of ctDNA in guiding a patient's post-operative surveillance and treatment decision-making process. In addition, this review focuses on the value of using multi-omics-based knowledge of a tumor's microenvironment to better predict how effective immunotherapy will be and support the effective combination of drugs for the treatment of GI cancers and how targeted therapy will broaden the clinical practice landscape for developing therapeutics containing new vulnerable targets. Finally, the review provides an overview of current barriers to the implementation of multi-omics and point out to future opportunities. Collectively, emerging omics data suggest a meaningful shift toward precision oncology in gastrointestinal cancers, though widespread clinical implementation remains an active area of investigation.

Keywords Gastrointestinal Cancer, Multi-Omics, Tumor Biology, Clinical Practice

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Introduction

Gastrointestinal (GI) cancers, which include tumors of the digestive system and its related organs, account for a large proportion of deaths and new cases worldwide with approximately five million new diagnoses annually and three million deaths each year [1]. There are indications that the high mortality rates associated with advanced GI tumors could be due to the continuing problems of delayed diagnosis combined with the fact that GI tumors exhibit extreme molecular variability. Even within a given histologic diagnosis, these tumors contain multiple cellular subclones and types of tumor microenvironment [2–4]. Though new therapeutic modes of treatment for these patients have emerged over the past several years, patient outcomes have continued to decrease at approximately the same rate as before. Until now, there have been insufficient advances in early detection of GI tumors, so there continues to be an unmet need for improved, lower-cost screening methods [5, 6]. Furthermore, as we have come to better appreciate, the cellular microenvironments of GI cancers are composed of heterogeneous populations of cancer cells and their respective stromal elements, which explain the varied response patterns of these cancers to various cancer therapeutic agents, create a highly variable landscape, even among histologically identical tumors. Advances in understanding the molecular basis for GI cancer initiation, progression and development of therapeutic resistance have been made through traditional methods of single-omic analysis and have led to many advances [7–10]; however, even using this method, we will not be able to achieve the level of understanding required to fully understand or develop strategies to overcome or mitigate the effects of multi-layered regulatory networks involved in GI cancer initiation, progression and development of therapeutic resistance.

The various molecular data types included in multiple omics assessments (genomics, epigenomics, transcriptomics, proteomics, metabolomics, and other molecules) represent an extremely exciting and powerful tool for examining the complexity of gastrointestinal malignancies. Multi-omics approaches enable us to correlate the changes that occur in multiple levels of DNA, RNA, proteins and metabolites, and provide an overall understanding of tumor biology. Where non-GI cancer studies are cited (e.g., methodological frameworks from breast or renal cell carcinoma), these are explicitly included as technical analogies whose relevance to GI oncology has been established or is under active investigation, rather than as primary evidence for GI-specific claims [11–14]. Thus, although a genomic mutation like the APC or KRAS mutation identifies a driver for colorectal cancer, multi-omics integrative analyses provide further insights into the downstream effects of this driver mutation on signaling pathways and metabolites, such as how APC loss activates Wnt/β-catenin signaling via genomics, which then promotes glutamine metabolic reprogramming via metabolomics through the up-regulation of glutamine synthetase [15–17]. These cross-omics analyses allow for a clearer connection between the genetic lesions and the resulting phenotypes, e.g. altered metabolism, immune evasion. Additionally, integrating multi-omics can identify common biomarkers or indicators across platforms, i.e. a protein marker that is supported by a corresponding change in the underlying DNA/RNA strands, which greatly increases confidence in the target and permits continuous monitoring of disease through minimally invasive means, such as liquid biopsy [18–20]. Indeed, the combination of circulating tumor DNA (ctDNA) mutations with protein markers in the blood of patients shows promise in the early detection of treatment resistance compared to standard imaging techniques; specifically, detecting both a KRAS mutation and elevated exosome EGFR phosphorylation in patients with metastatic colorectal cancer was predictive of cetuximab resistance approximately 12 weeks prior to clinical progression [21, 22]. As a result of applying the multi-omics framework, researchers have begun to change the way they view many areas of gastrointestinal oncology. For example, new forms of precise screening for at-risk populations, molecular subtyping of tumors, measuring minimal residual disease (MRD), predicting immunotherapy responses and identifying new therapeutic targets are all being developed utilizing these approaches [23–26]. This review will evaluate the continual development of omics across these different areas, as indicated up to the present day (Fig. 1). It will also discuss the various limitations that currently exist and the current barriers to widespread clinical use. Future directions will address how the continued development of technologies and AI may further improve multi-omics-driven personalized medicine for gastrointestinal cancers. The objective of this review is to comprehensively define the contemporary transformation of both gastrointestinal cancer biology and clinical practice through the utilization of multi-omics, while also identifying the remaining barriers to achieving widespread clinical utilization.

This narrative review was conducted through systematic searches of PubMed, Web of Science, and Scopus, covering publications from January 2015 through February 2026, with selected seminal studies included from before this window. Search terms included combinations of: "gastrointestinal cancer," "colorectal cancer," "gastric cancer," "hepatocellular carcinoma," "pancreatic cancer," "esophageal cancer," "multi-omics," "genomics," "epigenomics," "transcriptomics," "proteomics," "metabolomics," "liquid biopsy," "circulating tumor DNA," "minimal residual disease," "tumor microenvironment," "immunotherapy," and "targeted therapy." Inclusion

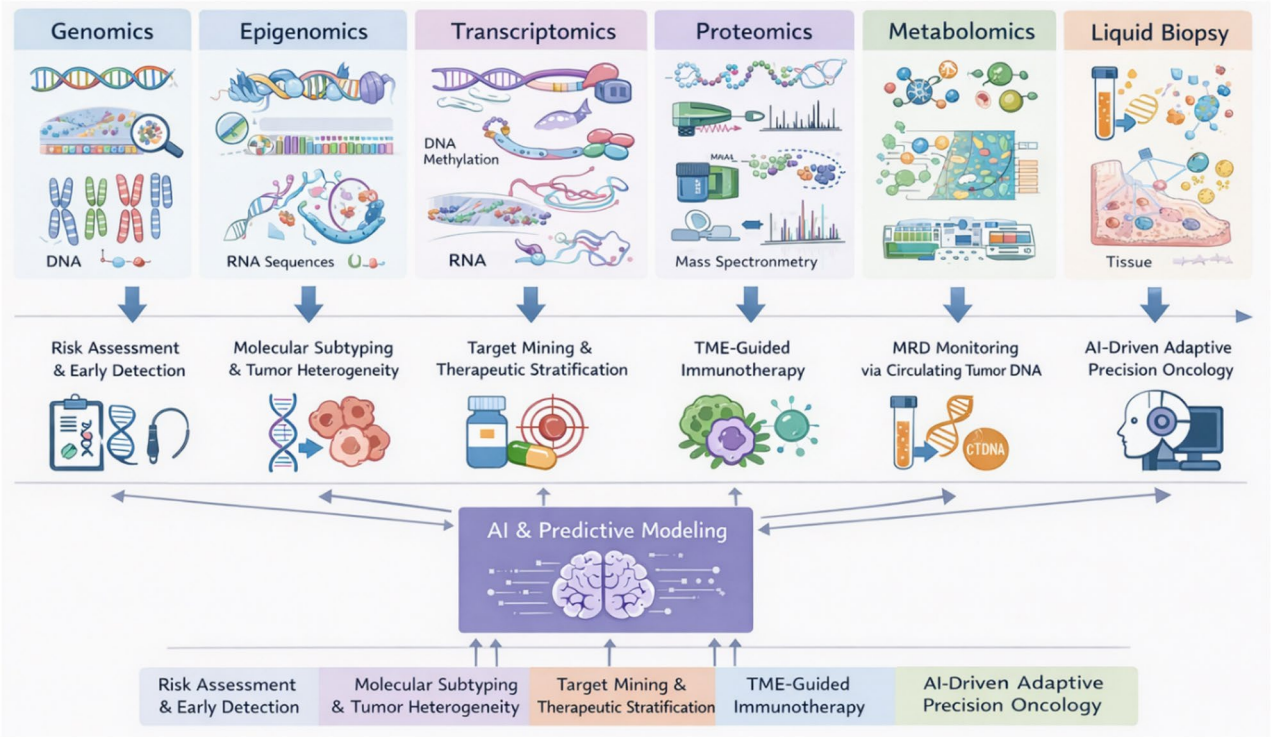

Fig. 1 Multi-omics–driven precision management across the gastrointestinal cancer continuum. By using multi-layered molecular profiles from genomics, epigenomics, transcriptomics, proteomics, metabolomics, spatial omics, and liquid biopsy, clinicians can conduct risk assessments and detect cancer at an earlier stage. Genomic sub-types can be identified, allowing for the best treatment strategy for the individual patient's unique characteristics and an opportunity to improve the understanding of how the various components of the tumor microenvironment interact with different therapeutics. Further, integrating these multi-omics methods allows the identification of actionable druggable targets, in addition to identifying potential new therapeutic strategies for fewer therapies targeting classical oncogenes and other well-characterized drivers. The creation of an MRD monitoring platform using these multi-omics methods to identify circulating tumor DNA that may lead to relapses and enable the development of an individualized adjuvant therapy plan can significantly impact overall patient outcomes. Finally, the application of Artificial Intelligence (AI) to combine multi-omics, imaging, and clinical data is at the forefront of developing predictive models for Adaptive Decision making and forecasting patient-specific disease trajectories, ultimately enhancing the precision medicine model for treating patients with GI cancers

criteria prioritized prospective clinical studies, large-scale genomic studies, and landmark translational analyses; single-case reports and underpowered studies were excluded. Non-GI cancer references are explicitly noted as methodological analogies when included.

