



Review Article



A Review and Update on Therapy of Gastrointestinal Tract Tumors: From the Bench to Clinical Practice

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Abstract

Gastrointestinal tract tumors, i.e., esophageal, gastric, small, and large bowel carcinomas, are some of the most frequent malignant neoplasms. The landscape of these neoplasms varies significantly. Although the incidence of esophageal carcinoma seems to be decreasing, gastroesophageal junction tumors are on the rise. The incidence of antral gastric carcinoma of the tubular type also seems to be decreasing, yet the prognosis remains largely unchanged, especially for advanced disease. Small bowel carcinomas are infrequent, but the prognosis is dismal. Colorectal carcinoma has become the second leading cause of cancer-related deaths in Western countries, and despite screening campaigns, many patients are still diagnosed with advanced or metastatic disease, leading to a poor prognosis. Unlike other tumors, breakthroughs in targeted therapies have not been so impressive in gastrointestinal tumors. Anti-HER2 drugs, immune checkpoint inhibitors, or drugs against claudin 18.2 only benefit small subsets of patients, and management in many cases is still based on conventional chemoradiation therapy. The future development of therapies for these tumors will depend on understanding the molecular basis of the disease. Many researchers are working to shed light on the molecular pathogenesis of gastrointestinal cancer. This review aims to summarize the main breakthroughs in the knowledge of the molecular basis of gastrointestinal cancers, focusing on those that could lead to significant changes in the management and prognosis of these prevalent and still lethal neoplasms.

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Introduction

Cancer, along with cardiovascular diseases, is one of the leading causes of death, especially in Western countries. Gastro-

intestinal malignancies rank among the most prevalent and lethal human tumors, representing more than 20% of all cancer cases worldwide. Some of these tumors have been among the main causes of cancer-related deaths for many years.¹ For many decades, cancer management was based on standard therapies that aimed to destroy tumor cells by exploiting their mitotic activity, namely chemotherapy and/or radiation therapy. However, these therapies were rather toxic and were associated with not only unwanted side effects but also the risk of developing second neoplasms in long-term survivors. In the 1970s and 1980s, there were some timid attempts to develop more personalized therapeutic strategies based on the molecular characteristics of tumors. In this sense, we must remember anti-estrogenic drugs for breast cancer and drugs targeting C-Kit for some leukaemias and gastrointestinal stromal tumors.² However, the lack of knowledge about the molecular basis of many human tumors hampered this approach for numerous types of cancer. It was not until the turn of the century that we witnessed a surge in molecular knowledge, paving the way for the modern approach to therapy known as personalized medicine.³

The shift in management paradigms has been founded on the dramatic advances in the technical means to extract well-preserved DNA and RNA from formalin-fixed paraffin-embedded tissues, and also on the widespread availability of high-output molecular techniques, such as next-generation sequencing that allows the identification of thousands of molecular abnormalities in human tumors using small DNA samples. The pharmaceutical industry has invested heavily in identifying drugs that selectively antagonize these molecular abnormalities, resulting in the appearance of new drugs that can destroy tumor cells with reduced toxicity. This revolution in targeted therapies is reshaping the landscape of cancer treatment and prolonging the survival of oncology patients.⁴ A simple Medline search of cancer and targeted therapies or cancer and molecular abnormalities will reveal hundreds of thousands of reports that describe potential predictive and prognostic factors. This review aims to summarize the most relevant findings for gastrointestinal tumors, focusing on those that have led to changes in patient management.

