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Tumor markers in colorectal cancer, gastric cancer and gastrointestinal stromal cancers: European group on tumor markers 2014 guidelines update

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Mini Review

Biomarkers currently play an important role in the detection and management of patients with several different types of gastrointestinal cancer, especially colorectal, gastric, gastro-oesophageal junction (GOJ) adenocarcinomas and gastrointestinal stromal tumors (GISTs). The aim of this article is to provide updated and evidence-based guidelines for the use of biomarkers in the different gastrointestinal malignancies. Recommended biomarkers for colorectal cancer include an immunochemical-based fecal occult blood test in screening asymptomatic subjects ≥50 years of age for neoplasia, serial CEA levels in postoperative surveillance of stage II and III patients who may be candidates for surgical resection or systemic therapy in the event of distant metastasis occurring, *K-RAS* mutation status for identifying patients with advanced disease likely to benefit from anti-EGFR therapeutic antibodies and microsatellite instability testing as a first-line screen for subjects with Lynch syndrome. In advanced gastric or GOJ cancers, measurement of HER2 is recommended in selecting patients for treatment with trastuzumab. For patients with suspected GIST, determination of KIT protein should be used as a diagnostic aid, while *KIT* mutational analysis may be used for treatment planning in patients with diagnosed GISTs.

In recent years, biomarkers have begun to play an increasingly important role in the detection and management of patients with gastrointestinal malignancies. This applies especially for colorectal cancer (CRC), gastrointestinal stromal tumors (GISTs), gastric and gastro-oesophageal junction (GOJ) cancers. In 2003 and 2007, the European Group on Tumor Markers (EGTM) published guidelines on the use of biomarkers in CRC.[1,2] The aim of this article is to update those guidelines as well as to provide new guidelines on the use of biomarkers in gastric and GOJ cancers and GISTs. The primary focus is on screening, prognostic, therapy predictive and monitoring biomarkers. Genetic susceptibility markers are not discussed in this article.

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The main targets of these guidelines include surgeons, physicians and nurses involved in the management of patients with gastrointestinal malignancies as well as laboratory professionals involved in the measurement of tumor biomarkers. The guidelines however, may also be useful for payers for healthcare, relevant policy makers, researchers and companies involved in the manufacture of tumor marker assays.

To prepare these guidelines, the literature relevant to the use of tumor markers in gastrointestinal cancers was reviewed. Particular attention was paid to systematic reviews, pooled or meta-analyses and to relevant guidelines issued by Expert Panels. For each guideline, we indicate the level of evidence (LOE)[3,4] and strength of recommendation (SOR)[5] for its clinical use (Table 1). In addition to reviewing clinical utility, we discuss cost-effectiveness of the recommended biomarkers and make suggestions for further research.

Colorectal Cancer

Use of fecal occult blood testing in screening for early colorectal cancer

Two types of FOBT are available, *i.e.,* the older guaiac test (gFOBT) which detects the pseudo peroxidase activity of

Table 1. Biomarkers recommended by EGTM for use in gastrointestinal malignancies

Marker	Cancer	Use	LOE	SOR
FIT-based FOBT	CRC	Screening	I	A
CEA	CRC	Prognosis, especially in stage II patients	III	A
CEA	CRC	Postoperative surveillance	I	A
CEA	CRC	Monitoring therapy in advanced disease	III	A
K-RAS[1]	CRC	Predicting response/resistance to anti-EGFR antibodies	I	A
MSI/dMMR	CRC	Prescreen for Lynch syndrome	I	A
MSI/dMMR	CRC	Prognosis, especially in stage II disease	I	A
HER2[2]	Gastric/GOJ	Predicting response to trastuzumab	I	A/B
c-KIT	GIST	Diagnostic aid	III	A
c-KIT[3]	GIST	Therapy decision making with imatinib	III	A/B

[1]Mutational status, *i.e.*, patients with specific mutations in *K-RAS* are unlikely to benefit from the anti-EGFR antibodies, cetuximab or panitumumab.
[2]Gene amplification or overexpression, *i.e.*, benefit from trastuzumab depends on *HER2* gene amplification or overexpression.
[3]Mutational status, *i.e.*, mutational status of c-*KIT* may be used to determine optimum dose of imatinib for patients with advanced GIST. Abbreviations: LOE, level of evidence[3,4]; SOR, strength of recommendation; [4]FIT, fecal immunochemical test; FOBT, fecal occult blood testing; dMMR, defective mismatch repair; FU, fluorouracil; GOJ, gastro-oesophageal junction; GIST, gastrointestinal stromal tumor.