Precise screening and ultrasound: low-cost, effective gi cancer risk assessment

Endoscopic and imaging-based approaches

The early detection of gastrointestinal cancers has an incredibly positive impact on the prognosis of those diagnosed with cancer. Conventional tools for detecting GI cancers through screening diagnostic testing tend to be much higher in cost, invasive procedure, and more difficult to access compared with standard tool [27]. Endoscopic procedures, such as colonoscopy for colorectal cancer (CRC) and upper endoscopy for gastric cancer (GC), are conventionally accepted as the "gold standards" for CRC and GC detection because of their accuracy and completeness in identifying cancer indicators [28–30]. However, these endoscopic procedures are expensive, require trained healthcare professionals to perform, and have potential risks associated with performing an invasive procedure, which discourages patients from participating in such procedures [31–33]. Because of these concerns, a significant proportion of eligible patients do not follow the recommended screening guidelines, especially for patients in resource-limited settings and asymptomatic patients, resulting in many missed opportunities for treatment. Therefore, significant research activity has focused on developing low-cost, early-stage, non-invasive screening technologies for early diagnosis of GI cancers. This has included additional forms of imaging (i.e., computer-assisted or conventional x-rays), blood-based testing of tumor-derived biomarkers; stool DNA testing; breath testing, etc. [34–39]. Furthermore, one of the primary methods for GI cancer screening at low cost is ultrasound. In the case of hepatocellular carcinoma (HCC), patients considered to be high-risk for developing HCC should undergo abdominal ultrasound every six months, as well as a blood test measuring alpha-fetoprotein (AFP) [40, 41]. This is because many early-stage

HCC tumors are likely diagnosed when curative treatment options can still be undertaken by semiannual ultrasound. Ultrasound has significant advantages, such as being relatively inexpensive and non-invasive. However, it does have moderate sensitivity and the ability for variability of imaging quality amongst operators. With regards to fatty and fibrotic liver tissue, small tumors (less than 2 cm) are likely to be missed during ultrasound examination [42]. To improve diagnostic accuracy and reliability in detecting HCC tumors, there are currently ongoing developments of new protocol techniques such as contrast ultrasound and artificial intelligence-assisted (AI) analysis of ultrasound images. For instance, AI (via convolutional neural networks) applied to ultrasound images has increased the ability to accurately identify HCC tumors and classify HCC with sensitivity and specificity rates ranging from approximately 80%–95% [43]. AI applications for ultrasound generate higher-than-average intra-operator consistency, assist operators during ultrasound imaging analysis of HCCs and provide live previews of images and HCC input while allowing medicine a more patient safety guarantee for HCC detection in real time [44]. These findings are exploratory and require prospective validation in diverse populations before routine clinical adoption. Future applications of ultrasound using additional inexpensive imaging along with AI analysis would be possible to continue to improve upon potential detection yield of early-stage or asymptomatic HCC cancers in the general population (Fig. 2).

Stool-based, liquid biopsy, and multi-omics molecular screening

Multi-omics, beyond ultrasound, are developing as new biomarkers for gastrointestinal cancers and to screen for these cancers (Fig. 3; Table 1). Many of the non-invasive tests use tumor-derived molecules detected in bodily fluids. For example, stool-based DNA tests have now been approved as an option for screening for colorectal cancer (CRC) [36]. One of the examples of a stool-based test is the multitarget stool DNA test (Cologuard) [45, 46]. Cologuard identifies a range of DNA mutations and the presence of blood in stool from colorectal cancers. The multitarget stool DNA test has about a 92% detection rate for CRC and about an 87% specificity [45, 47]. Recently, blood tests that identify the presence of ctDNA or other signatures used to develop blood tests for CRC have been developed [48]. A blood test developed by Shield looks at cancer-specific changes in methylation patterns and mutations using plasma DNA [49, 50]. Clinical trial data demonstrated that Shield has 83% sensitivity and 90% specificity for detecting advanced neoplasms associated with CRC [50, 51]. The performance of these blood tests is now comparable to that of colonoscopy for CRC detection, and studies are being conducted to assess the performance of blood tests among

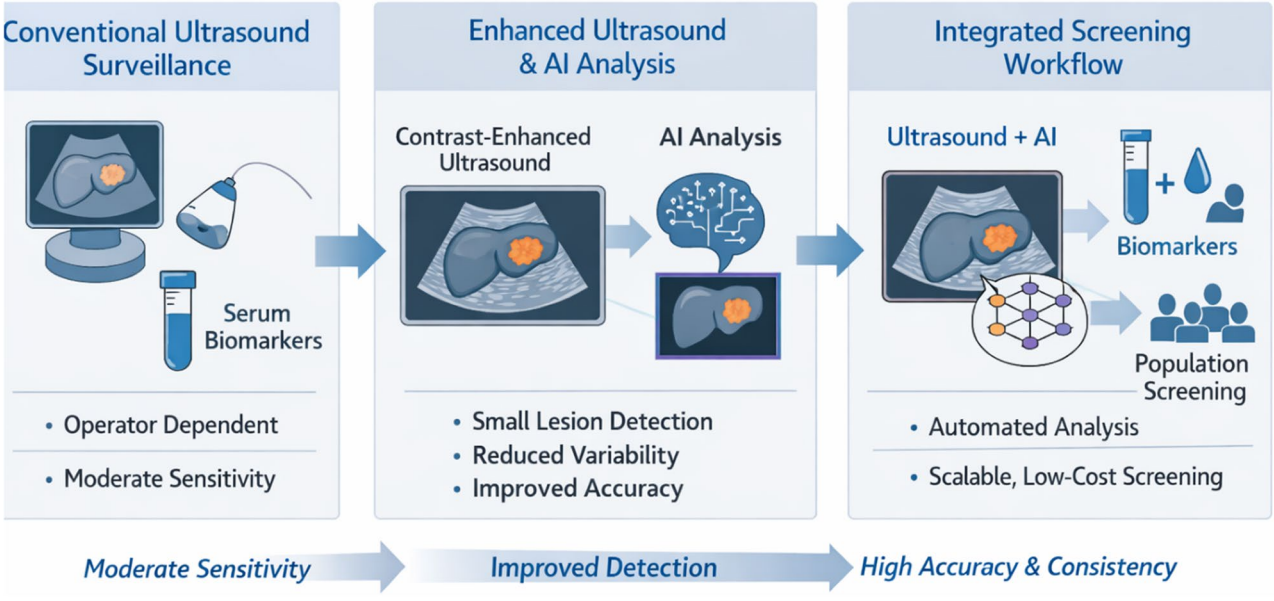

Fig. 2 Ultrasound-based and imaging-enhanced strategies for early detection of gastrointestinal cancers. Hepatocellular carcinoma (HCC) is used as an example. Standard surveillance tools for high-risk individuals remain the conventional abdominal ultrasound, generally combined with serum blood tests, but they have been shown to have moderate sensitivity and significant operator dependence. Improvements in technology including contrast-enhanced ultrasound and deep learning-assisted image assessment, provide improved lesion detection; reduced variability in lesion detection; and improved diagnostic accuracy. A combination of ultrasound, automated image assessment, and a panel of complementary serological markers provides a feasible and reliable way to perform early population-wide cancer screening programs using affordable means of imaging and assessment