Esophagus

Esophageal carcinoma ranks among the ten most prevalent tumors worldwide and contributes a large burden to cancer mortality. Despite wide geographic variations, the most frequent tumor type overall is squamous cell carcinoma (85% of the cases), although the rate of adenocarcinomas aris-

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ing in Barrett's esophagus is increasing, mainly in Western countries.^{5,6} In the last tumor node metastasis recommendations, Siewert type III adenocarcinomas have been considered equal to gastric tumors in terms of prognosis and therapy and it seems esophageal adenocarcinomas share many features with gastric adenocarcinomas. While surgery is the cornerstone of therapy for early-stage disease,⁷ therapy for advanced squamous cell carcinoma is still based on cytotoxic therapy. Esophageal carcinomas were among the first tumors treated with neoadjuvant schemes, mainly to ease surgical resection.⁸ Despite wide variations in incidence and mortality, the prognosis remains poor. Even with adjuvant cytotoxic therapy, mortality of squamous cell carcinoma remains high, and few patients (less than 15%) are free of disease at 5-year follow-up.⁵

Recent reports have broadened knowledge about the genetic changes underlying esophageal squamous cell carcinoma.⁹ Whole-genome and whole-exome sequencing and clustered regularly interspaced short palindromic repeat technologies among others, are helping define the genetic landscape of squamous cell carcinomas. Although some abnormalities, such as Wingless integrated (*WNT*)/Notch1 downregulation or the chemokines ligand 2-chemokines receptor 2 axis, are promising candidates for targeted therapies, to date no targeted therapy has shown clinical utility.¹⁰ The lack of success in phase 3 trials employing EGFR inhibitors underscores the importance of defining predictive markers that allow a more personalized approach to therapy in the near future.¹¹

The most promising strategy for squamous cell carcinoma to date is programmed death ligand 1 (PD-L1) inhibition, combined or not with cytotoxic therapy.¹² Therapy selection depends on the immunohistochemical expression of PD-L1. It is important to note that several antibodies against PD-L1 are marketed, and they can predict the antitumor activity of different drugs (pembrolizumab, atezolizumab, nivolumab). Besides, PD-L1 expression in this setting has been variably measured in tumor epithelial cells and/or the inflammatory cells associated with the tumor and, expressed as either the tumor proportion score (TPS) or the combined positive score (CPS). A recent meta-analysis of clinical trials employing PD-L1 inhibitors as first or second-line agents and using CPS or TPS has shown that tumors with less than 1% TPS do not significantly benefit from first-line PD-L1 inhibitor therapy in terms of overall survival (OS) and that patients with less than 10% CPS benefit little but significantly from this therapeutic approach.¹³ It remains to be proved if tumor mutation burden or mismatch repair deficiency will improve patient selection of patients.

Screening patients with Barrett's esophagus via serial endoscopy and local therapy for premalignant and early-stage adenocarcinoma is feasible in Western countries and can lead to improved prognoses for these aggressive lesions. In advanced unresectable gastroesophageal adenocarcinoma, significant progress has been made with immunotherapy, both for human epidermal growth factor receptor 2 (HER-2) positive and negative cases, either alone or in combination with cytotoxic therapy. However, there have been controversies regarding criteria for indicating this therapy; in the USA, immune checkpoint inhibitors can be used without a CPS cutoff point, while in Europe, a cut-off value of CPS \geq 5 has been settled for nivolumab as first-line therapy in this setting.¹⁴ In patients with HER-2 positive tumors, clinical trial results confirm the efficacy of combined therapy with first-line chemotherapy, immunotherapy, and trastuzumab.¹⁵ The options in this field are expanding, with numerous clinical trials exploring different combinations of novel drugs targeting the HER2 and PD1 pathways (double targeting).¹⁶

The tight junction protein claudin-18 isoform 2 (*CLDN* 18.2) has recently emerged as a potential target in many human neoplasms,¹⁷ including gastric and gastroesophageal carcinomas, with some series indicating up to 30% of cases with high expression of this protein. Targeted drugs against *CLDN* 18.2, such as zolbetuximab, have shown success in clinical trials and are becoming realistic therapeutic options for patients with HER-2 negative gastric and gastroesophageal adenocarcinomas, in both early and advanced stages.^{18,19}

Based on the dramatic results shown in other tumors (such as cholangiocarcinoma), fibroblast growth factor receptor inhibitors (such as bemarizumab or futibatinib) have been investigated in advanced gastroesophageal carcinoma with limited success to date,²⁰ a fact that can be attributed to the different genetic abnormalities affecting this gene (such as fusions and mutations). It remains unclear whether double inhibitions or a combination of fibroblast growth factor receptor (*FGFR*) inhibitors with other targeted drugs can improve these results and overcome the development of resistance to these drugs.²¹