Table 2. Advantages of FITs compared to gFOBTs

• FITs have better analytical sensitivity and specificity than gFOBTs[1]
• FITs have greater sensitivity for advanced adenomas than gFOBTs
• Use of FITs leads to higher participation rates than use of gFOBTs
• In contrast to gFOBTs, FITs can be automated
• Use of FITs require fewer stool samples than gFOBTs
• FITs are quantitative or at least semi-quantitative
• FITs provide an adjustable cut-off point
• With FITs, no dietary or medication restriction is necessary
• FITs are more cost-effective than gFOBTs

Summarized from refs. 10–15.
[1]gFOBTs detect the presence of any blood, FITs are specific for human blood.

hemoglobin and the newer fecal immunochemical test (FIT) which detects the globin component of hemoglobin.[6,7] Although extensively validated for reducing mortality form CRC,[8,9] the gFOBT has many limitations as a screening test for CRC.[10–15] These limitations include lack of specificity for human hemoglobin, (certain foodstuffs and medications may interfere with test) and relatively low clinical sensitivity and specificity for colorectal neoplasia. Furthermore, it is difficult to automate, rendering it unsuitable for large population-based screening.[10]

Because of these limitations, the use of gFOBT, as a screening test for CRC, is gradually being replaced by FITs.[10–15] As summarized in Table 2, FITs possess many advantages over gFOBTs.[10,12–15] Because of their superior performance, the EGTM panel have recommended that a FIT should be used in new centers embarking on FOBT screening. Specifically, the panel recommends use of a quantitative FIT, with an adjustable cut-off concentration.[10] Recently published European Union guidelines for quality assurance in CRC screening and diagnosis also recommend use of FIT rather than gFOBT.[15] All FOBTs however, lack specificity for colorectal neoplasia. Positive tests must therefore be followed-up with colonoscopy.[10]

An important consideration in introducing any new diagnostic procedure, especially disease screening, is cost-effectiveness. Indeed, the World Health Organization has stated that screening should only be implemented when a "good balance" exists between costs and benefits.[16] In the context of CRC, several studies have concluded that when compared to no screening, all the widely investigated screening tests including FOBT offers additional years of life at a cost that is considered acceptable by most advanced countries and indeed may be cost-saving.[17–25] Thus, in five cost-effectiveness analyses, the estimated mean cost per life-year gained from annual screening of subjects 50 years or older with a specific gFOBT ranged from $5,691 to $17,805.[18] These costs are substantially less than the cost-effectiveness thresholds commonly used to evaluate medical interventions (*e.g.,* ~€30,000 to €40,000 per quality life-year (QALY) gained in the EU, and $50,000–$100,000 in the USA).

The EGTM panel recommends that screening for CRC and advanced colorectal adenomas be performed with a FOBT.[2,10] For new centers undertaking screening, the panel recommend use of a quantitative FIT that posses an adjustable cut-off point. Results using FITs should be expressed as micrograms of hemoglobin per gram of feces.[26] Work to improve the standardization of FIT assays would be highly desirable,[11] as would further research into DNA-based tests,[27] including automation and cost reduction.[10]

CEA in determining prognosis and staging

A multiplicity of studies carried out over the last 30 years have addressed the prognostic impact of CEA levels at initial presentation in patients with CRC (reviewed in Refs. 28,29).

Table 3. Recent studies reporting a prognostic impact of preoperative CEA in patients with CRC cancer

No. of patients	Tumor stages	Key findings	Ref.
9083	I-IV[1]	CEA an independent prognostic marker, prognosis was worse in high CEA patients with a lower stage compared to low CEA patients with a higher stage. High CEA as strong as nodal positivity for predicting poor outcome.	(30)
474	I-III	CEA an independent prognostic marker, CEA prognostic in stage II patients.	(31)
1637	I-III	CEA an independent prognostic marker in total population, as well as in patients with either stages II or III disease	(32)
1263	I-III	CEA an independent prognostic marker in total population, as well as in patients with either stages II or III disease	(33)
82	IIA	CEA prognostic in stage IIA patients	(34)
2230	I-IV	CEA an independent prognostic marker	(35)
572	II	CEA prognostic in stage II patients	(36)

[1]Investigated colon cancer patients only.