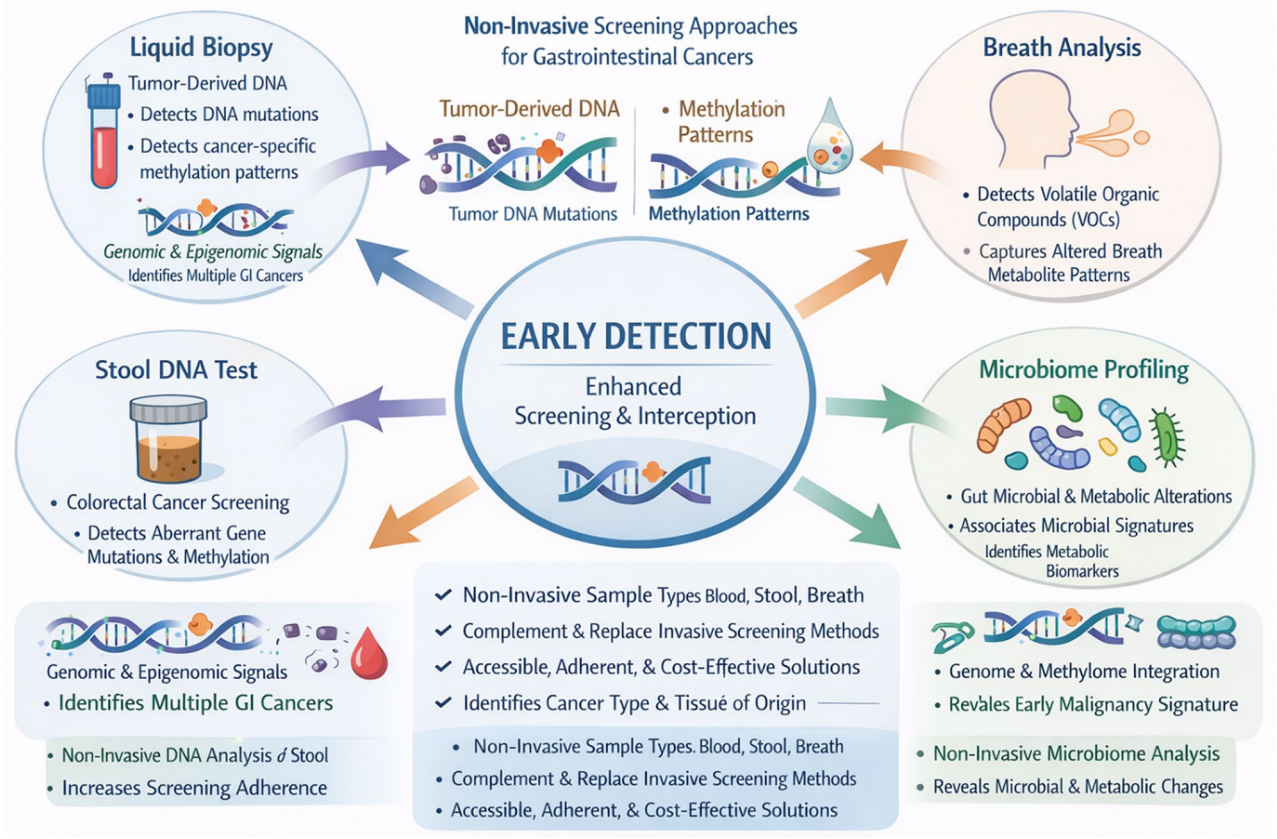

Fig. 3 Multi-omics–based non-invasive screening approaches for gastrointestinal cancers. Emerging non-invasive screening methods using advanced medical technology, such as liquid biopsy assays, stool-based DNA screening tests, breath testing for volatile organic compounds and microbiome profiling, to identify tumors at an earlier stage, will help medical professionals identify tumors quickly and efficiently, thereby eliminating the need for invasive procedures such as colonoscopies. Multi-cancer early detection (MCED) blood tests measure epigenetic and genetic markers to detect the presence of cancer in a person's blood, as well as their location within the body. These multi-omics screening methods provide new alternatives to the existing methods of detecting and diagnosing GI cancers by, increasing access to screening, increasing medical professional's adherence to screening regimens and improving opportunities for users to detect GI cancer at an earlier stage

average-risk populations for CRC. Researchers are also investigating blood test detection methods for gastric cancer using both serum markers and microbial signatures obtained through oral samples [52, 53]. In preliminary studies, one blood test, CancerSEEK (using both the ctDNA mutations and protein biomarkers), had a sensitivity of approximately 70% and a specificity greater than 99% for gastric cancer detection [54–56]; and analyzing the profiles of patients with gastric cancer showed that it could accurately differentiate between the two with about a sensitivity of 85% by looking for changes in their bacterial populations [57, 58]. These methods may help to identify patients at high risk for gastric cancer by using non-invasive tests and provide a means of mass screening for gastric cancer in patients who do not have access to endoscopic procedures at this time. For esophageal cancer, particularly with Barrett's esophagus, novel non-invasive approaches to screening esophageal cancer are also under development. A cytosponge, designed to be inserted into the esophagus and use biomarkers to identify Barrett's esophagus or dysplasia, was invented to screen esophageal cancer [59, 60]. Breath testing has been shown to be effective for identifying volatile organic compounds (VOCs) produced by tumor metabolism for early identification of esophageal cancer [61, 62]. A small pilot breath test of five VOCs indicated a sensitivity for detecting esophageal cancer of better than 94% and specificity of 96% [62]. Similarly, the cytosponge would allow a low-cost, easily deployable method of screening for esophageal cancer. Like this testing, capsule endoscopy can be used to screen esophageal cancer and small intestinal tumors [63]. The results are hypothesis-generating and require replication in larger cohorts. Currently, most of the use of capsule endoscopy is for the diagnosis of small intestinal disease, with ongoing research to provide early detection of cancer by integrating capsule endoscopy with molecular sensors or AI to improve the accuracy of diagnosis [64, 65].

One of the most innovative approaches to cancer screening is the multi-cancer early detection (MCED)

Table 1 Summary of key multi-omics-based non-invasive detection approaches in GI cancers

Test	Cancer Type	Omics Layer(s)	Key Study (Design, *n*)	Sensitivity/Specificity	Validation Status	Key Limitations
Multitarget stool DNA (Cologuard)	CRC	Genomics (mutations) + Epigenomics (methylation)	DeeP-C trial (prospective, *n* = 9,989)	92%/87%	FDA-approved; guideline-recommended	High false-positive rate; requires follow-up colonoscopy
Blood-based cfDNA (Shield)	CRC	Epigenomics (methylation) + Genomics (mutations)	SHIELD trial (prospective, *n* = 7,861)	83%/90%	FDA-approved (2024)	Lower sensitivity for early-stage lesions
CancerSEEK (ctDNA + proteins)	Multi-cancer incl. gastric	Genomics (ctDNA) + Proteomics (8 proteins)	DETECT-A (prospective, *n* = 9,911)	70% GC/69% overall	Clinical validation phase	Low sensitivity for early-stage GI; high cost
Galleri MCED	Multi-cancer (50 + types)	Epigenomics (cfDNA methylation profiling)	PATHFINDER (prospective, *n* = 6,621)	76% (all cancers)/99% specificity	Clinical validation; FDA Breakthrough	Variable sensitivity by stage/cancer type
Oral microbiome profiling	Gastric cancer	Metagenomics (16 S rRNA sequencing)	Retrospective cohort (n = ~400)	85%/~80%	Exploratory; requires prospective validation	Non-standardized collection; susceptible to diet/antibiotic effects
Cytosponge + TFF3	Barrett's esophagus/EAC	Proteomics (TFF3 biomarker) + Cell morphology	BEST3 trial (RCT, *n* = 1,429)	73% (BE)/94%	Regulatory review (UK NICE)	Endoscopic confirmation required; does not detect EAC directly

blood test. The MCED test employs several blood-based molecular techniques—primarily cell-free DNA (cfDNA) methylation profiling (an epigenomic layer), targeted somatic mutation detection from cfDNA (a genomic layer), and, in some platforms, circulating protein biomarkers (a proteomic layer)—to simultaneously identify multiple cancer types through integrated analysis of these signals [66, 67]. In a recent trial of such an MCED test, Galleri, the MCED test was able to successfully identify a cancer risk signal from a GI cancer, such as colon, stomach or liver cancer, in asymptomatic individuals and often able to accurately identify the tissue type associated with each signal, although work is still ongoing to improve the sensitivity of these tests to detect very early-stage disease [66, 68, 69]. Economic models predict that if these tests demonstrate great sensitivity for CRC (> 90%) with reasonable cost they are likely to be cost-effective compared to single cancer screening programs [70]. However, as of today, there is a lack of real-world data and regulatory approval for these tests and no MCED test has yet received regulatory approval for population screening. The future of GI cancer screening appears to be headed toward increasingly less invasive, multi-omics approaches utilizing established methods combined with novel technologies. For example, while imaging is critical to diagnose certain cancers, as well as enhancing the imaging through AI, liquid biopsies and other molecular diagnostic tests represent enormous new opportunities to diagnose cancers at earlier stages by identifying tumor signals in blood, stool and even breath [71]. Individually, these testing strategies will very likely expand the screening reach and efficacy significantly. There are significant challenges to meeting these aims, including increased sensitivity for early tumors; decreased false-positive results to prevent unnecessary diagnostic procedures; evidence of cost-effectiveness in large-scale populations; and building trust and understanding of these new tests by the public. If the above challenges can be met, then in the next decade GI cancer screening may shift dramatically from reliance upon invasive diagnostic testing to the routine use of non-invasive, multi-omics assays enabling early interception of cancers prior to their advancement.

Molecular typing: multi-dimensional revealing of disease complexity

Gastric cancer: TCGA molecular classification

GI cancer is composed of multiple subtypes, each exhibiting biological diversity and very different clinical behaviors and therapeutic responses (Table 2). The traditional classifications by the anatomical site or cellular structure alone i.e., Lauren classification for gastric cancer, TNM staging systems, are not sufficient to capture the molecular mechanisms that determine prognosis and treatment responses [72, 73]. Therefore, it is important to use a

Table 2 Major multi-omics–defined molecular subtypes of gastrointestinal cancers and their therapeutic implications