Overall, it seems patients with high risk of microsatellite instability (MSI-H) esophageal adenocarcinoma can benefit more from tailored targeted therapies.²²

Stomach

Gastric cancer (GC) ranks as the fifth most common tumor and the fourth leading cause of cancer mortality worldwide.¹ Its incidence is highest in Eastern Asia and some Eastern European countries while being less common in Western countries. It is noteworthy that there are significant differences not only in incidence but also in the implementation of screening programs, clinical characteristics, prognosis, histological and molecular characteristics, and patient management between Asian and Western regions.^{23,24} Advances in understanding the molecular basis of cancer have identified numerous molecular alterations in GC, some of which hold prognostic or therapeutic value, and the development of molecular classifications, among which stand out those published by The Cancer Genome Atlas (TCGA) and the Asian Cancer Research Group (ACRG).

However, despite recent advances, the prognosis for GC remains poor, especially in Western countries where it is diagnosed at advanced stages, resulting in high mortality rates with less than 30% survival at 5 years.²⁵ The only curative treatment is surgery, with total or subtotal gastrectomy and D2 lymphadenectomy being the most common approach, although endoscopic techniques can be performed in the early stages.²⁶ In advanced stages, treatment is based on chemotherapy, and the only approved targeted therapies for GC currently include anti-vascular endothelial growth factor (*VEGF*) and its receptor (*VEGFR*) (such as ramucirumab and apatinib) and anti-HER2 drugs (such as trastuzumab).²⁷ Additionally, in 2017, the U.S. Food and Drug Administration approved pembrolizumab for advanced solid tumors with MSI-H or deficient-mismatch repair tumors (d-MMR), an approval that included GC.²⁸ One of the emerging and promising targets is claudin 18.2 (*CLDN* 18.2), already described in esophageal junction adenocarcinoma. Phase II and III clinical trials have confirmed the benefits of anti-*CLDN* 18-2 drugs (such as zolbetuximab) associated with chemotherapy in the first-line setting, although there is no consensus about the cut-off points to define positivity.²⁹ The FAST clinical trial established a cut-off point of at least 40% of tumor cells, while the SPOTLIGHT clinical trial established a cut-off value of 75% or more of moderate to strong membrane *CLDN*18 staining.³⁰ There is a clear need to settle common cut-off

points to compare study results and to develop new strategies for detecting overexpression, different from immunohistochemistry, such as immunoPET.³¹ Besides, therapy might be based not only on targeted single drugs but also on T chimeric antigen receptor technologies, double inhibition, and other strategies currently under development.

Due to the limited improvement in the prognosis of GC patients, there is a need to identify new molecular alterations that allow better stratification of patients in both clinical practice and clinical trials. This also involves developing new targeted therapies to increase the currently available therapeutic options. In this context, researchers have analyzed both isolated molecular alterations and pathways in GC, and genomic and transcriptomic information to establish molecular categorizations. Despite these efforts, this knowledge has not yet been translated into clinical practice, unlike what has been observed in other tumor types. In this section of the review, we will focus on the two most important molecular classifications published in GC. These classifications group common molecular alterations, correlate with clinical, histological, and prognostic factors, and hold potential therapeutic value. The classifications published by TCGA and ACRG divide GC into groups that have shown prognostic value in several studies, although other researchers have reported contradictory results.^{29,32,33} The TCGA system establishes four categories: GC with MSI, positive for Epstein-Barr virus (EBV), genomically stable (GS), and tumors with chromosomal instability (CI).³⁴ On the other hand, ACRG determined similar but not entirely equivalent groups to TCGA, dividing GC into mesenchymal-like (epithelial-mesenchymal transition), MSI, *TP53*-active (with intact p53 activity), and *TP53*-inactive.³⁵ Overall, the most common alteration in GC is *TP53* mutation. As for the main molecular changes of GC subtypes, MSI tumors display high mutation rates, EBV-related tumors are associated with PD-L1 and PD-L2 overexpression, GS and mesenchymal-like GC show cadherin 1 mutation, and *TP53*-inactive or CI tumors are enriched in gene amplifications and *TP53* mutation.³⁵ Mesenchymal-like and GS subtypes show the worst prognosis, while GC with MSI according to ACRG and EBV-positive GC according to TCGA show the best prognosis.³⁶ Additionally, the ACRG study observed that GS tumors had a higher recurrence rate and a tendency for peritoneal extension, while the MSI group showed more liver-limited metastasis. Meanwhile, *TP53*-active subtypes according to ACRG and MSI tumors according to TCGA had an intermediate prognosis but were better than *TP53*-inactive and CI subtypes.