Although these different studies varied with respect to the specific CEA assay used, cut-off point for CEA, number of patients included, follow-up period and whether or not adjuvant chemotherapy was used, almost all concluded that elevated preoperative CEA levels were associated with adverse outcome. Indeed, several of these studies showed that CEA was an independent prognostic factor and, importantly, predicted outcome in patients with stage II disease.[30–36] Key findings from the more recent and larger studies on the prognostic value of CEA in CRC are summarized in Table 3.

In agreement with other organizations,[37–42] the EGTM recommends measuring preoperative CEA levels in newly diagnosed CRC patients.[1,2] Preoperative levels provide prognostic information as well as a baseline value for interpreting subsequent levels. No study however, has shown that CEA can be used to select those patients with stage II CRC who would benefit from adjuvant chemotherapy.

For future research, the EGTM recommends that preoperative levels of CEA be included for risk stratification in clinical trials evaluating new adjuvant systemic treatments for patients with CRC. We also suggest that the prognostic impact of CEA be compared with other emerging prognostic markers for CRC such as microsatellite instability (MSI) and gene expression profiling (see below). In the context of biological/molecular prognostic biomarkers for CRC, measurement of CEA is likely to be considerably simpler and less expensive than determination of tissue-based biomarkers.

CEA in postoperative surveillance

At least eight published randomized controlled trials, including almost 3,000 patients in total, have addressed the impact of intensive postoperative surveillance on outcome in patients who have undergone curative surgery for colorectal cancer (for review, see Ref. 43). These randomized trials varied with respect to intensity of follow-up and diagnostic modalities used, and most were statistically underpowered to detect a significant effect of surveillance on survival. Furthermore, many were carried out prior to the use of adjuvant chemotherapy for CRC and availability of modern and more effective systemic treatment for recurrent CRC.

Nevertheless, meta-analyses of these trials[44–49] showed that intensive follow-up resulted in a reduction of 20–30% in the hazard rate for all cause mortality.[43] However, due to the different follow-up strategies used in both the intensive and nonintensive follow-up arms, it was not possible to draw conclusions about the best combination of tests or the frequency of their performance. Despite this, regular measurement of CEA, as part of an intensive follow-up regime, appears to be necessary to achieve significant improvement in survival.[44,48,49]

Compared to other available diagnostic modalities, serial CEA determinations appear to be the most sensitive for the detection of early recurrent disease (*i.e.,* provide the first indication), especially liver metastasis.[50–53] Thus, in a recent large randomized prospective trial comparing laparoscopic-assisted colectomy with open colectomy in patients with curable colon cancer, serial CEA measurements outperformed other diagnostic modalities for patients with both early stage (stage I and IIa) and late stage disease (stage IIb and III).[53] For the 537 patients with early stage CRC, CEA detected 29.1% of the first recurrences compared with 23.6% by CT scan, 12.7% for colonoscopy and 7.3% for chest X-ray. For the 254 patients with late stage disease, CEA detected 37.4% of the first recurrences, CT scan 26.4%, chest X-ray 12.1% and colonoscopy 8.8%.[53]

Similar to the situation with CRC screening, intensive follow-up after curative surgery for CRC has been shown to be cost-effective.[54,55] Based on data from five randomized trials, Renehan *et al.*[55] calculated that the number of years gained through intensive surveillance over 5 years was between 0.73 and 0.82. The adjusted net cost for each patient was £2479 and for each life year gained was £3402. Although the most cost-effective strategy is unknown, measurement of CEA appears to be one of the least expensive tests performed as part of an intensive follow-up strategy.[56] Thus, in an early study carried out in the US, the cost per recurrence detected was $5,696 using CEA, $10,078 with chest X-ray and $45,180 using colonoscopy.[56] Although the absolute costs of these tests are likely to have increased since publication of this report, the relative costs are likely to be the similar.