Cancer type	Molecular subtype	Primary Omics Layers	Key molecular features (multi-omics)	Prognostic features	Therapeutic implications
Gastric cancer	EBV-positive	Epigenomics (CIMP methylation); Genomics (viral integration); Transcriptomics	EBV infection; extreme DNA hypermethylation (CIMP); recurrent *PIK3CA* mutations; amplification of *PD-L1/PD-L2*, *JAK2*; immune infiltration	Generally favorable; immune-active	Immune checkpoint inhibitors (PD-1/PD-L1); potential PI3K pathway targeting
	MSI	Genomics (MMR deficiency, TMB); Epigenomics (MLH1 promoter methylation)	Mismatch repair deficiency; high TMB; *MLH1* promoter methylation; strong neoantigen load	Favorable; high immunogenicity	Immune checkpoint inhibitors
	Chromosomal instability (CIN)	Genomics (aneuploidy, RTK amplifications); Proteomics (ERBB2 overexpression)	Aneuploidy; focal RTK amplifications (*ERBB2*, *EGFR*, *MET*); TP53 mutations	Intermediate	Targeted therapy (e.g. anti-HER2); combination RTK inhibition
	Genomically stable (GS)	Genomics; Transcriptomics; Epigenomics (hypomethylation)	Diffuse-type histology; *RHOA* mutations; *CLDN18–ARHGAP* fusions; cell adhesion pathway activation	Poor	Claudin18.2-targeted therapy; novel invasion/adhesion pathway targeting
Colorectal cancer	CMS1 (MSI-Immune)	Transcriptomics; Genomics (BRAF, high TMB); Epigenomics (CIMP)	MSI; high TMB; immune infiltration; *BRAF V600E*; CIMP	Poor in metastatic setting	Immune checkpoint inhibitors; anti-angiogenic combinations
	CMS2 (Canonical)	Transcriptomics; Genomics (WNT/MYC activation, CIN)	WNT/MYC activation; chromosomal instability; epithelial phenotype	Favorable (early stage)	Anti-EGFR therapy (RAS/BRAF WT); standard chemotherapy
	CMS3 (Metabolic)	Transcriptomics; Genomics (KRAS); Metabolomics	KRAS mutations; metabolic dysregulation (glutamine, lipid metabolism)	Intermediate	Metabolic targeting strategies; chemotherapy
	CMS4 (Mesenchymal)	Transcriptomics; Genomics (TGF-β pathway); Spatial omics (mesenchymal signature)	TGF-β activation; stromal invasion; CAF-rich TME; angiogenesis	Worst prognosis	TGF-β, stroma, or CAF-targeted therapies; immunotherapy combinations
Pancreatic ductal adenocarcinoma (PDAC)	Classical (progenitor)	Transcriptomics; Genomics (GATA6 expression); Proteomics	Differentiation markers (*GATA6*, *PDX1*); KRAS signaling dependency	Relatively better	Standard chemotherapy (e.g. FOLFIRINOX); KRAS-pathway strategies
	Basal-like (squamous)	Transcriptomics; Genomics (TP53, CDK2NA loss)	Mesenchymal/squamous programs; MYC activation; loss of lineage identity	Poor	Epigenetic therapy; YAP/TAZ or stroma-targeting strategies
Esophageal cancer	Adenocarcinoma	Genomics (TP53, RTK amplifications, CIN); Epigenomics	TP53 mutation; CIN; *ERBB2* amplification; gastric-like features	Variable	Anti-HER2 therapy; chemotherapy ± immunotherapy
	Squamous cell carcinoma	Genomics (CCND1, SOX2); Epigenomics; Transcriptomics	Squamous lineage programs; TP53 alterations; immune signaling	Variable	Immunotherapy; chemoradiotherapy
Hepatocellular carcinoma	Proliferation subtype	Genomics (IDH1/2, FGFR2 fusions); Transcriptomics; Epigenomics	TP53 mutation; FGF19–FGFR4 activation; cell-cycle signaling	Poor	FGFR4 inhibitors; multi-kinase inhibitors
	CTNNB1 subtype	Genomics (KRAS, TP53)	Wnt/β-catenin activation; well-differentiated phenotype	Better	Limited ICB response; pathway-specific targeting
Cholangiocarcinoma	FGFR2 fusion-positive	Genomics (HBV/HCV integration); Epigenomics; Transcriptomics	FGFR2 rearrangements; distinct transcriptomic profile	Intermediate	FGFR inhibitors
	IDH1/2-mutant	Metabolomics; Genomics (CTNNB1, TERT); Transcriptomics	Oncometabolite production; epigenetic reprogramming	Intermediate	IDH inhibitors
Pan-GI (subset)	NTRK fusion-positive	Genomics (NTRK fusions); Transcriptomics	Rare gene fusions; strong oncogenic dependency	Favorable with therapy	TRK inhibitors (tumor-agnostic)

multi-omics approach as the principal method to stratify GI tumors into biologically meaningful subtypes by using genomic aberrations, transcriptomic profiles, epigenetic regulation, and various other molecular levels.

The use of multi-omics-based molecular taxonomies provides a rational framework to understand the heterogeneity of GI tumors and improve the ability to develop personalized treatment strategies. One of the most significant examples of this is the classification of gastric adenocarcinomas by The Cancer Genome Atlas (TCGA). Using an integrated approach to the analysis of multiple datasets, TCGA identified four distinct molecular subtypes of gastric adenocarcinoma: Epstein–Barr virus (EBV) positive, microsatellite instability (MSI), genomic stable (GS) tumors and chromosomal instability (CIN) [74, 75]. Each unique molecular subtype has its own specific molecular and clinical characteristics. Tumors that are EBV positive characterized by DNA hypermethylation (epigenomics), PIK3CA mutations (genomics), and amplified PD-L1/PD-L2 expression (transcriptomics/proteomics, making them highly sensitive to immune checkpoint blockade therapy [76, 77]. Tumors that exhibit MSI have a high number of mutations due to the lack of DNA repair mechanisms and will also respond to immunotherapy [78]. For CIN tumors, the most significant feature is widespread aneuploidy and amplification of receptor tyrosine kinases such as ERBB2, which are likely candidates for targeted therapies such as anti-HER2 therapy [79]. Conversely, GS tumors been shown to have aberrations in the cell adhesion and cytoskeleton pathways, which will require the use of alternative therapies aimed at claudin 18.2 and RhoA signaling pathways [80]. Therefore, this classification system developed by the TCGA serves as a direct link that illustrates how multi-omics-based molecular classifications inform therapeutic strategies.

Colorectal cancer: consensus molecular subtypes

Integration of transcriptomic data in CRC has resulted in the development of the consensus molecular subtype (CMS) classification system, which identifies four distinct categories of CRC tumors with unique biological and clinical characteristics [65, 81, 82]. CMS1 tumors are defined by MSI-H status and BRAF mutations (genomics), immune infiltration (transcriptomics), and are associated with poor overall survival in the metastatic setting, despite showing sensitivity to immunotherapy [83–85]. CMS2 tumors are also a major determinant of survival, which are highly epigenetically stable, differentiated toward an epithelial lineage, and characterized by activation of WNT/MYC signaling [86]. Consequently, CMS2 tumors exhibit favorable clinical outcomes, particularly at early stages of disease. CMS3 tumors, referred to as metabolic tumors, are characterized by frequent KRAS mutations and activation of metabolic pathways [85]. CMS4 tumors display strong TGF-β signaling, marked immunosuppression, and are associated with poor prognosis [87]. Therapeutic implications for CMS3 and CMS4 remain largely hypothesis-generating at this stage, with prospective subtype-directed trials ongoing. These insights have driven the development of subtype-directed clinical trials across gastrointestinal cancers.

Pancreatic, esophageal, and hepatocellular carcinoma subtypes

Historically, pancreatic ductal adenocarcinoma (PDAC) was considered a molecularly homogeneous disease. However, two major PDAC subtypes defined by lineage transcription factor expression (transcriptomics) and differentiation status (proteomics): the classical (pancreatic progenitor) subtype and the basal-like (or squamous-like) subtype [88]. Classical tumors express lineage-specific transcription factors, show better differentiation, and respond more favorably to gemcitabine-based chemotherapy (evidence primarily from retrospective translational studies; prospective validation in multi-omics-defined PDAC subtypes remains ongoing), whereas basal-like tumors exhibit mesenchymal features, aggressive behavior, and relative resistance to standard chemotherapy regimens. Multi-omics studies further demonstrated intratumoral heterogeneity, with dominant cellular phenotypes correlating with clinical outcome [89]. These findings suggest that subtype identification may guide treatment selection, with classical tumors responding better to standard regimens such as FOLFIRINOX, while basal-like tumors may require targeting of epigenetic regulation, YAP/TAZ signaling, or the tumor microenvironment [90].

Similar multi-omics studies in esophageal cancer have defined two distinct biological entities: adenocarcinoma (AC) and squamous cell carcinoma (SCC). The molecular features of AC closely resemble those of the chromosomal instability (CIN) subtype of gastric cancer, including frequent TP53 mutations and ERBB2 amplification [91]. In contrast, esophageal SCC shares molecular similarities with head and neck SCC. Multi-omics analyses of HCC and have further identified molecular subtypes associated with specific oncogenic pathways and etiologies, such as WNT/β-catenin activation and FGF19–FGFR4 signaling, thereby creating opportunities for targeted therapeutic development [92, 93].

