

Position Paper

Adequacy criteria and reporting for prognostic and predictive profiling of biopsies from gastrointestinal neoplasia: A position paper from the Italian group of gastrointestinal pathologists, section of Italian society of anatomic pathology and cytology (GIPAD-SIAPeC-IAP)



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ARTICLE INFO

Article history:

Received 26 January 2026

Accepted 1 February 2026

Available online 16 February 2026

Keywords:

Gastrointestinal neoplasia

HER2

Microsatellite Instability (MSI)

Mismatch repair (MMR)

PD-L1

Claudin18.2

ABSTRACT

Precision oncology relies on precision diagnostics, and histopathological diagnosis, along with biomarker evaluation, currently represents the cornerstone for personalized treatment. In gastrointestinal neoplasms, diagnostic assessment and molecular profiling are often performed on biopsy tissue, which may be quantitatively/qualitatively limited. Therefore, appropriate sample management is essential to avoid unnecessary waste and to obtain all the information necessary for treatment planning. Several factors may significantly impact biomarker testing: (i) pre-analytical issues; (ii) heterogeneity in biomarker expression; (iii) lack of standardization in biomarker testing and evaluation. Moreover, in the metastatic setting, inadequate/incomplete clinical information can lead to inappropriate sample handling, with negative implications. The application of appropriate guidelines in testing and reporting biomarker status according to clinical context is, therefore, strongly encouraged. In this position paper, the Italian Group of Gastrointestinal Pathologists (GIPAD), a section of the Italian Society of Pathological Anatomy and Cytology (SIAPeC-IAP), aims to summarize all the clinical and pathological requirements for adequate assessment of prognostic and predictive biomarkers in the gastrointestinal oncology patient, from biopsy acquisition to diagnostic reporting.

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1. Introduction

The therapeutic revolution in gastrointestinal (GI) oncology, which has gathered momentum over the past decade, has led to the introduction of several new prognostic and predictive biomarkers into clinical practice [1,2]. Consequently, the pathologist has assumed a leading role in the identification of the optimal therapeutic strategy for each patient [3].

In most cases, predictive biomarker assessment should be performed at the time of diagnosis, to enable the adoption of new targeted neoadjuvant treatments where available (e.g., immunotherapy in mismatch repair-deficient [dMMR] colorectal cancer [CRC]) [4,5]. In the metastatic/advanced clinical setting, the only available tissue is often represented by biopsy/cytology samples, containing small amounts of neoplastic cells [6]. The pathologist's primary objective in this setting is to reach a diagnosis and, in case of tissue from metastases, to verify the clinical suspicion regarding the primary site of the tumor, with targeted ancillary tests if necessary. Accurate diagnostic interpretation may, in fact, require immunophenotyping of tumor cells to identify their histotype and origin, thus limiting the amount of tissue available for subsequent molecular profiling. According to Guidelines, each primary tumor requires the evaluation of different prognostic and predictive biomarkers, crucial for personalized therapy [7–12]. Furthermore, prognostic and predictive factors can be investigated with different, and sometimes complementary, techniques, such as immunohistochemistry (IHC), *in situ* hybridization (e.g., fluorescent [FISH] and chromogenic [CISH]), and molecular assays such as next-generation sequencing (NGS), depending on the primary tumor (for example, IHC in gastric cancer [GC] and NGS in biliary tract cancers).

For these reasons, it has become imperative to manage small tissue samples most appropriately, to provide a correct diagnosis and adequate molecular profiling, avoiding inappropriate and time-consuming testing, which could result in the need for re-biopsy in (often unfit) patients (Fig. 1).

This paper aims to summarize the state-of-the-art in prognostic and predictive biomarker testing in GI neoplasms, with particular focus on appropriate tissue handling. Moreover, for each prog-

nostic and predictive factor, we report evidence-based data from the Literature regarding the 'adequate' amount of tissue for reliable biomarker assessment. Finally, we discuss the concept of 'adequacy' for biopsies from metastatic sites, with particular reference to the implications for biopsy handling in the absence of adequate clinical, radiological, and laboratory information.

2. Pre-analytical, analytical, and post-analytical factors affecting biomarker evaluation

Several factors limit the diagnostic adequacy of routine tests for determining predictive biomarkers in clinical practice. We attempted to summarize the most important factors (this is by no means an exhaustive treatise), that pathologists should be aware of when evaluating biomarkers (Table 1). Examples from inadequate pre-analytical phase affecting biomarkers evaluation are showed in Fig. 2.

2.1. Cold ischemia time

Cold ischemia time is defined as the interval between tissue removal from the patient and its stabilization in formalin, and it is a crucial pre-analytical factor affecting protein preservation [13]. Fixation delays of two to three hours have been shown to reduce HER2 immunohistochemical staining and FISH signals [14]. In tonsillar tissue, cold ischemia time over three hours increases PD-L1 suboptimal staining and false-negative results, with marked deterioration after six hours [15]. Notably, non-refrigerated samples are more affected by prolonged cold ischemia time compared to refrigerated ones [16]. In addition to refrigeration, shipping and storing the sample under vacuum contributes to better preservation of both nucleic acids and proteins [17]. Since no universal cutoff applies to all tissues or analytical platforms, maintaining a cold ischemia time of one hour or less is widely recommended [13,18,19].