Because of its ease of measurement, relatively low costs and sensitivity for early metastasis, most expert panels recommend regular CEA measurements during the follow-up of

patients following curative surgery for CRC.[1,2,37–40] According to the EGTM panel, CEA should be measured at baseline and then every 2–3 months for at least 3 years after diagnosis in patients with Stage II or III CRC who may be candidates for further intervention (*e.g.,* liver resection or systemic treatment) in the event of recurrent disease. After 3 years, CEA may be measured approximately every 6 months for 5 years.[2] Any increase in levels must be confirmed with a second sample prior to undertaking further investigations. As different CEA assays may give different results, ideally, the same assay should be used throughout. Broadly similar recommendations have been published by other expert panels including the American Society for Clinical Oncology (ASCO),[37] the National Comprehensive Cancer Network (NCCN),[38] the National Academy for Clinical Biochemistry (USA) (NACB)[39] and the European Society for Medical Oncology (ESMO).[40] Caution however, should be exercised in interpreting increases in CEA levels as certain benign diseases may also increase its concentration.[57–59]

Monitoring systemic therapy in advanced disease

Radiology has been and remains the "gold standard" for evaluating response to systemic therapy in patients with advanced CRC.[60] For most patients however, good agreement has been found between radiological and CEA-defined responses.[61–63] Thus, in a recent study using a well defined population of patients with isolated liver metastasis from CRC, de Hass *et al.*[61] reported agreement between CT scan and CEA response in >90% of cases. For patients with radiological response or stable disease, agreement with CEA response was found in 94% of cases, while in patients with radiological evidence of disease progression, agreement was present in 95% of cases. Based on these findings, the authors concluded that use of CEA is as accurate as CT imaging for assessing response of colorectal cancer liver metastasis to chemotherapy.

Most expert panels, including the EGTM, currently recommend measurement of CEA in monitoring patients with advanced CRC receiving chemotherapy.[1,2,37–40] As previously pointed out however,[1,37] caution is necessary when interpreting serial CEA levels shortly after initiation of specific cytotoxic therapies, as spurious or transient rises may occur.[64,65] These transient increases occur in 10–15% of patients with advanced CRC receiving chemotherapy,[64,65] and appear to be associated with a favorable outcome.[64,65] They have been attributed to apoptosis and/or necrosis of tumor cells caused by the cytotoxic agents.

DNA mismatch repair and microsatellite instability

As a prescreen for lynch syndrome in patients with colorectal cancer. Lynch syndrome (LS) is an autosomal dominant disorder, associated with a predisposition to multiple types of malignancy including cancers of the colon, rectum, endometrium, stomach and small bowel.[66,67] Approximately 3% of all CRC are LS-related. This syndrome is due to germline loss-of-function mutations in the mismatch repair (MMR) genes, *MLH1, MSH2, PMS2* and *MSH6*. Loss of these genes results in defective MMR (dMMR), which in turn results in microsatellite instability (MSI).

As MSI or dMMR is present in >90% of cases, their detection is used as an initial test in the detection of LS in patients with CRC. If MSI/dMMR is present, further investigations including mutational analysis of the *MSH2* and *MLH1* genes should be performed. The absence of MSI or dMMR makes the presence of LS unlikely. Although MSI and MRR protein status are relative sensitive tests for LS, they are not specific, as 12–17% of all CRCs exhibit these defects, the majority of which are sporadic. In sporadic CRC however, MSI generally results from loss of MLH1 and PMS2 proteins.[68] Loss of *MLH1* expression in sporadic CRCs is due to epigenetic silencing by hypermethylation of CpG nucleotides in its gene promoter region.

Establishing a diagnosis of LS in patients with CRC is important, as these subjects are at increased risk of developing other cancers. In addition, since some family members will have inherited LS, they are also at high risk of developing CRC and possible other malignancies. Although randomized trials have not been reported, several observational studies suggest that close surveillance of Lynch syndrome subjects decreases both cancer rates and mortality.[69–74]

While traditionally, MSI/MMR protein measurement in CRC was limited to subjects fulfilling specific clinical characteristics such as the Amsterdam and Bethesda criteria,[75–77] more recently several expert panels and some individual investigators have recommended that all patients with CRC should undergo such testing.[78–82] Advantages of a universal testing approach include cost-effectiveness[80,82] and increased sensitivity for detecting mutation carriers.[78,79] Limited resources may however, restrict universal testing in some countries.