Importantly, as molecular subtype, molecular classification is increasingly influencing clinical practice, reflecting how multi-omics findings can inform treatment selection—though the degree of clinical implementation varies considerably across cancer types and is currently supported by guideline endorsement only for specific biomarker-treatment pairs. As demonstrated by the

tissue agnostic approval of immune checkpoint inhibitors for patients with MSI-high tumors that are located throughout the gastrointestinal tract, the molecular characteristics of a tumor are now able to dictate how a patient should be treated without having to consider the tumor location. Similar to the approval of immune checkpoint inhibitors, the identification of FGFR2 fusions in patients with intrahepatic cholangiocarcinoma and rare NTRK fusions in patients with GI malignancies has also led to the development of targeted therapy for the molecularly defined subset of patients [94]. As multi-omics testing becomes more widely available and, for select approved assays such as genomic profiling panels and MSI/TMB testing, more cost-accessible in certain healthcare settings, physicians will have increasing access to molecular subtype-specific vulnerabilities that can help improve outcomes through correct treatment choices. The evolution of molecular subtype healthcare will continue to transform the field. The recent technological advancements in single-cell and spatial transcriptomics will provide insight into intratumoral heterogeneity; distinct subtypes will be found in different regions within the same tumor [95]. This heterogeneity, and the fact that clinicians are going to have to diagnose a patient based upon local histopathology and then use multi-omics to determine how to specifically treat that patient, demonstrates that the future of diagnosis is going to involve the integration of histopathology and multi-omics profiling to identify the predominant subtype as well as the minority subtypes within individual tumors. As the development of molecular subtypes continues to develop in the future, it will become standard care for GI oncologists, providing better quality of patient care and allowing for more personalized care of individual patients.

MRD monitoring: precise postoperative surveillance for recurrence

ctDNA-based MRD detection: clinical evidence in gi cancers

Even when treatment appears to have cured a patient, many patients with GI cancer will experience disease relapses due to minimal residual disease (MRD) that is not fully removed during surgical resection and potentially further mitigated through adjuvant therapy [96, 97]. MRD is generally defined as the remnant of what could be considered cancer, i.e. any remaining microscopic tumor deposits or circulating cancer cells left over from initial treatment which subsequently begin seeding into new sites of metastases. In addition to this, the traditional methods of monitoring patients for post-operative disease relapse, which are based primarily on periodic imaging and serum tumor markers, such as carcinoembryonic antigen (CEA) or carbohydrate antigen 19 – 9 (CA19-9), are only capable of detecting disease relapse after macroscopic metastases have occurred and do not have sufficiently adequate sensitivity and specificity [98]. As such, ultra-sensitive sequencing approaches classified within the domain of "ctDNA analysis," have been deployed as transformative tools for the early diagnosis of MRD and risk-stratification of these types [99].

Tumors are constantly releasing small pieces of their DNA into the bloodstream with current ultra-sensitive sequencers and digital PCR machines. It is now possible to detect very low levels (0.1%) of mutations specific to the tumor. Detection of ctDNA in blood post-surgery, even though the tumor should have been completely removed, provides strong evidence that there is still some cancer remaining in the patient. There is mounting evidence from many studies in colorectal cancer, which illustrates that the presence of ctDNA following surgical resection represents one of the single most significant predictors of subsequent disease recurrence [97, 100]. For example, when reviewing data from stage II colon cancer patients following surgery, by contrast with those individuals who tested positive for having ctDNA circulating within their bloodstream during the early post-operative period had a dramatically increased risk of having disease recur, whereas individuals that were thusly defined as testing negative for ctDNA were shown to have a very low risk of disease recurrence [101]. Further, the presence of ctDNA will often be detectable many months prior to the appearance of radiological evidence of disease recurrence. Thereby, providing clinicians with the ability to intervene therapeutically during a time frame prior to developing a significant level of cancer recurrence [101]. The early detection of MRD has substantial clinical implications. Patients who test positive for ctDNA following surgery may be considered for adjuvant chemotherapy or intensified systemic therapy despite their negative imaging results; while similarly, patients testing negative for ctDNA, particularly if they have other known, quantifiable intermediate-risk features; may be able to avoid unnecessarily and possibly unwanted adverse effects from treatment [100, 102]. This has been well established in prospective trials conducted in patients with colorectal carcinoma as well as ctDNA-guided adjuvant therapy studies, which demonstrate that there is substantial potential to reduce the number of patients treated with chemotherapy, while also providing comparable rates of survival when comparing the chemotherapy-treated cohort to the non-treated cohort of patients, represent the beginning of a new way of managing postoperative patients based on their MRD status. In addition to colorectal cancer, the application of monitoring MRD is quickly becoming commonplace for management of other GI malignancies, for example, in the case of pancreatic ductal adenocarcinoma, evidence that detection of ctDNA prior to and following surgical

resection strongly correlates to the likelihood of achieving a long-term disease-free survival following treatment, while further, the continuation of positive ctDNA after surgical resection is a strong indication for initiating early treatment in patients who run a very high risk of having recurrent disease within a years' time following initial treatment [103]. Emerging data continue to emerge and point toward the same conclusion, with regard to gastric cancer as well as esophageal cancer whereby ctDNA as well as other circulating markers may serve as a means to detect for disease recurrence and the potential development of peritoneal and potentially micro-metastatic disease long before patients will show any overt clinical evidence of disease recurrence, thereby justifying the need for continued research efforts [104, 105]. As an example, tumor-specific, methylated DNA signatures have been associated with the identification of patients at risk for eventual recurrent peritoneal findings following subtotal gastrectomy procedures performed on patients with stomach cancer [106, 107].

Tumor-informed versus tumor-agnostic MRD assays

The design of an assay will greatly affect the performance of the assay in detecting MRD. The first step in tumor-informed assays is to characterize the mutational landscape of a given patient's tumor (e.g., by next generation sequencing) and then develop personalized mutation panels to track in the patient's plasma (the maximum sensitivity of which is achieved for that patient) [99, 103]. On the other hand, tumor-agnostic assays are dependent on mature mutation and methylation panels that were created for the same mutations across many or all patients regardless of tumor type [98, 108, 109]. They are particularly valuable when there is no tumor tissue available for use in the assay. Both assay designs have been validated as having robust prognostic capabilities and are evolving toward increasingly incorporating multiple analytes. Multi-omics liquid biopsy platforms combining ctDNA mutations (genomics), DNA methylation (epigenomics), circulating tumor cells, and protein biomarkers (proteomics) outperform assays that utilize only one analyte by significantly increasing their sensitivity and thus capturing cases that may have been missed using a single analyte [98, 110–112]. In addition to providing continuous information on the presence or absence of MRD, monitoring MRD through the sequencing of plasma samples over time enables the analysis of clonal evolution as well as the emergence of alterations conferring resistance to therapy prior to the development of clinical or radiographic progression, allowing the potential for proactive changes and modification to therapy [113, 114]. In CRC, studies have demonstrated that the clearance of ctDNA during adjuvant chemotherapy is associated with the patient's favorable outcome; persisting ctDNA indicates that the patient has a high likelihood of having a poor therapeutic response and may benefit from alternative or experimental treatments [100, 115]. With respect to rectal cancer, liquid biopsy strategies are being assessed to determine the molecular complete response to chemoradiation therapy and thus ultimately provide patients with an opportunity to be cured without surgery in certain circumstances [98]. The continued evolution of multi-omics technologies will continue to expand the potential range of MRD biomarker options beyond ctDNA, as there are several different types of circulating microRNAs and methylation panels that are being studied for their utility in esophageal and gastric cancers [104, 116–119]. With hepatocellular carcinoma, multiple recent studies have demonstrated that composite blood-based scores and methylated DNA panels may be predictive of the recurrence of hepatocellular carcinoma several months earlier than conventional imaging, which suggests that it may be possible to complement or even surpass conventional MRD diagnostic biomarkers using modern molecular surveillance technologies [120].

Barriers to clinical implementation of MRD monitoring

MRD monitoring has a lot of potential, but there are still some obstacles that need to be overcome before it becomes widely used. For example, not all patients will have easy-to-monitor tumor alterations. Currently, broad sequencing makes it possible to identify these changes in most CRC cases, but there are still many tumors that do not produce detectable alterations. Also, false positives from clonal hematopoiesis should be eliminated with great care because they could lead to over-treating patients, and it will be necessary to standardize when blood samples are collected for testing against the timing of surgery and adjuvant therapy [121]. Fortunately, growing experience with designing assays and bioinformatic correction is helping alleviate some of these problems. However, the emerging technology of multi-omics-based MRD monitoring centered around ctDNA liquid biopsy is an exciting breakthrough in the post-operative care of GI cancers through its ability to provide individualized risk assessments [110, 122]. This process allows for escalation of therapy for patients with evidence of residual disease at the molecular level and de-escalation of therapy for those who appear to be cured. As a proactive, molecularly-directed approach to the treatment of colorectal cancer has been validated and is now being widely expanded to other GI malignancies, we anticipate that with continued refinement of technology and prospective clinical validation, MRD-directed care will join the increasing number of elements of precision oncology as part of the standard of care in the adjuvant setting.