2.2. Fixation time

Under-fixation and over-fixation are known sources of issues in both IHC and sequencing methods [18]. In CRC specimens, under-fixation (<20 h) and over-fixation (>90 h) have been associated

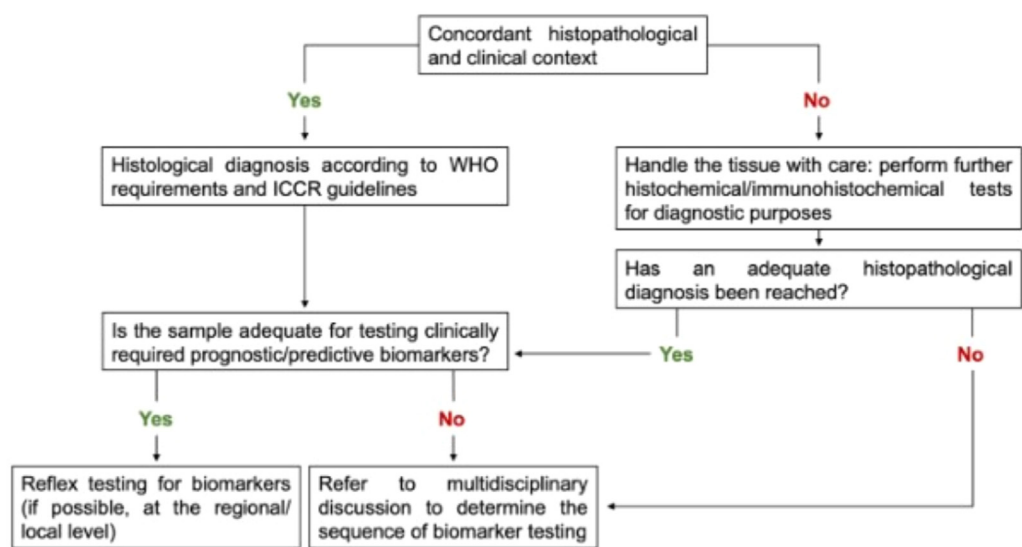


Fig. 1. Recommended algorithm for proper sample management and biomarkers testing.

Table 1
Pre-analytical, analytical, and post-analytical recommendations for the evaluation of prognostic/predictive biomarkers in clinical practice.

Pre-analytical phase	Analytical phase	Post-analytical phase and reporting
Limit cold ischemia time to ≤60 min.	For IHC, use ~4 µm thick sections (with a range from 3 to 5 µm) mounted on positively charged slides.	Evaluate only the infiltrative neoplastic component.
Avoid under- or over-fixation (10 % neutral buffered formalin for approximately 24 h, with a range from 6 to 48 h).	For fusion genes testing, prefer alteration-specific IHC, FISH, and RNA-based NGS.	For endoscopy biopsies, report the number of neoplastic fragments analyzed (especially for HER2/PD-L1/CLDN18.2 testing).
Avoid testing FFPE samples older than 3 years (especially for PD-L1 evaluation).	Consider at least 5–8 biopsy samples representative of the invasive tumor.	Report the neoplastic cellularity (especially for PD-L1 and MMR testing).
	Avoid performing IHC MMR testing on pre-treated tumors.	Report the sensitivity/specificity of the adopted method of analysis for mutational profiling.
	Reassess biomarker status in metastatic or post-treatment samples if clinically indicated.	Report the quality of the nucleic acid (e.g., DIN/RIN) used for molecular testing.

Abbreviations: CLDN18.2: Claudin-18.2; DIN: DNA Integrity number; FFPE: Formalin fixed/paraffin Embedded; FISH: Fluorescent *in situ* hybridization; IHC: Immunohistochemistry; µm: micrometer; MMR= Mismatch Repair; NGS: Next Generation sequencing; RIN: RNA Integrity Number.

with a higher number of inadequate results for MMR protein evaluation by IHC, particularly for MLH1 and PMS2 staining [20]. Nucleic acid degradation, fragmentation, and sequence alteration following excessive fixation have been reported [13,21]. Formalin cold fixation has been shown to preserve nucleic acids and proteins better than room temperature fixation [22,23], also demonstrating better results in the assessment of MMR proteins by IHC [20]. Types of DNA damage known to occur in formalin-fixed paraffin-embedded [FFPE] tissues include (i) formaldehyde-induced cross-links; (ii) DNA fragmentation; (iii) deamination of cytosine bases producing C-to-T mutations; and (iv) formation of abasic sites [13,24]. These DNA damages can significantly interfere with both polymerase chain reaction (PCR) and NGS [13,25,26].

For biomarker testing in clinical practice, we strongly recommend following the standard procedures proposed by the College of American Pathologists (CAP) Preanalytics for Precision Medicine Project Team guidelines [13]. Specifically, the CAP guidelines recommend a fixation time between 6 and 36 h, with 24 h being optimal for most tissues, and up to 48 h for those with a high fat content (e.g., colectomy specimens) (range of 12–24 h for biopsy and 24–48 h for surgical specimens) [13].