In summary, GC cases with MSI or EBV positivity are associated with a good prognosis, while GS or mesenchymal-type tumors, often of the discursive type, have the worst prognosis. Furthermore, among the remaining tumors with an intermediate prognosis, those with *TP53* mutation show a worse prognosis.

However, translating these molecular classifications into clinical practice presents one major limitation, which is —the need to perform multiple and complex molecular determinations requiring specific and costly equipment. Therefore, some authors have attempted to develop molecular classifications in GC using immunohistochemistry as a surrogate marker, which has also shown prognostic value.^{37,38} It is worth noting that some of the mentioned molecular alterations, beyond HER2 amplification or PD-L1 expression, such as EBV infection or MSI, can be studied in isolation and are available in most pathology laboratories.

Other alterations traditionally associated with a worse prognosis include modifications of matrix-degrading enzymes, angiogenic factors (such as *VEGF*, interleukin-8, ba-

sic fibroblast growth factor, platelet-derived endothelial cell growth factor, or telomere-related genes (such as protection of telomeres).³⁹ Using the TCGA and ACRG databases, He *et al.* identified a set of immune-related genes with prognostic value, including *FABP4*, *LBP*, *LCN1*, *CMA1*, *INHBA*, *ANGPTL1*, *ACKR1*, *GHR*, and *OGN*. Finally, epigenetic events, such as aberrant DNA methylation or histone modification, have also been associated with a worse prognosis in GC.³⁹

The molecular changes identified by TCGA and ACRG also have therapeutic value. It is noteworthy that TCGA identified an increased expression of mitotic network components including Aurora kinase (AURKA/B), transcription factor *E2F*, Forkhead Box M1, and Polo-kinase 1 signaling, and DNA damage response pathways across all tumor types. Consequently, GC emerges as a significant candidate for treatment with targeted drugs against family members of the aurora or polo-like kinases.³⁷ GS tumors exhibit these alterations to a lesser extent. In respect of specific molecular changes, GC with MSI and EBV-positive cases are significant candidates for immunotherapy. Tumors with amplification of receptor tyrosine kinase genes are amenable to blockade with multiple drugs currently in use or the developmental phase, and those with amplification of cell cycle mediators can be treated with cyclin-dependent kinase inhibitors. Examples include the potential use of dovitinib for GC with *FGFR2* amplification or crizotinib and foretinib for cases with proto-oncogene mesenchymal epithelial transition amplification.⁴⁰ Finally, previous studies have found that constitutive activation of the phosphatidylinositol 3/mammalian target of the rapamycin pathway predicts GC response to everolimus and that tumors with cadherin 1 alteration show a reduced response to current targeted and conventional therapy.⁴¹ Unfortunately, beyond the limited number of approved targeted therapies, a clear survival benefit from recently studied molecular drugs has not been observed.⁴² One of the major obstacles in this regard is the high molecular heterogeneity in this aggressive disease. Improving patient stratification in research studies and utilizing model systems representative of GC subtypes could enhance the available therapeutic armamentarium.⁴³