Immunotherapy: tumor microenvironment (TME) signatures predicting immune checkpoint blockade response and resistance

TME composition and immune checkpoint predictive biomarkers

Immunotherapy, which primarily involves immune checkpoint blockade (ICB) that also targets PD-1/PD-L1 or CTLA-4, has changed the way cancer is treated [123–125]. However, the rates of success in using it to treat GI cancers vary greatly. Although some subsets of GI cancers, including microsatellite instability-high (MSI-H) colorectal and gastric cancers and select HCC patients, have shown sustained responses, most GI cancers have shown limited benefit [26, 126]. One of the major challenges to utilizing ICB is identifying the patients who would benefit the most from it, along with developing ways to bypass resistance to treatment [127, 128]. Multi-omics analyses of the tumor microenvironment (TME) have been one way to meet this challenge, as it captures the diverse interactions taking place between tumor cells, immune cell types found within the tumor tissue, stroma, signaling molecules, and microbiome [129–132]. On a genomic level, MSI-H status and high total tumor mutation burden (TMB) are the two most predictive biomarkers of ICB response. These tumor types contain many neoantigens and typically contain many cytotoxic T cells (CTL) infiltrating the tumor. Thus, PD-1 inhibitors have shown a much higher rate of response in patients with these tumors as well [133–135]. Most GI cancers are classified as microsatellite stable (MSS) and have intermediate TMB levels, indicating that using genomic analysis alone will not provide sufficient information to accurately predict responses [136, 137]. Investigators have therefore turned to transcriptomic profiling of the TME for additional insight into this area. Across multiple studies that evaluated the use of ICB in the treatment of esophageal, gastric, and colorectal cancers, the tumors that exhibited high levels of CD8 + T-cell markers and high levels of interferons responsive genes and cytotoxic activity were determined to have a much higher rate of ICB response compared to tumors that did not, and tumors that displayed transcriptional signatures consistent with the lack of immune infiltration or immunosuppression had low ICB response rates [138–143]. The use of computational deconvolution methods to evaluate bulk RNA-Seq data was used to identify that both an abundance of effector T cells and M1-like macrophages positively correlates with ICB benefit and that a high percentage of cancer-associated fibroblasts (CAFs), M2 macrophages, and TGFβ signaling negatively correlate to ICB benefit [144–148]. Proteomics provided an additional layer of data by providing information on specific immune checkpoint and activation proteins. Using multiplex immunohistochemistry and mass cytometry, it has been shown that in addition to an abundance of PD-L1, the cellular distribution of PD-L1 is important [149, 150]. Tumors with PD-L1 expressed on both the cancer cells and the myeloid cell types, as well as on PD-1 expressing exhausted T cells, are more likely to respond to immune checkpoint blockade, consistent with a "reversible exhaustion" state [151, 152]. Conversely, tumors containing no immune infiltrating cells ("cold tumors") have an extremely low rate of ICB response. Taken together, composite or integrated biomarkers that measure TMB, PD-L1 levels, and immune gene expression signatures are more predictive of ICB benefit than any single biomarker alone. In patients with HCC, combining spatial heterogeneity of PD-L1 with genomic and transcriptomic features improved the predictive ability for ICB responses [153].

Mechanisms of acquired immune resistance

Acquired resistance to therapy has been further elucidated. Longitudinal clinical sampling and single cell sequencing have demonstrated that the tumors of patients who do not respond to therapy, or who relapse, frequently display a redistribution of their TME, which includes significant exhaustion of T-cells; increased expression of alternate immune checkpoint markers (e.g., TIM-3, LAG-3) [154, 155]; and metabolic reprograming, which suppresses their immune function [156]. For example, gastric cancer tumors that are resistant to treatment contain significantly more terminally exhausted $CD8^+$ T-cells and produce increased lactate due to elevated levels of LDHA, which has created an acidic, immune suppressive, microenvironment [157, 158]. As previously discussed, these findings support the rationale for combining (i.e., using together) immune checkpoint inhibitors with agents that modulate the metabolic pathway, or using next generation immune targets and modulation agents. For example, the immunologically restrictive nature of PDAC offers an example of how multi-omics analysis can inform the development of innovative therapies. When KRAS/TP53 mutant PDAC is analyzed together with the patient's immune response, researchers find that the PDAC's microenvironment offers an explanation for the design of new combination trials with agents that combine immune checkpoint blockade with agents that target myeloid cells or stromal components, with the goal of converting "cold" PDAC tumors into "permissive" tumors that can allow for the development of a patient's immune response [159]. Another important consideration in the future design of ICB therapy is the human gut microbiome [160]. The metagenomic and metabolomic assessment of the gut microbiome demonstrates that the microbial composition and metabolic output of the gut microbiome can influence both systemic and intratumoral immunity [161–163]. Microbiomics and metabolomics of MSI-H GI cancer non-responders

(to PD-1 blockade) demonstrate distinct microbial and metabolite signatures, including reduced levels of short-chain fatty acid producing microbes and an increased presence, as a class, of microbes producing immunosuppressive metabolites [164–167]. Experimental studies of the human gut microbiome with fecal microbiota transplantation have provided some evidence of causality between the observed differences in the microbiome, the metabolic output of the microbiome, and the resulting patient's immune response to PD-1 blockade [168, 169].

Spatial multi-omics and the tumor-immune interface

The emerging field of spatial multi-omics evaluation will provide further insight into the influence of cellular organization (not solely the quantity of immune cells) on clinical outcomes. For example, spatial transcriptomic and proteomic studies of CRC show that in many CRC, CD8 T-cells are found predominantly at the invasive margin of the CRC, and they are unable to penetrate deep into the tumor nests, which indicate an immune-excluded phenotype [170]. Quantitative spatial metrics positively correlate with response to PD-1 blockade [171]. Similar patterns have been described in gastric cancer patients with effective responses to PD-1 blockade, as these patients demonstrate an intimate mixing of their immune cells with the tumor islets compared to the complex collagen barriers and stromal separation from the tumor associated with resistance [172, 173]. As advancements in genomics, transcriptomics, proteomics, and metabolomics continue their integration into the clinic through the creation of new diagnostics, therapeutic agents, and prognostic indicators for patients with GI cancers, there is now greater awareness that these have potential benefits beyond what traditional single-omics tests could offer. Recently developed assays that combine multiple types of omics data into a single "ImmunoScore" are examples of how researchers have begun using AI-based prediction algorithms based on multi-dimensional data sets [174–176]. AI allows researchers to analyze vast amounts of data and determine the relationships between them, leading to more accurate predictions about how patients will respond to immunotherapy due to these characteristics in their patient's TME. Establishing TME-related biomarkers is being aided by using sophisticated and validated algorithms that can identify patient subpopulations using their TME-specific characteristics [176]. For example, TME-guided approaches and combinations (Atezolizumab + Bevacizumab) exemplify how targeting angiogenesis-mediated immunosuppression could create a synergistic relationship with ICB therapy [177, 178]. Ongoing clinical trials studying advanced colorectal and advanced gastric cancer, as well as many others targeting stromal cells, TGF-Beta signaling pathways, and alternative immune checkpoint pathways, will help define future treatment algorithms for these patients based upon TME and omics characteristics [179, 180]. In summary, multi-omics profiling of TME creates a more accurate, in-depth view of how to create predictive models for patients living with GI cancer and how to effectively treat them using immunotherapies. Creating predictive models requires knowledge of a patient's multiple omics characteristics, including genomic instability, gene expression, the presence of immune checkpoint alterations, as well as how the patient's tumor architecture and the microbial environment affect the efficacy of immunotherapy in these patients. Ultimately, the ability to perform Immunophenotyping, the overall geographic location, and molecular characteristics of a patient's TME will lead to more precise patient selection for immunotherapy. To implement personalized, appropriately administered immunotherapies for patients with GI cancers, there will need to be a clear understanding of the TME and multi-omics profile of each GI cancer patient (Fig. 4).

Target mining: deep exploration of potential drug targets in GI cancer

Druggable protein targets: receptor overexpression and pathway co-activation

The integration of multi-omics has become a powerful engine for therapeutic target discovery in GI oncology, extending far beyond traditional mutation-centric approaches. By jointly interrogating genomic, transcriptomic, proteomic, metabolomic, epigenomic, and microenvironmental data, multi-omics enables the identification of genes, pathways, or cellular interactions that are essential for tumor survival and progression, and thereby expands the landscape of actionable targets.

A major contribution of multi-omics has been the systematic identification of druggable protein overexpression linked to underlying genomic or transcriptomic alterations. In gastric cancer, integrative analyses demonstrated that approximately 20% of tumors harbor ERBB2 (HER2) amplification with concordant protein overexpression, directly supporting the clinical success of trastuzumab in this molecularly defined subgroup [181, 182]. Beyond single-gene targeting, multi-omics revealed that HER2-amplified tumors frequently exhibit co-activation of the PI3K/AKT pathway through concurrent mutations or copy number alterations, providing a strong rationale for combination strategies targeting both HER2 and downstream signaling [183–185]. Similarly, in CRC, proteomic and phospho-proteomic profiling uncovered substantial heterogeneity in downstream signaling among KRAS-mutant tumors, with subsets preferentially activating MAPK, PI3K, or RAL pathways [186]. These differences suggest that KRAS-mutant CRC is not biologically uniform and may be vulnerable to pathway-specific combination therapies, such as PI3K/