The recommended fixative is 10 % pH-neutral phosphate-buffered formalin [13]. Fixation efficiency depends on temperature and specimen thickness; at room temperature, formalin penetrates tissues at approximately 1 mm per hour [27]. To ensure uniform fixation, specimens should be fully immersed in formalin with a

ratio of fixative-volume to tissue-mass of at least 4:1, ideally 10:1 [13,28]. To preserve the integrity of mucosal and neoplastic tissue and to avoid the degradation of proteins and nucleic acids, organs/parts of organs with lumens (e.g., stomach and colon) should be opened as quickly as possible, and solid surgical specimens (e.g., pancreas and liver) should be cut to a thickness of no >10–15 mm before fixation. Finally, for adequate morphological and biomarker evaluation, sampling from surgical specimens should not exceed 4–5 mm in thickness [13,28].

2.3. Storage and use of archival FFPE blocks

The age of microtome sections and of FFPE tissue blocks may affect IHC and molecular analyses [29,30]. Older FFPE samples can show reduced staining intensity, especially for membrane (e.g., PD-L1) and nuclear proteins [29,31–33]. For this reason, the use of FFPE blocks older than 3 years and the use of sections older than 30 days should be discouraged [29,30].

2.4. Choice of antibody clones

Different antibody clones can be used to test the same biomarker, and can be required as companion diagnostic assays for the adoption of different targeted therapies [34]. An important example in the GI tract is represented by the several commercially

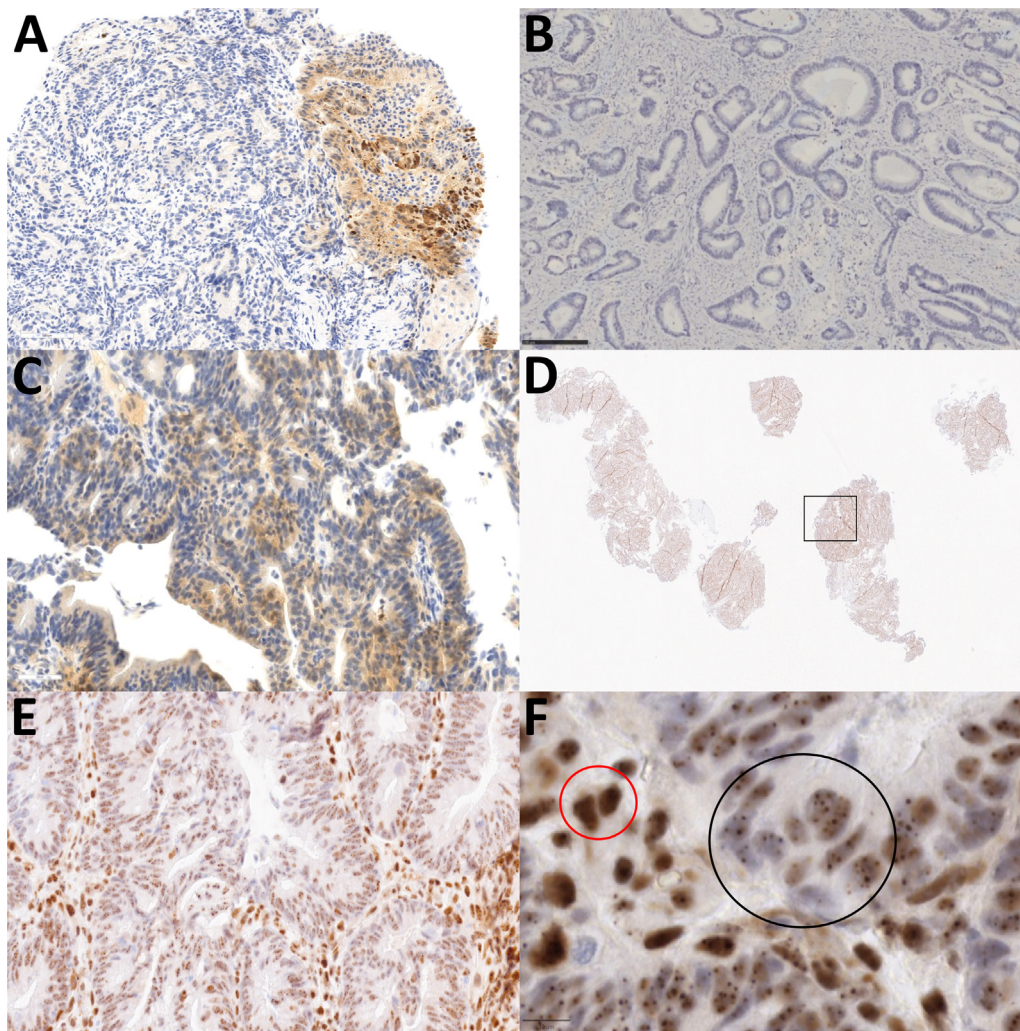


Fig. 2. Biomarkers' expression artifacts from pre-analytical phase. A: Marginal and cytoplasmic PD-L1 immunostaining, due to sampling crash; B: MLH1 inadequate immunostaining (artifactual loss in internal controls) due to over fixation-time; C: Cytoplasmic HER2 immunostaining; D-E-F: MMR punctuate immunostaining can mislead pathologists evaluation both at low- (D; black square identifying area showed in E) and medium-power (E) resulting positive/expressed, whereas only careful high-power evaluation (F) reveals "false-positive" punctate pattern of tumor cells (black circle) as opposed to true diffuse positive nuclear staining of inflammatory cells (red circle).

available IHC assays for the evaluation of PD-L1, of which those approved for use in clinical practice are the Dako 28–8, Dako 22C3, and Ventana SP263 clones [34]. The 28–8 and 22C3 clones bind to the extracellular domain of PD-L1 (while SP263 binds to an intracellular domain), and for this reason, they are more susceptible to deglycosylation and structural conformational changes [35]. Especially in the case of suboptimal decalcification or fixation, this can lead to a partial reduction or a complete loss of IHC staining [35] and may result in a discordant PD-L1 evaluation depending on the IHC assay adopted [36,37]. In the setting of Claudin18.2 (CLDN18.2), the Ventana CLDN18 (clone 43–14A) assay is now commercially available for clinical practice as a companion diagnostic assay [38].