Small bowel

Small bowel carcinoma is rare, representing less than 5% of all GI malignant neoplasms.¹ Often diagnosed in advanced stages, the prognosis for small bowel carcinoma remains poor. Besides, the incidence appears to be rising, a fact that can be at least partially attributed to the widespread use of imaging techniques that lead to the diagnosis of these mainly subclinical tumors. Conventional adenocarcinoma represents only 30–40% of malignant neoplasms in the location, with the small bowel frequently involved by neuroendocrine neoplasms, malignant lymphoma, and some sarcomas, mainly gastrointestinal stromal tumors. The frequency of small bowel adenocarcinoma decreases from proximal to distal, with most cases arising in the proximal duodenum, including the ampulla. This predilection for the proximal duodenum has been linked to the carcinogenic action of biliary salts. The ampulla of Vater is a complex anatomical area where the common bile duct drains into the duodenal lumen after receiving secretions from the pancreatic duct. In the ampullary region, the World Health Organization Blue Book recognizes several types of carcinoma, mainly those originating in the duodenal lining or the ampulla (referred to as periampullary tumors), which are conventional intestinal-type adenocarcinomas, and those originating in the ampulla or the distal common bile duct, which are similar to their pancreatic counterparts, such as carcinomas originating in mucinous

papillary intracystic neoplasms and ductal carcinomas. Small bowel adenocarcinoma is linked to some familial syndromes (such as adenomatous familial polyposis, Peutz-Jeghers syndrome, and Lynch syndrome), and some cases are associated with long-standing Crohn's disease (although lesions in these patients tend to arise in the distal small bowel) and celiac disease (through mechanisms that are not yet fully understood).⁴⁴

The genetic profile of small bowel carcinoma seems to be different from that of colorectal carcinoma (CRC) despite morphological similarity. Adenomatous polyposis coli mutations are less common in small bowel carcinomas, while rapidly accelerated fibrosarcoma (*RAF*) mutations and microsatellite instability are more frequent. However, the V600E *RAF* mutation is not as predominant as in CRC. Oncogenic pathways in small bowel carcinoma have not been completely clarified, but recent reports have linked the risk of small bowel carcinoma to four target genes (*APOA4*, *APOB*, *COL1A2*, and *FN1*) and have even proposed genetic differences among the different segments of the small bowel.^{45,46} For ampullary tumors, there seems to be a clear genetic difference between intestinal and pancreatic tumor types, which can influence prognosis and correlate relatively well, although not perfectly, with histopathologic type.⁴⁷

Like other tumors, TNM staging is one of the most important prognostic factors in small bowel carcinoma, with a significantly worse prognosis for stage III or IV disease. The ratio of positive to negative lymph nodes is another important prognostic factor and most guidelines suggest that a minimum number of 8 lymph nodes is necessary for accurate staging. Besides the stage, several histopathological factors influence prognosis, including the tumor's location, histologic grade, and lymphovascular invasion. Microsatellite instability/mismatch repair gene status is important both as a prognostic and predictive factor. It seems clear from the literature that MSI-H tumors show a better prognosis, and some reports have advocated the use of MSI status to select adjuvant therapy in stage II disease. However, some other reports do not confirm the potential negative effect of chemotherapy in MSI-H stage II patients.

As for targeted therapy, the low relative incidence of these neoplasms makes it difficult to define the molecular basis of the disease and also to perform adequately powered clinical trials with new drugs. There have been attempts at therapy targeting angiogenic pathways and the epidermal growth factor receptor (EGFR). However, to date, platinum-based chemotherapy seems to be the most effective therapy for these tumors.⁴⁸ A recent review of 18 phase II/III clinical trials, including anti-VEGF and anti-EGFR drugs and immunotherapy, concluded that there is currently no evidence supporting these therapies in small bowel adenocarcinoma to date.⁴⁹