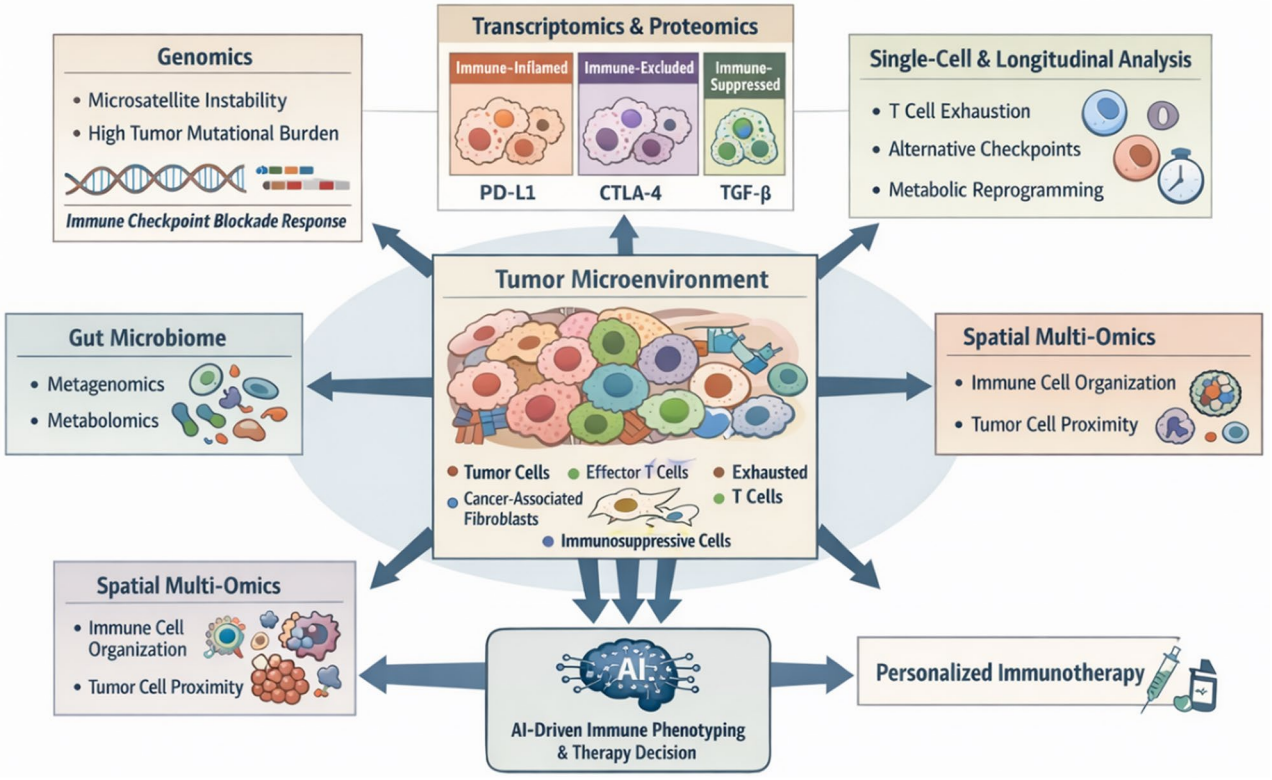

Fig. 4 Multi-omics profiling of the tumor microenvironment enables precision immunotherapy in gastrointestinal cancers. Graphical representation of the integrated multi-omics-Tumor Microenvironment (TME) to predict the immunotherapeutic response and resistance of GI malignancies. High tumor mutational burden (TMB), and microsatellite instability (MSI) are examples of genomic determinants that can predict the effectiveness of immune checkpoint inhibitors (ICB). Transcriptomics and proteomic analysis can be utilized to determine the characteristics of either immune inflamed or immunosuppressive TMEs. These TME types may exhibit characteristics such as the presence of activated effector T cells or cancer-associated fibroblasts (CAFs) as well as expression of the checkpoint proteins that contribute to the efficacy of ICB. Single cells and longitudinal analysis reveal mechanisms of primary and acquired resistance to ICB therapy including profound exhaustion of T cells, alternative mechanisms of checkpoint activation, and metabolic reprogramming. By utilizing spatial multi-omics studies to determine the organization and spatial location of immune cells about the tumor cells, researchers can observe how this organization may influence the efficacy of therapy. Metagenomic and metabolomic profiling of the gut microbiome may add an additional layer of regulation to the modulation of systemic and TME immune responses. Combining molecular, spatial, and microbial analyses with the aid of artificial intelligence is providing a new approach for the composite immune phenotyping and design of rational combination strategies for personalization of immunotherapy in GI cancer patients

AKT or IGF1R/mTOR inhibition in selected contexts. Multi-omics has also highlighted cell-surface proteins as highly attractive therapeutic targets. Transcriptomic profiling identified claudin 18.2 (CLDN18.2) as selectively overexpressed in a subset of gastric cancers, particularly the diffuse subtype, leading to the development of CLDN18.2-targeted monoclonal antibodies and cellular therapies that have shown promising clinical activity [187, 188]. In CRC, integrative analyses revealed Guanylyl Cyclase C (GUCY2C) as broadly expressed in intestinal tumor cells but largely absent from extraintestinal tissues, motivating the development of vaccines and antibody–drug conjugates targeting GUCY2C [189, 190].

Gene fusions, rare drivers, and actionable alterations

Multi-omics contributed another transformation in the identification of actionable gene fusions and rare driver alterations. For example, using comprehensive sequencing of whole exome sequencing (WES), transcriptome sequencing (RNA-seq), and Genomics of Drug Sensitivity in Cancer (GDSC), NTRK gene fusions were identified in small but significant subpopulations of colon, pancreatic and biliary cancers, with dramatic durable responses seen from the use of TRK inhibitors in each of the above types of cancers [191, 192]. The same sequencing technology also identified FGFR2 fusions in intrahepatic cholangiocarcinoma (ICC) that led to the approval of FGFR inhibitors for patients with ICC [193, 194]. In addition to using genomics to identify potential targeted therapies for CRC based on ALK and ROS1 gene fusions and BRAF V600E mutations, actionable amplifications such as FGF19 and the pathways that activate them through FGFR4 and Wnt/beta-catenin signaling pathways in HCC have led to the establishment of multi-target clinical trials of each of these drugs in these molecularly defined patient populations [195, 196]. In addition,

the information gained from these studies, which go beyond the scope of genomic identifications, provides another avenue for developing therapeutic agents based on cancer metabolism, and the application of metabolomics or through aggregating metabolic data with the use of integrated metabolic and onco-metabolic analyses. The relationship between altered APC/Wnt signaling in CRC and increased glutamine utilization and glutamine synthetase levels implicate that the Wnt pathway could be an avenue for therapeutic development [197]. Findings from CRC single-cell assays of tumor cells have demonstrated that the glutaminase (GLS1) enzyme is selectively overexpressed in cancer stem cells [198, 199]. These findings have allowed for the identification of promising therapeutic avenues to selectively target cancer stem cells. Additionally, multi-omics studies of PDAC have demonstrated a metabolic separation of PDAC tumors that utilize oxidative phosphorylation versus glycolysis [200, 201]. This has given the field greater insight into the varied metabolic characteristics of PDAC tumors. Findings from many of these studies have pointed to a potential use of metabolic therapy for treating patients with PDAC. It has been shown that lipid metabolism has a role in the development of cancers through the overexpression of CPT1A, and functional analyses of gastric cancer cell lines support the use of CPT1A inhibitors in gastric cancer treatments [202].

AI-driven network analysis and systems-level target identification

Currently, the use of the interconnectedness of proteins and AI-based multi-omics approaches has provided access to additional cellular biology-based therapeutic target opportunities that have not previously been considered due to how they have previously been identified through non-intuitive methods [203]. Correlating the analyses of RNA expression patterns, mortality data, and the use of protein-protein interaction networks has provided the data necessary to identify the immune-modulating agents that could be developed using cancer-specific cytokines. In gastric cancer patients, B7-like protein 6 (NCR3LG1) has been suggested as a potential immune modulator, and the data resulting from these analyses has allowed for the development of antibody-drug conjugates (ADCs) and CAR-T therapies for patients with gastric cancers [204]. These new avenues of research and insights into the linkage of the various types of omics studies have made it possible to identify very important targets that may have otherwise been missed by using traditional single-omics data analyses and frequency-based analyses. Epigenomic profiling has added complexity to tumor biology by identifying the master regulators of the oncogenic transcription programs [205]. Novel experimental techniques, including ATAC-seq and ChIP-seq, have identified specific transcription factors and chromatin regulatory proteins associated with super-enhancers, which are critical in a number of GI cancer subtypes [206, 207]. The work to identify these factors has led the field to begin developing testing for epigenetic therapies in subsets of patients with GI cancers that can be selected for such treatments using the data obtained from multi-omics profiling. In addition, the identification of other potential therapeutic targets in tumors and tumor microenvironments through the identification of the role of tumor stromal and immune support networks has supplied additional therapeutic target opportunities for clinical trials to evaluate. One example is in PDAC, where ongoing analyses of the integrated examination of the tumor stroma and immune support networks have identified the CCL2-CCR2 and CXCL12-CXCR4 axes as critical driver mechanisms for supporting the recruitment and establishment of immunosuppressive macrophages and fibroblasts in PDAC [208, 209]. Consequently, this information has allowed for the initiation of clinical testing of inhibitors targeting interactions within this tumor-stroma environment. Finally, in CRC, the profile of the metastatic niche has identified the angiogenic mediators VEGF and ANGPT2, thereby providing a rationale for the combination of these two approaches to anti-angiogenic therapy of patients with CRC [210, 211].