2.5. Minimum number of biopsy samples and neoplastic cells

Endoscopists and radiologists must be aware of the minimum number of samples (e.g., from primary GC) or neoplastic cells (e.g., in liver biopsy) required for a reliable diagnosis and biomarker assessment. It is important to note that even if the tissue is satisfactory for histopathological diagnosis, it is not always sufficient or adequate for complete molecular characterization.

GI tumors, and in particular gastric/gastroesophageal junction cancers (GC/GEJCs), are burdened by a high level of histological

and molecular intra-tumor heterogeneity [33,39–43]. Furthermore, not all biopsy samples are composed of invasive neoplasia: some fragments may have dysplasia (not suitable for biomarker evaluation), while others are composed of ulcer or granulation tissue or even of normal mucosa [44]. For this reason, the analysis of multiple biopsy specimens is required for an adequate evaluation of biomarkers.

Specifically, the adequacy criteria for the assessment of certain biomarkers, such as HER2 and CLDN18.2, refer to the minimum number of biopsies representative of the invasive neoplasia. For the assessment of MMR and PD-L1, however, adequacy relates to the minimum number of neoplastic cells from the invasive component. At least 6–8 endoscopic biopsies representative of the invasive tumor should be obtained for the assessment of HER2 and CLDN18.2, and at least 100 neoplastic cells for the assessment of MMR and PD-L1, ideally from at least 5 different biopsy samples [41–43,45,46].

However, whether this is sufficient remains a matter of debate, given that the number of biomarkers required for appropriate management is increasing and is expected to grow further soon. Furthermore, criteria for the adequacy of HER2 and CLDN18.2 have not yet been explored and/or established in the metastatic setting (e.g., liver biopsies).

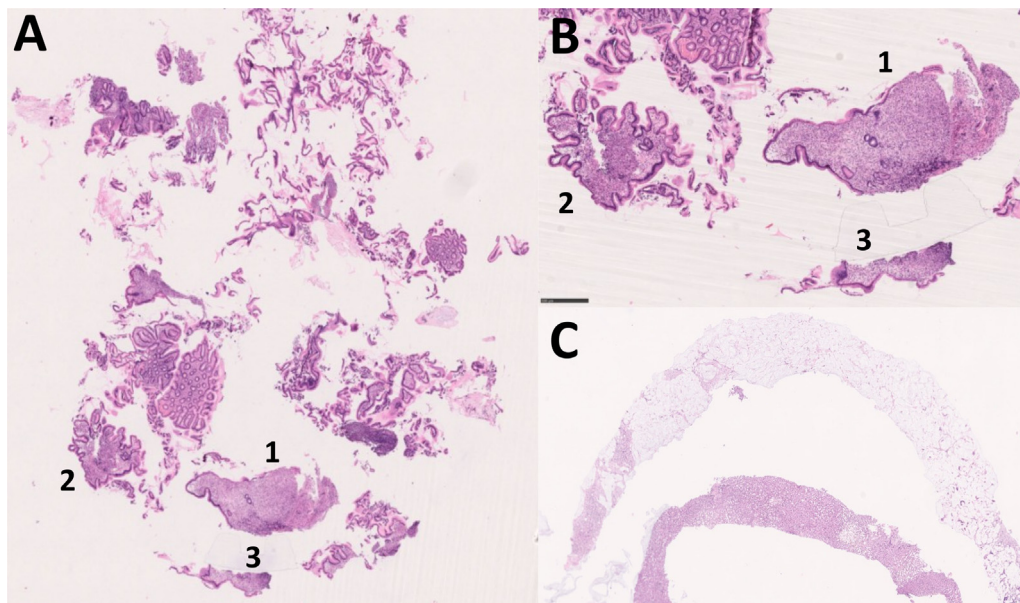


Fig. 3. Examples of inadequacy for biomarker assessment in primary and metastasis. A (5x), B (10x) Sampling from gastric cancer with only 3 fragments representative of infiltrating carcinoma; C: Hepatic localization of mucinous adenocarcinoma in absence of clinical information.

Awareness of adequacy criteria by endoscopists and radiologists can lead to improved sampling techniques, thus avoiding repeat esophagogastroduodenoscopy (EGD)/liver sampling to obtain additional tissue, resulting in reduced healthcare costs, patient discomfort, and time-to-treatment.

In any case, a statement on the adequacy of the tissue for biomarker assessment, describing the number of fragments available, should be included in the pathology report e.g., six or more fragments representative of the invasive component/100 or more neoplastic cells *versus* less than six fragments/<100 neoplastic cells [46] (Fig. 3).

With regard to re-biopsy, it is not the pathologist's role to recommend obtaining additional tumor samples in the pathology report; rather, discussion at a multidisciplinary meeting is advised in such cases, as all factors, including the patient's condition, should be taken into consideration.