Large bowel

CRC has become the third most common malignant neoplasm and the fourth leading cause of cancer-related death, with a tendency to increase, mainly in Western countries. This increase can be attributed in part to the implementation of screening programs for precursor lesions based on fecal occult blood.^{1,50} The risk factors for CRC development are well-defined and include lifestyle factors, dietary habits, age, and body mass index, among others.⁵¹ It is well known that the microbiota is implicated both in the development and response to therapy of CRC through a chronic inflammation-mediated mechanism.^{50,52} Therapy is based on surgical resection of early-stage lesions, but the prognosis

for locally advanced or metastatic cases remains poor and therapy is based on a multimodal approach that includes platinum-based chemotherapy and some monoclonal antibodies targeting EGFR (namely, cetuximab and panitumumab) and vascular endothelial growth factor receptor (VEGFR) (namely, bevacizumab).^{53,54}

Most CRCs are sporadic, but there are also familial and hereditary cases linked to some genetic abnormalities.^{55,56} Similar genetic abnormalities are present both in sporadic and familial/hereditary cases. The proposal of the two-hit hypothesis for the development of hereditary tumors by Knudson at the end of the 20th century led to a better comprehension of tumor pathogenesis linked to suppressor genes,⁵⁷ although recent decades have witnessed a refinement of this theory with increasing knowledge of tumor epigenetics.⁵⁸ There are at least three oncogenic pathways leading to CRC. One of these oncogenic pathways is linked to the adenomatous polyposis complex syndrome⁵⁹; the second is related to deficiencies in mismatch repair genes/microsatellite instability (MMR/MSI, associated with Lynch syndrome),⁶⁰ and the third is related to inflammatory bowel disease and seems to be related pathways linked to p53 mutations and gastric metaplasia of the large bowel epithelium.⁶¹

CRC is a multistep process in which several abnormalities involving the *KRAS*, *BRAF*, and *PIK3CA* genes occur. Abnormalities in these genes are also well-known prognostic and predictive factors. The increasing knowledge on the molecular basis of CRC has led to a proposed classification of tumors into four molecular subtypes, namely consensus molecular subtypes (CMS) 1 (hypermutated, MSI-immune, 14%), CMS2 (canonical, high in somatic copy number alterations (SCNA) and activation of *WNT* and *MYC*, 37%), CMS3 (metabolic, SCNA low, with *RAS* mutations, 13%) and CMS4 (mesenchymal, high in SCNA and tumor growth factor- β activation, 23%) with different biological behaviors and prognoses, which could inform the design of future clinical trials aimed to improve therapy.^{62,63} Nevertheless, clinical use of this classification is hampered by the lack of adequate immunohistochemical surrogate markers and the need for complex molecular analysis, which may not be immediately available in all laboratories. Besides, almost 25% of CRCs are not adequately classified and belong to a mixed category that requires further refinement.

Many scientific societies worldwide involved in CRC management have issued guidelines recommending essential tests for this tumor. In clear contrast to other tumors (such as breast carcinoma, genitourinary neoplasms, and or melanoma), less than 5% of metastatic CRC have molecular abnormalities that can be candidates for selective antagonism, and therapy remains mainly based on MSI testing, *RAS* mutations, and *RAF* mutations.^{64–67}

Although polymerase chain reaction can be used, MSI status is usually analyzed with immunohistochemistry targeting MSH2, MSH6, MLH1, and PMS2 proteins as a surrogate marker.⁶⁸ Normal tissue is used as a positive control. Lack of MSH2 and/or MSH6 expression is considered indicative of MSI-H and patients should undergo genetic counseling. The lack of MLH1 and PMS2 expression should prompt the determination of *BRAF* mutations and the methylation status of the promoter region of MLH1, mainly to determine the familial risk of disease.^{69,70} MSI determination is essential not only for estimating familial disease risk but also as the primary criterion for indicating immunotherapy in CRC.^{28,71} The clinical use of PD-L1 immunohistochemical expression in CRC remains unclear, but a recent meta-analysis suggests it as a marker of poor prognosis, although not widely used for therapy selection.⁷² Immunotherapy has been incorporated

Table 1. Summary of the number of clinical assays in gastrointestinal tract cancer

Location	Looking for participants	Active, not recruiting	Completed	Terminated
Esophagus	722	181	973	221
Stomach	959	218	1,227	214
Small bowel	111	37	233	48
Large bowel	1,494	402	2,962	520