The integration of artificial intelligence and computational informatics represents an increasingly important dimension of multi-omics-driven target mining in GI oncology. Machine learning algorithms—including graph neural networks applied to protein–protein interaction networks, and deep learning models trained on multi-omic datasets—can identify non-obvious synthetic lethal interactions and predict candidate drug targets with higher throughput than conventional experimental approaches. For example, network-based integration of transcriptomic, proteomic, and phosphoproteomic data in CRC has revealed hub kinases not identified by mutational frequency analysis alone, several of which are now under evaluation as therapeutic targets [212]. Complementarily, multi-omics data has enabled systematic drug repurposing strategies in GI cancers: by mapping existing approved drugs' mechanisms of action against multi-omics-defined tumor dependencies, investigators have identified repositioning candidates such as metformin (targeting metabolic vulnerabilities in KRAS-mutant pancreatic cancer) [213], statins (exploiting mevalonate pathway addiction in select GC subtypes) [214], and anthelmintics (leveraging Wnt pathway suppression in CRC) [215]. These computational approaches reduce time-to-candidate and capitalize on known safety profiles of repurposed agents, representing a cost-effective complement to de novo drug discovery. Graph-based deep learning models applied to protein-protein interaction

networks have predicted candidate synthetic lethal interactions in CRC and PDAC that were subsequently explored in preclinical cell-line and organoid models [216]. Beyond de novo target identification, multi-omics integration with drug sensitivity databases (e.g., GDSC, CCLE, PRISM) supports systematic drug repurposing strategies, where existing compounds approved for non-oncologic indications or other cancer types are identified as candidate agents for GI malignancies [217–219]. For example, integrated transcriptomic and metabolomic profiling in cholangiocarcinoma has suggested potential repositioning of metabolic enzyme inhibitors, illustrating how cross-modal data integration may accelerate repurposing candidate identification and reduce de novo drug development timelines [220]. It should be noted, however, that most AI-driven target predictions remain at the preclinical validation stage, and prospective clinical evaluation in GI cancer patient cohorts is required before actionability can be established.

Functional genomics, synthetic lethality, and drug repurposing

Functional genomics defines "functional" within the context of how to prioritize targets for drug development. Using CRISPR-based gene editing tools and RNA interference (RNAi) technology in combination with other data sets, functional genomics define the importance of identifying synthetic lethality. For example, studies demonstrated a synthetic lethal interaction between KRAS mutation in CRC cells and RAC1 [221]. Conversely, APC mutation CRC demonstrated an exogenous reliance on certain metabolic enzymatic activities, demonstrating the importance of additional biological context when conducting functional screens [221]. Multi-omics data sets can facilitate repurposing existing drugs and developing therapies with rational combinations of existing drugs. In adding multi-omics with molecular profiling of Epstein-Barr Virus-positive gastric cancer, JAK2 and PD-L1 were overexpressed, suggesting that inhibition of these proteins may be beneficial [222]. Similarly, in CRC, multi-omics-based definitions of metabolic subtypes have demonstrated that these metabolic subtypes are sensitive to many non-oncologic drugs currently used to target Gln metabolism [223, 224]. Additionally, multi-omics based integrated data from therapy resistant tumors shows the presence of MAPK reactivation following anti-EGFR therapy and activation of PI3K in JAK-resistant gastric cancer and can inform combination strategies currently being clinically evaluated [225]. Thus, multi-omics approaches provide a framework to identify therapeutic targets for the treatment of GI cancers and define the vulnerabilities across the intrinsic pathways of cancer cells, metabolic pathways, epigenetics, and the tumor microenvironment. Target prioritization that is based on functional relevance or clinical relationships rather than mutation frequency alone will expand the possible targets to develop therapies to include those beyond classic oncogenes. The expected future development of drug discovery technologies will continue to use these insights to drive a more rational drug discovery approach, and to improve the outcomes of patients with many of the most challenging GI malignancies.

Limitations and future perspectives

Multi-omics technologies have significantly enhanced our understanding of gastrointestinal cancer biology. However, despite the advances in our understanding, there are still important barriers that limit the application of these technologies and therefore limit their clinical use. The primary barriers to clinical use exist primarily due to sample requirements, analytical complexity, costs of implementation, and resource availability [226, 227]. Together, these four elements restrict the scalability and routine clinical application of multi-omics technologies. Sample collection and quality control are the most significant barriers [228]. Most omics platforms need high-quality biospecimens, specifically, genomics and transcriptomics require high-quality, fresh frozen tissue or high-integrity nucleic acids. In contrast, most of the clinical practice uses either formalin-fixed paraffin embedded samples or small biopsy specimens. These impediments to clinical implementation are most pronounced in gastrointestinal cancers, particularly pancreatic and cholangiocarcinoma, because, when compared to other cancers, these cancers often require limited amounts of tissue for evaluation. There are emerging techniques, such as single-cell and spatial omics that place even higher demands on sample acquisition as these approaches also require rapid processing of viable fresh-tissue specimens. Furthermore, proteomic and metabolomic analyses are sensitive to pre-analytical variables such as temperature, ischemia time, etc. As a result, without standardization and adherence to collection and storage protocols by various institutions, there is an increased prevalence of batch effects and problems with reproducibility. These same factors contribute to the increased difficulty of interpreting results from bulk, heterogeneous samples, which typically contain mixtures of varying amounts of malignant cells, stromal cells, and immune cells that can dilute out specific cancer signals. While microdissection, deconvolution by computational means, and single cell methods mitigate some of these difficulties, they add financial and technical complexities. The second significant barrier is the complexity of data analysis and interpretation; multi-omics datasets are also, by definition, high-dimensional, heterogeneous, and noisy and each platform contains distinct technical biases [229, 230]. To integrate discrete genomic alterations with

continuous transcriptomic, proteomic, and metabolomic datasets, sophisticated modelling, either through statistical or machine learning approaches will need to be followed. The potential for batch effects, false correlations, and overfitting should be closely monitored and addressed in the context of the limitations associated with small sample sizes. Barriers extending beyond the data are the lack of a comprehensive knowledge base for the biological interpretation of the multi-omics data; a significant proportion of the variants detected using multi-omics technologies are still not well defined and continue to remain functionally unannotated and poor characterization of genes, metabolites, and proteins that aren't well-characterized. Lastly, as a result of these biological limitations, the identification of truly actionable signals and the risk of premature clinical translation is increased. The additional costs and resource constraints for large-scale, comprehensive multi-omics profiling limits the feasibility of conducting multi-omics studies. However, while the cost of sequencing has been declining, current reimbursement policies support a very limited number of genomics tests with established clinical utility, and the majority of multi-omics technologies remain tools for research purpose only, and thus, lack a clinical application. Furthermore, regulatory requirements for the validation and quality control of new assays impose more obstacles to clinical utilization. However, the future of multi-omics for gastrointestinal cancer is highly promising primarily because new developments in the fields of standardizing, automating, and using AI to analyze multi-omics data will continue to remove the technical and analytical barriers that limit the realization of these technologies for clinical use. AI-based integration of multi-omics datasets has enabled a more accurate prediction of clinical outcomes, while the development of generative models for lost data has provided a new framework for augmenting multi-omics datasets and creating models of disease progression [231]. Emerging applications have also been identified, including the utilization of real-time molecular profiles, the integration of real-time molecular profiles into the design of adaptive clinical trial models, and using single-cell and spatial data for more accurate patient stratification as the cost of conducting these analyses decreases. Multi-omics remain restricted to research settings due to current constraints; therefore, there remains significant opportunity for growth within these domains as technological advancements and AI are driving increased feasibility and clinical applicability. Continued validation and decreased costs will allow multi-omics to flourish as a core component of predictive/adaptive/personalized precision oncology for GI cancers.

A particularly critical practical barrier—relevant across both research and clinical translation contexts—concerns the requirement for paired or matched multi-omics data from the same clinical specimen across different molecular modalities. In routine clinical practice, most tumor samples yield only partial omics coverage: surgical specimens may enable genomic and transcriptomic profiling, but proteomic and metabolomic analyses often require additional fresh tissue that is unavailable from standard biopsy specimens. This modality mismatch means that truly integrated multi-omics datasets are predominantly derived from well-resourced research cohorts, limiting the representativeness of multi-omics-derived findings and their generalizability to broader patient populations. Strategies to address this limitation include computational imputation of missing omics layers, modality-specific biomarker prioritization, and multi-institutional data-sharing consortia—each of which represents an active area of methodological development with implications for future clinical utility.

Conclusion

The multi-omics strategy is creating new ways to approach GI cancers by simultaneously bringing together biological data from several systems involved in creating cancer cells. By using this information, doctors can diagnose GI cancers earlier, better define a patient's individual cancer type, monitor for the best treatment method, determine a patient's chances of responding to specific immunotherapies, and find other possible treatment methods for that specific type of cancer. While there are still limitations due to differences in the quality of samples, issues integrating different types of data, and the cost of conducting multi-omics research, advancements in AI and the push for "standardization" of approaches to multi-omics research will continue to allow us to make advances toward using multi-omics as part of the clinical management of GI cancers. Multi-omics approaches hold substantial promise for improving diagnostic accuracy and enabling more individualized treatment planning for patients with GI cancers, though widespread clinical implementation will depend on continued prospective validation, cost reduction, and structured integration into standard care pathways.

Authors' contributions

J.P.S., Q.L., Y.Q. and X.W. designed and wrote the manuscript. X.W., J.F.Z and J.L.C. did literature searching and wrote the manuscript. J.F.Z., J.L.C. and Q.Z. did literature searching in drug targets section. J.L.Z. and X.W. authored the chapter on radiomics of ultrasound. J.P.S., Q.L., Y.Q. and R.H.L. prepared the table and Figures. All authors listed have made a substantial contribution to the work. All authors have read and approved the article.

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Data availability
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Declarations

Ethics approval and consent to participate
Not applicable.

Competing interests
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