2.6. Biomarkers heterogeneity: where to test, primary tumors and/or metastases?

Recent studies on intratumor heterogeneity and clonal evolution in metastases suggest reconsidering biomarker profiling strategies in advanced/metastatic disease.

Although the 2016 guidelines recommended testing a single site (primary or metastatic) to assess HER2 status in GC/GEJC [47], according to the more recent Literature, the discordance for HER2 status between primary tumors and distant metastases ranges from 0 % to 26 % [48–62]. Significant spatial heterogeneity between primary GC/GEJC and paired lymph nodes and distant metastases has also been reported for PD-L1 [63–66] and CLDN18.2 expression [43,63,67]. These findings may explain the failure of several targeted therapy trials in metastatic GC/GEJC [68,69] and suggest that testing only the primary tumor or the metastatic site may lead to suboptimal therapeutic selection.

On the other hand, high concordance of MMR profile and EBV status between primary GCs and paired peritoneal metastases has recently been reported [63].

In patients with advanced CRC, high concordance rates have been reported for *KRAS*, *NRAS*, *BRAF*, and *PIK3CA* status between primary tumors and distant metastases, ranging from 93 % to 100 %

[70,71]. Instead, little is known about the concordance of MMR/MSI status between primary tumors and paired metastases. However, one study reported a discordance rate ranging from 0 % to 16 % between primary mucinous colorectal adenocarcinomas and paired synchronous metastases, depending on the testing method adopted [72]. Whether this finding is due to true biomarker discordance or solely secondary to the different sensitivity of tests is up for debate.

2.7. Timing of biomarker assessment and biomarker prioritization

The timing of biomarker assessment in clinical practice may vary depending on the organization of the national healthcare system, the type of healthcare facility, and reimbursement scenarios. Biomarker status can be assessed at the time of diagnosis as a reflex-test, upon clinical request, or following a step-by-step evaluation. As most targeted therapeutic options have been approved as first-line treatments and several combination regimens are under investigation or have already been successfully tested in clinical trials, the reflex-testing option appears to be the most appropriate way to optimize tissue utilization and reduce the time-to-treatment. However, reflex-testing is not always feasible according to regional/national organization and, therefore, a strict collaboration with clinicians is fundamental.

In the advanced/metastatic setting, most of the available tissue samples are represented by endoscopic biopsies [6]. Consequently, the quantity of tumor tissue available is often insufficient to evaluate multiple biomarkers, and it is necessary to implement innovative tissue-sparing and reflex-testing strategies, as already introduced for the molecular profiling of non-small cell lung cancer (NSCLC) [73]. First and foremost, the pathologist must correctly diagnose invasive carcinoma, distinguishing the invasive components from pre-invasive areas, which should not be considered when evaluating biomarker. The pathologist must also order only the IHC stains strictly necessary for the diagnosis. A definitive diagnosis can often be reached on hematoxylin and eosin (H&E) stained slides, although sometimes histochemical stains (e.g., mucin stains such as Alcian Blue PAS) or IHC stains (e.g., pan-cytokeratins, tissue-specific transcription factors) may be necessary for the correct interpretation in undifferentiated histology. The

pathologist must be fully aware that, once the diagnosis is made, the residual tissue is usually necessary for biomarker testing and that this evaluation is as important as the diagnosis itself. In cases of very low neoplastic cellularity, the choice of the biomarker testing sequence should be discussed/debated with the oncologist in order to prioritize the assessment of biomarkers required at the beginning of the therapeutic algorithm.

2.8. Clinical requirements for sample evaluation and adequacy assessment

To ensure proper sample handling and evaluation, treating physicians must provide the pathologist with adequate clinical information. Essential information includes oncological history, imaging, laboratory data (including tumor markers), any previous surgical procedures and histopathological diagnoses, and the presence of any predisposing conditions favoring tumor-onset. All this information must be provided on the histology request form.

Adequate clinical information is especially critical in the metastatic setting, where knowing the location, histotype, and grading of the primary tumor is essential. In fact, metastases from different primary sites can exhibit highly similar histological features (e.g., mucinous adenocarcinomas and signet ring carcinomas; Fig. 3) and, depending on their origin, may require distinct biomarker profiling strategies (e.g., tissue fragmentation for NGS in lung cancer; tissue preservation for different IHC staining in GC/GEJC, CRC or breast cancer). Without adequate clinical information, attempting to identify the primary site can result in the waste of tissue without necessarily being able to reach a definitive diagnosis (as no IHC panel is sufficiently sensitive/specific for the identification of the primary tumor). Furthermore, performing NGS on a liver metastasis clinically defined as 'lung primary' will result in unnecessary waste of tissue if a 'true' gastric primary, is present, which needs IHC for treatment planning.

In addition, neoadjuvant therapy has been associated with treatment-induced alterations in prognostic and predictive biomarkers. An example in the GI tract is the decreased staining intensity and increased focal/inconclusive staining of MMR proteins in CRC following neoadjuvant treatment [74–76]. For this reason, the biomarker status as assessed on pre-treatment biopsy should be provided in patients undergoing surgery after neoadjuvant therapy. Obtaining previous histology reports from external institutions is, therefore, particularly relevant, as they provide "documented/reliable" sources on the type of surgery and on the location, histology, and IHC profiling (if performed) of the tumor.