Data summarized from ClinicalTrials.gov, last accessed 3/3/2024.

into the therapeutic armamentarium for CRC with dramatic results for MSI-H tumors, yet resistance to therapy remains a challenge for the future. *K-RAS* mutations are the most frequent molecular abnormalities in CRC, present in over one-third of cases. These mutations usually involve the codons 12 and 13 and can be easily diagnosed by polymerase chain reaction. To date, *RAS* mutations primarily guide therapy, predicting resistance to anti-EGFR drugs and also indicating a poor prognosis according to a recent meta-analysis.⁷³ It seems clear from the literature that *RAS* status remains fairly constant in both primary tumor and metastasis, making it a valuable biological marker for liquid biopsy during the follow-up of the patients.⁷⁴

B-RAF mutations are not as common, but they represent a group with prognostic and predictive implications.⁷⁵ The most frequent pattern of *B-RAF* alteration is V600E, which is associated with poor survival, with less than 1 year of survival after therapy failure. In contrast to other tumors, the response to *B-RAF* inhibitors (such as encorafenib) has not been encouraging so far, probably due to the reactivation of the pathway through EGFR signaling. Combined therapy, including anti-EGFR and conventional chemotherapy, can improve these results in the near future.⁷⁶

Avian erythroblastic leukemia viral oncogene homolog 2 (*ERBB2*; also known as *HER2*) amplification is present in nearly 3% of metastatic CRC, mainly in patients with *KRAS* and *BRAF* wild-type lesions. *ERBB2* status can be determined with immunohistochemistry as a screening tool and confirmed with polymerase chain reaction in doubtful cases.⁷⁷ It seems *ERBB2* amplification is predictive of resistance to anti-EGFR drugs and also indicative of the highly aggressive behavior of the tumor.⁷⁸ Most clinical trials suggest that the best therapeutic effect in advanced and metastatic *KRAS* wild-type CRC is gained from the combination of trastuzumab and either lapatinib, tucatinib, or pertuzumab, with approval of the combination of trastuzumab and tucatinib in the USA.⁷⁹

Neurotrophic tropomyosin receptor kinases (*NTRK*) targeted drugs (such as larotrectinib and entrectinib) have emerged as agnostic targeted therapies that can have an effect on any tumor showing rearrangement of this gene.⁸⁰ *NTRK* fusions are very uncommon in colorectal carcinoma, making it difficult to test these drugs in conventional clinical trials. However, clinical practice has confirmed the efficacy and safety of these drugs in CRC.⁸¹ Again, they might find indications in selected cases combined with conventional or targeted therapy in future therapeutic protocols that still need to be refined.

Several targeted drugs against different pathways are being explored, although they have not yet shown clinical efficacy. These include mesenchymal epithelial transition, Wnt, Hedgehog or tumor growth factor, to name a few.⁸²

Table 1 summarizes the total number of clinical trials in different phases for gastrointestinal tract tumors. Table 2 summarizes the results of just a few relevant clinical trials.^{13,19,20,83-88} Table 2 is not intended to be exhaustive due

to the large number of clinical trials but offers a perspective on the results of some targeted therapies in different tumor types.

Future perspectives

The development and implementation of next-generation sequencing and all the omics can significantly influence the understanding of the mechanisms underlying human malignant neoplasms,^{89,90} including gastrointestinal carcinoma. This information can be especially relevant for comprehending the mechanisms underlying resistance to conventional and targeted therapy, a very frequent and challenging situation in clinical practice. In this sense, there is a growing interest in cancer stem cells and the molecular pathways that favor drug efflux,⁹¹ as well as in the interactions between epithelial malignant cells and the microenvironment, which can promote metastatic potential and resistance to therapy.⁹² This knowledge can also lead to the discovery of new targeted drugs that can improve the prognosis of oncologic patients.