In agreement with other organizations,[37–39] the EGTM recommends measurement of MSI or MMR proteins as prescreens for LS in patients with CRC.[2] If MSI is present or MMR enzyme loss is detected, subjects should be offered genetic counseling and undergo germline gene testing for LS. Subjects with MSI-positive tumors that are negative for MLH1 protein may be considered for *BRAF* mutation and/or *MLH* promoter methylation testing, prior to further genetic testing. Future research should focus on the optimum and most cost-effective approach for LS prescreening.

Prognosis and therapy prediction. In addition to being used as a prescreen for LS, MSI/MMR status may also have use as a prognostic marker in CRC, as several studies have shown that the presence of MSI or defective MMR activity is a marker of favorable outcome. Two separate pooled analysis,[83,84] as well as several large randomized trials have shown that the presence of MSI/dMMR was associated with a favorable outcome, especially in patients with Stages II and III colon cancer.[85–90] All these studies when taken together provide strong evidence that MSI/MMR status is a prognostic

biomarker for Stages II and III colon cancer. Additionally, MSI status is now recommended in the WHO classification of mucinous-type CRC, with high MSI indicating good prognosis and low or absent MSI suggesting poor outcome.

Several studies including two randomized trials,[85,86] a retrospective case study[91] and a systematic review[92] also suggest that MSI/MMR status may be a predictive biomarker for adjuvant 5-FU-based therapy, *i.e.,* the presence of MSI/dMMR is associated with lack of benefit.[85,86,91]

Although most studies have shown a relationship between MSI/dMMR and lack of benefit from adjuvant 5-FU, some have not confirmed these findings.[87,93,94] Possible reasons for the conflicting data include the different protocols used for determining MSI/MMR status, especially the number of microsatellites measured when assessing MSI status, the number of patients investigated in the various studies, inadequate randomization and length of follow-up.

Because of the multiplicity of studies linking MSI/dMMR with good prognosis, the EGTM states that these parameters may be measured in patients with Stage II colon cancer who are under consideration for adjuvant 5-FU-based therapy. Patients with MSI/dMMR may not require such therapy as their prognosis is likely to be favorable. MSI-positive patients with adverse prognostic features such as pT4 stage or lymphangio-invasion however, should not be excluded from receiving chemotherapy.[87]

Future work should compare the prognostic impact of MSI/MMR status in stage II CRC with that of CEA and the various multigene profiles currently undergoing evaluation (see below). Further research is also required to investigate whether the MSI/dMMR status is of value in predicting benefit from other chemotherapeutic drugs such as platinum salts (oxaliplatinum) and topoisomerase inhibitors (irinotecan).

K-RAS for predicting response to anti-EGFR antibodies

Cetuximab and panitumumab are monoclonal antibodies that bind to the extracellular domain of EGFR, thereby inhibiting downstream signaling and resulting in decreased cell proliferation and migration.[95,96] Early clinical trials using these antibodies, either alone or in combination with chemotherapy, to treat unselected patients with advanced CRC gave response rates of only 10–15%.[97–100] More recently, retrospective analysis of randomized controlled trials has shown that patients with specific mutations in codons 12 of the *K-RAS* gene almost never benefited from treatment with these antibodies. However, 15–20% of patients with wild-type *K-RAS* show an objective response with antibody alone and 35–40%, when treated with cetuximab and irinotecan.[97–101]

While almost all of the known *K-RAS* mutations at codons 12 are associated with lack of benefit from cetuximab or panitumumab, a specific mutation at codon 13, *i.e.,* G13D may be an exception. Thus, in two trials, administration of cetuximab to patients with this mutation was associated with a significantly better outcome than that seen in patients with other types of *K-RAS* mutations.[102,103] Indeed, patients with the G13D mutation appeared to benefit to approximately the same extent as patients with *K-RAS* wild-type tumors from the addition of cetuximab to first-line chemotherapy.[103] Clearly, these findings require validation in a large prospective randomized trial.