If 'liver lesion, NOS (not otherwise specified)' is reported on a liver biopsy histology request form, with no additional clinical information, only a morphologic diagnosis should be performed (e.g., mucinous adenocarcinoma), and any IHC testing and biomarker profiling should be deferred until adequate clinical information has been obtained (Fig. 1).

2.9. Evaluating the infiltrative neoplastic component

A significant proportion of GC/GEJCs occur within a multistep carcinogenic cascade related to gastric *Helicobacter pylori* infection (Correa's cascade) [77] or long-standing gastroesophageal reflux disease (Barrett's carcinogenesis) [78]. Therefore, dysplastic lesions are often present in biopsy specimens obtained during endoscopic mapping of the invasive lesion. Due to their neoplastic nature, dysplastic lesions (both low-grade and high-grade) may be associated with driver molecular alterations that can result in difficult or incorrect biomarker evaluation.

For example, HER2 amplification has been extensively documented as an early event in esophageal and gastric carcinogene-

sis [42,79]. An analysis of HER2 status in pre-invasive and invasive gastric lesions reported a HER2 positivity in 12.9 % of high-grade dysplastic lesions compared to 20.2 % of invasive carcinomas, with three cases (33.3 %) of HER2 overexpression/amplification limited to the dysplastic lesion and not found in the invasive component [80]. Similarly, PD-L1 can also be expressed in preinvasive gastroesophageal lesions, and a significantly higher prevalence of PD-L1 positivity has been reported in esophageal Barrett's dysplasia compared to gastric dysplasia [81]. A discordant PD-L1 status between the dysplastic lesion and the paired invasive component has been reported in 40 % of cases, with 6.7 % of PD-L1-positive dysplastic lesions associated with a PD-L1-negative invasive component [81]. Finally, the dMMR profile in preinvasive gastroesophageal lesions has been found in 11.5 % of patients with gastroesophageal adenocarcinoma, and 16.7 % of these were associated with a synchronous MMR-proficient (pMMR) adenocarcinoma [82].

Therefore, dysplasia should not be considered in biomarker assessment, and samples consisting only of dysplastic lesions should be considered 'inadequate' [81–83].

The evaluation of the invasive component in endoscopic biopsy tissue is subject to significant inter-pathologist variability [82,84]. When possible, biomarker interpretation should be performed by dedicated gastrointestinal pathologists, and in complex cases, IHC/ISH evaluation with the corresponding H&E section should be performed jointly with a second pathologist. This is important to avoid incorrect biomarker assessment, which could have significant adverse consequences on the therapeutic decision-making process.

2.10. Adequacy for molecular profiling

The amount of neoplastic nucleic acids available for molecular profiling depends on several factors (e.g., tumor type, specimen size, neoplastic cellularity, and percentage of neoplastic cells), and it is well known that a low DNA yield can negatively affect genomic assays [3,75,85]. For this reason, samples from pre-treated tumors are often not ideal for molecular analyses, due to the presence of extensive necrotic or mucinous tissue and depletion of tumor cells [86]. In samples with a high non-neoplastic component, microdissection or macrodissection should be performed to obtain a minimum tumor fraction of approximately 20 %, trying to avoid areas of necrosis and mucus [86,87]. The neoplastic percentage in the selected area must be estimated to identify the most appropriate molecular assay (e.g., PCR, Sanger sequencing or NGS) and ensure that the sample meets the limit of detection for the chosen method [86]. Furthermore, a poor quality input material can significantly impact the analysis results [85]. For instance, a DNA Integrity Number (DIN) <4 has been associated with a poorer analytical performance when using the TapeStation 4200 platform to evaluate MSI status [88].

2.11. Cytology samples and molecular characterization

Whenever possible, cell blocks (CBs) should be obtained from cytology samples (serous cavity fluids and fine-needle aspiration [FNA] specimens). CBs are an optimal method for preserving cytology specimens and performing IHC, for which smears are not suitable [89]. According to the guidelines from the College of American Pathologists, CBs are an acceptable alternative to tissue specimens for HER2 IHC testing in gastroesophageal adenocarcinomas [47]. Although specific recommendations are still lacking, recent studies have also found a good level of concordance for MMR and CLDN18.2 evaluation between CBs and tissue specimens [90,91].

The strategic management of cytology specimens in advanced/metastatic tumors includes prioritizing CBs for the histotype definition and IHC testing, and cytology smears for molecular analyses [92,93].

3. Predictive biomarkers for gastrointestinal tumors

3.1. Esophageal carcinoma

According to the current ESMO guidelines, immunotherapy is recommended both in the first-line and in second- or later-line settings in advanced/metastatic esophageal squamous-cell carcinoma (SCC) [94,95]. IHC evaluation of PD-L1 with semi-quantitative immunoscores is required to assess the eligibility of patients to immune checkpoint inhibitors (ICIs) therapy [33,96].

The tumor proportion score (TPS) is the percentage of viable neoplastic cells with partial or complete PD-L1 membrane staining at any intensity [33,95], and a prolonged Overall Survival (OS) has been reported in patients with $TPS \geq 1$ treated with ICIs [97].