The field of artificial intelligence is growing at an incredible rate, and it will undoubtedly modify our clinical practice in the very near future. Algorithms and nomograms can help predict the risk of lymph node metastasis in early lesions arising in adenomas or predict response to therapy.^{93,94} There are also deep learning algorithms that can predict the molecular profile and make a molecular classification of the tumors from hematoxylin-stained slides.⁹⁵

Conclusions

In summary, pathologists will continue to play crucial roles in the field of targeted therapies and must adapt to the new tools that are beginning to reshape cancer management and diagnosis. There is no need to fear the future; undoubtedly, our role will remain vital in the era of artificial intelligence. Our goal is to cure cancer or at least transform it into a chronic controllable disease, therefore reducing the burden it still imposes on lives at this moment. Cancer is a dreadful enemy and all available weapons we can use are more than welcome in this ongoing battle, which we are confident will ultimately be won.

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Table 2. A few selected clinical trials in gastrointestinal tract tumors

Name (reference)	Phase	Type of tumor	Line of therapy	Comparator	Outcome
CheckMate 649 ¹³	3	Non-Her2 positive GC, EAC, GEJC	First	Nivolumab + Chemo vs Chemo	HR for OS for nivolumab: 0.71 (95% CI 0.59–0.86); p < 0.0001 HR for PFS for nivolumab: 0.68 (0.56–0.81); p < 0.00001 The cut-off point for CPS 5 or more
Attraction 283	3	GC, GEJC	Third (heavily pretreated)	Nivolumab vs Placebo	ORR 16.7%; median PFS 1.6 mo; median OS: 8.1 mo for nivolumab ORR: 0%; median PFS 1.6 mo; median OS: 6.5 mo for placebo
KeyNote 590 ⁸⁴	3	ESCC	First	Pembrolizumab + chemo Placebo + chemo	OS 13.9 vs. 8.8 mo (p < 0.0001) PFS 6.3 mo vs. 5.8 mo; p < 0.0001 The cut-off point for CPS 10 or more
Spotlight ¹⁹	3	Unresectable GC or GEJC	First	Anti-CLDN18 (Zolbetuximab) + mFOLFOX6 Placebo + mFOLFOX6	Median PFS 10.61 mo vs. 8.67 mo (p = 0.0006) Significant effect of anti-CLDN18 drugs
Fight ²⁰	2	Her2 negative GC or GEJC		Bemarituzumab + mFOLFOX6 Placebo + mFOLFOX6	Median PFS 9,5 mo vs. 7.4 mo (p = 0.07) No significant effect of FGFR inhibition
Jacob ⁸⁵	3	Her2 positive metastatic GC or GEJC		Pertuzumab + trastuzumab + chemo Vs. Placebo + trastuzumab + chemo	Median OS 17.5 mo vs. 14.2 mo. (p = 0.005) No significant influence of double inhibition of Her2
ROAR ⁸⁶	2	BRAFV600E mutated small bowel adenocarcinomas		Dabrafenib + trametinib	ORR 67% (3 small bowel adenocarcinomas) Basket trial
BEACON ⁸⁷	3	BRAFV600E mutated metastatic CRC	Second	Encorafenib + cetuximab	ORR 19.5% Median OS 9.3 After promising results, BREAKWATER clinical trial to compare this double therapy with and without chemo is ongoing
KRYSTAL 1 ⁸⁸	1/1b	Advanced KRASG12C mutated CRC		Adagrasib Adagrasib + cetuximab	OR 19% vs. 46% Median PFS 5.6 mo vs. 6.9 mo Better results for the combination

chemo, chemotherapy; CI, confidence interval; CLDN18, claudin 18; CPS, combined positive score; CRC, colorectal carcinoma; EAC, esophageal adenocarcinoma; ESCC, esophageal squamous cell carcinoma; GC, gastric adenocarcinoma; GEJC, gastroesophageal junction carcinoma; HR, hazard ratio; OR, odds ratio; ORR, objective response rate; OS, overall survival; PFS, progression free survival.

Author contributions

Design, review, and writing (MJFA, CDA), design, review, and critical reading (DH). All authors have made a significant contribution to this study and have approved the final manuscript.

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