As with several of the biomarkers discussed above, an economic benefit for *K-RAS* testing prior to prescribing anti-EGFR antibodies for patients with metastatic CRC has been demonstrated.[104] Using modeling data, Vijayaraghavan *et al.*[104] calculated that administration of anti-EGFR antibodies only to patients with wild-type *K-RAS* would result in a net saving of €3,900–€9,600 in Germany and $7,500–$12,400 in the US. For these calculations, it was assumed that all patients had previously received at least one line of chemotherapy treatment.

Because of its clinical and economic benefit, EGTM recommends mutation testing of *K-RAS* prior to administering cetuximab or panitumumab to patients with advanced CRC. Patients with specific activating mutations especially at codon 12 should not be treated with anti-EGFR antibodies. Patients with the G13D mutation may however, benefit from combined cetuximab and chemotherapy but this remains to be confirmed. It is important that the laboratory report indicate the specific *K-RAS* mutation analyzed and detected as well the methodology used. Recommendations for performing *K-RAS* gene mutations testing in CRC have recently been published.[105]

Future research should aim to standardize assays for assessing the mutational status of *K-RAS* in CRC. Research should also focus on the identification and development of additional biomarkers in order to increase the positive predictive value for response to anti-EGFR antibodies. This should focus on validating preliminary findings suggesting a predictive or prognostic value for mutations in *BRAF, PIK3CA* and *N-RAS,* loss of *PTEN* and levels of EGFR ligands.[106–108] Finally, as mentioned above, further work is necessary to establish which patients with which G13D mutations are likely to benefit from treatment with anti-EGFR antibodies.

Other therapeutic targets as well as emerging therapeutic targets for the treatment of CRC are listed in Table 4. Apart from the anti-EGFR antibodies discussed above, validated predictive markers are currently unavailable for the drugs inhibiting these targets.

Gastric and Gastro-Oesophageal Junction Cancers

HER2 for predicting response to trastuzumab

As with breast cancer, patients with gastric and GOJ cancers overexpress *HER2* in 10–25% of cases.[119] Consistent with this finding, HER2-positive cell lines are sensitive to trastuzumab,[120,121] while patients with advanced HER2-positive gastric and GOJ tumors benefit from treatment with the anti-HER2 antibody.[122] Based on these findings, EGTM state that for patients with advanced gastric or GOJ under consideration for systemic therapy, measurement of HER2 should be

Table 4. Established and emerging therapeutic targets for the treatment of colorectal cancer

Target	Drug	Phase of Development	Ref.
EGFR	cetuximab, panitumumab	In clinical use	98–101
VEGF	Bevacizumab, aflibercept	In clinical use	109–111
Multi kinases[1]	Regorafenib	In clinical use	112,113
BRAF (mutant)	Vemutafenib, dabrafenib	In development	114
MEK	Selutmetinib, pimasertib	In development	115,116
mTOR	Evorilimus	In development	116,117
PI3K	LY294002, GDC0941	In development	116,118

[1]Regorafenib inhibits VEGFR1, VEGFR2, VEGFR3, PDGFRbeta, Tie-2, FGFR1, RET and BRAF.

Table 5. Emerging biomarkers for gastrointestinal cancers

Marker	Cancer	Potential use	Ref.
Multigene profiles[1]	CRC	Determining prognosis	140–142
TIMP-1	CRC	Prognosis	143
CA 19-9	CRC	Postoperative surveillance	144
Stool DNA profiles	CRC	Screening	145
Septin 9	CRC	Screening	146
TFAP2E	CRC	Chemoprediction	147
CA 242	Gastric	Prognosis, monitoring therapy	148
DOG1	GIST	Diagnostic aid	149

[1]Amongst the best-validated multigene signatures are the ColoPrint test (Agendia) (129), 634-geneColDx (Almac) (130), and the Oncotype DX colon cancer assay (Genomic Health) (131).
Abbreviations: CRC, colorectal cancer; GIST, gastrointestinal cancer.

performed using immunohistochemistry and/or FISH. Patients with HER2-positive disease are candidates for receiving combined trastuzumab and chemotherapy. HER2 staining and scoring in gastric and GOJ tumors should be performed and scored as recently recommended.[123] Because the expression of HER2 in gastric and GOJ cancer may be heterogenous, multiple biopsies are necessary in order to obtain a reliable indication of the oncoprotein status.[124]