The combined positive score (CPS) is the number of tumor cells, lymphocytes, and macrophages with PD-L1 membrane staining divided by the number of viable tumor cells and multiplied by 100 [33,95]. The anti-PD-1 antibody pembrolizumab has been associated with a prolonged OS in patients with $CPS \geq 10$ [98,99].

The tumor area positivity (TAP) is the percentage of neoplastic cells and immune cells with PD-L1 membrane staining on the whole tumor area [100]. The tumor area is defined as the region occupied by all viable tumor cells and tumor-associated stroma; non-neoplastic tissue is included in the tumor area only if bordered by neoplastic tissue on both sides of a 10x field, while regions with necrosis, crush, and cautery artifacts are excluded [100]. Intra-luminal macrophages are included in the score only if they fill the luminal space and are in contact with tumor cells, while immune cells located within vessels are not considered [100]. The anti-PD-1 antibody tislelizumab has recently been approved by the European Medicines Agency (EMA) for esophageal SCCs with a TAP score of $\geq 5\%$ [94].

In advanced esophageal SCCs, all three scores (TPS, CPS, and TAP) should be performed on the same PD-L1-stained slide and included in the pathology report [46]. For an adequate PD-L1 evaluation, at least 100 viable tumor cells and at least 5 tissue biopsies should be assessed [33,46,101]. If fewer than 100 viable tumor cells are present, the evaluation should be performed in another block or at a deeper level of the same block [33].

Notably, a recent multicenter Italian study reported heterogeneity in PD-L1 assessment using CPS, TPS, and TAP in esophageal cancer and GC/GEJC between different Institutions [102]. This heterogeneity was significantly associated with the PD-L1 workload, the choice of antibody clone, and tissue aging, highlighting the need to address these issues to improve the consistency of PD-L1 evaluation in routine testing [102]. Esophageal and GEJ adenocarcinomas should be profiled as GC [46].

3.2. Gastric adenocarcinoma

Advanced or metastatic unresectable GC should be tested for HER2, PD-L1, MMR, and CLDN18.2 status; the same applies to esophageal and GEJ adenocarcinomas [10,46,103]. Additional biomarkers are currently under investigation and may be introduced into clinical practice in the near future [46,104].

In GCs, HER2 IHC staining is classified according to the Hofmann scoring system as follows: absent (0); faint/barely perceptible (1+); weak to moderate (2+); or moderate to strong (3+) [105,106]. Membranous staining must be present in at least 10 % of tumor cells in surgical specimens and in a cluster of at least 5 tumor cells in biopsy samples; otherwise, the score is 0 regardless of staining intensity [1]. Tumors with a score 3+ are regarded as HER2-positive, whereas cases with a score 2+ are equivocal and re-

quire further testing with *in situ* hybridization (e.g., FISH or CISH) [1]. A HER2/CEP17 signal ratio ≥ 2 is considered positive [1]. Pre-neoplastic lesions can also express HER2, although only the invasive component should be evaluated [1,79]. Given the spatial heterogeneity of HER2 expression, at least 5–6 biopsy samples are required to perform a reliable assessment [1,41].

In GC, PD-L1 expression has traditionally been evaluated using CPS [107]. Since this evaluation is time-consuming and often poorly reproducible [108], the TAP score has recently been introduced [100]. Studies have reported a good level of concordance between the CPS and TAP score [109,110], and both should be included in the pathology report. In GC, PD-L1 positivity is generally limited to the immune component and is only rarely observed in neoplastic cells [46]. To accurately assess PD-L1, at least 5 biopsy samples and 100 viable neoplastic cells should be examined [33,111].

The deficit of the MMR system (dMMR profile) and the resulting microsatellite instability (MSI) status are well-known predictors of response to immunotherapy [112]. MSI tumors represent approximately 22 % of all GCs [113], but as they are associated with a good prognosis, they represent <5 % of tumors in the metastatic setting [114]. dMMR status is assessed by IHC evaluation of the MMR proteins MLH1, PMS2, MSH2, and MSH6 [1]. The expression is classified as retained in case of moderate/strong nuclear staining in $\geq 10\%$ of tumor cells, lost if nuclear expression is absent, or indeterminate if the staining intensity of tumor cells is lower than the internal control or nuclear staining is found in <10 % of tumor cells [1]. In cases with indeterminate IHC results, is recommended to perform MSI testing by NGS or PCR [1]. In the absence of IHC positivity in the internal controls (i.e., non-neoplastic mucosa, lymphocytes, and/or fibroblasts), the sample is considered 'inadequate' for MMR proteins assessment, and MSI testing by molecular assays is recommended.

Since the loss of MLH1 and MSH2 leads to the degradation of PMS2 and MSH6, respectively, a cost-effective approach could be to initially test PMS2 and MSH6 only. If loss of one of these is observed, the expression of MLH1 and/or MSH2 should be evaluated. Although a meta-analysis reported the validity of this two-antibody approach [115], it is not recommended by ESMO guidelines, due to the potential impact of pre-analytical and analytical issues [75,112].

Although specific guidelines for GC are lacking, we suggest evaluating at least 5–6 biopsy samples for MMR status [46].