Gastrointestinal Stromal Tumors

KIT as a diagnostic aid

Gastrointestinal stromal tumors (GISTs) although rare are the most common mesenchymal tumor found in the gastrointestinal tract (for review, see Refs. 125,126). At a molecular level, GISTs are characterized by the presence of KIT protein and mutations in the *KIT* gene. Thus, the KIT protein is found in >95% of GISTs, while mutations in the *KIT* gene are present in ~80–85% of cases.[125,126] Most of the mutations in the *KIT* gene are found in Exon 11 and consist of point mutations and deletions. Less frequent mutations are present in Exons 9, 13 and 17. Five to 10% of GISTs have mutations in the homologous gene, *PDGFRA*.[125,126] Mutations in *PDGFRA* are mostly found in Exons 12 and 18 and appear to be mutually exclusively with mutations in *KIT*. Overall, 80–95% of GISTs have mutations in either the *KIT* or *PDGFRA* gene.

Because the *KIT* gene is almost universally expressed and/or mutated in GISTs, it has been extensively investigated as a biomarker for this disease[125–127] and immunostaining for KIT protein is used as a diagnostic aid for GISTs. For the small proportion of GISTs that fail to express KIT protein (~5%), mutational analysis of the *KIT* and *PDGFRA* genes may confirm the diagnosis. However, although KIT protein is rarely detected in other abdominal tumors, it may be present in some nonabdominal tumors, including melanomas, breast cancers, and seminomas.[125,128] Other markers that may aid the diagnosis of GISTs include DOG1, CD34, S100, desmin, PS100 and smooth muscle actin.[125,128] Measurement of markers however, complements but does not replace histopathology in the diagnosis of GIST.

A number of expert panels including ESMO,[129] a French National Consensus Group,[130] and NCCN[131] recommend measurement of KIT as an aid for diagnosis of GIST. Both the ESMO and NCCN Panels also state that *KIT* and *PDGFRA* mutation testing should be considered for KIT protein-negative tumors that are suspected to be GISTs.[129,131]

In agreement with these groups, EGTM recommend that staining for KIT protein should be used as a diagnostic aid for GIST. However, its absence does not exclude GIST. Mutational analysis of *KIT* or *PDGFR* genes may be considered, if sample is KIT protein-negative.

KIT in predicting benefit from Imatinib

Imatinib is a tyrosine kinase inhibitor which blocks KIT and PFGFRA as well as BCR-ABL. The use of imatinib has revolutinized the treatment of patients with GISTs in recent years.[125] Response to imatinib however, depends on the mutational status of the *KIT* gene. Thus, patients with advanced GISTs exhibiting mutations in Exon 11 of *KIT* had a better outcome than those with Exon 9 mutations or those without a detectable *KIT* mutation.[132–138] Based on the above, several expert panels currently recommend *KIT* and *PDGFFRA* mutational analysis prior to prescribing imatinib to patients with GISTs.

Although the use of imatinib in patients with GISTs was originally restricted to patients with advanced disease, a recent provisional clinical opinion published by a European consensus group recommended adjuvant imatinib in patients with *KIT* exon 11 mutations. However, adjuvant imatinib was not recommended for patients with primary GISTs containing PDGFRA D842V mutations.[139]

In agreement with other expert panels,[129–131] EGTM state that mutational analysis of *KIT* and *PDGFRA* should be

considered prior to administering imatinib to patients with GIST.

Emerging Markers

Table 5 lists some promising new biomarkers and multigene profiles for gastrointestinal cancer. None of the listed markers/profiles however, can currently be recommended for routine clinical utility.

Conclusion

It is clear from the above that several biomarkers are now integral to the management of patients with different types of gastrointestinal cancers (Table 1). We should point out, however, that the guidance published here and elsewhere should not replace physician judgment in specific patients. Furthermore, as new data becomes available, recommendations based on existing evidence may change. It is therefore vital that physicians using these biomarkers and the laboratories performing the assays keep up-to-date with new findings. Finally, laboratories performing the recommended assays should participate in external quality assessment schemes, be accredited by appropriate regulatory organizations and be staffed and managed by an appropriately trained work-force.

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