Targeted therapy directed against CLDN18.2 represents the most recent addition in the treatment landscape of GC [38]. The monoclonal antibody zolbetuximab is currently recommended in addition to chemotherapy as the first-line treatment in CLDN18.2-positive tumors, defined as moderate to strong IHC membrane staining (complete, basolateral, or lateral) in at least 75 % of tumor cells [38,103]. As cutoff values may change over time with the introduction of new agents, we recommend reporting the percentage of 2+/3+ tumor cells, rather than simply indicating whether the tumor is CLDN18.2-positive or -negative. Pre-neoplastic lesions should not be considered in the evaluation, and at least 6 biopsy samples and 50 tumor cells should be assessed [38].

FGFR2 is a member of the FGFR family, and its splice isoform FGFR2b is expressed in almost 40 % of GCs [116]. FGFR2b-positive tumors have been associated with diffuse histology and a worse prognosis [117–119]. The monoclonal antibody bemarituzumab, directed against the FGFR2b isoform, has shown promising results in the phase II FIGHT trial [120]. However, the phase III FORTITUDE-101 study did not confirm the efficacy of bemarituzumab in patients with FGFR2b 2+/3+ IHC staining in $\geq 10\%$ of tumor cells [121]; future studies are needed to better tailor anti-FGFR2b-targeting.

3.3. Small bowel adenocarcinoma

Small bowel adenocarcinoma (SBA) is a rare malignancy, associated with a worse prognosis than CRC [122,123]. Only a limited number of studies have explored the role of predictive biomarkers and targeted treatments in advanced/metastatic SBAs [46]. dMMR/MSI status has been described in approximately 11 % of metastatic SBAs, with a higher prevalence compared to CRCs [124–126]. Since these tumors may benefit from immunotherapy [124,127,128], dMMR/MSI status should be assessed [46].

HER2 positivity has recently been described in approximately 8 % of advanced SBAs [126], and there is evidence of the efficacy of HER2-targeted treatments in this context [129–131].

In addition, NGS can identify other actionable alterations, including *BRAF* V600E, *KRAS*, and *POLE/POLD1* mutations, *RET* or *NTRK* fusions, and high tumor mutational burden (TMB) [46,132].

3.4. Colorectal adenocarcinoma

In metastatic CRC, MMR and HER2 status should be evaluated by IHC, along with molecular characterization of *KRAS* (exons 2, 3, and 4), *NRAS* (exons 2, 3, and 4), and *BRAF* (exon 15) [9,46]. In selected cases, multigene tumor NGS may be indicated to detect *KRAS*, *BRAF*, and *POLE* mutations, *ERBB2* amplifications, *NTRK* and *RET* fusions, MSI, and high TMB [8]. If tissue biopsies are not available, circulating tumor DNA (ctDNA) assays represent a valid alternative for the molecular characterization of tumors [133].

The evaluation of MMR/MSI status should be performed in all CRC patients at the time of diagnosis in the context of the universal screening for Lynch syndrome (LS) [134,135]. LS is caused by germline mutations in the DNA mismatch repair genes (*i.e.*, *MLH1*, *MSH2*, *MSH6*, and *PMS2*) or in *EPCAM* (leading to epigenetic silencing of *MSH2*) [134], and it is associated with an increased risk of developing several malignancies, including colorectal, endometrial, ovarian, gastric, small bowel, and urinary tract cancers [136]. dMMR/MSI tumors represent approximately 15 % of all CRC, and 20 % of these arise in the context of LS [45]. As part of the universal screening for LS, patients with loss of *MSH2* and/or *MSH6* expression or isolated loss of *PMS2* expression should undergo genetic testing [134,135]. In patients with *MLH1* and *PMS2* expression lost, genetic testing is recommended only in cases without *BRAF* V600E mutation and/or *MLH1* promoter hypermethylation, which are commonly found in sporadic dMMR tumors [134,135].

Localized dMMR/MSI tumors are associated with a better prognosis but a worse response to fluoropyrimidine therapy compared to pMMR/Microsatellite stable (MSS) ones; therefore, MMR testing is useful to identify a subgroup of stage II CRC patients in which adjuvant treatment is not recommended [137]. In the metastatic setting, dMMR/MSI tumors represent 5 % of cases [45]. These tumors are associated with good responses to ICLs [138,139]. Recent studies have also demonstrated excellent responses to neoadjuvant immunotherapy in locally advanced CRC [4,140,141], highlighting the importance of assessing MMR status in biopsy specimens before surgery and neoadjuvant therapy [45].

MMR protein expression is evaluated by IHC, followed by MSI analysis (by PCR or NGS) in case of indeterminate/inadequate results [45,75]. An adequate assessment requires the analysis of at least 50 viable tumor cells and at least 5 biopsy specimens [45,46,75].

Mutations in exons 2, 3, and 4 of *KRAS* and *NRAS* (found in approximately 40 % and 4 % of CRCs, respectively) are negative predictive factors for response to anti-epidermal growth factor receptor (EGFR) targeted therapies [1,9]. Therefore, *RAS* analysis by PCR or NGS is mandatory before starting treatment with the anti-EGFR monoclonal antibodies cetuximab and panitumumab [9,46]. In addition, the *KRAS* G12C mutation, which is found in approximately

4 % of CRCs, has recently emerged as a potential drug target [142]. A recent phase III trial showed that the combination of the *KRAS* G12C inhibitor sotorasib plus panitumumab resulted in prolonged progression-free survival (PFS) compared to standard therapy [143]. This combination has been approved by the FDA as a second-line treatment for *KRAS* G12C-mutated metastatic CRC.

BRAF mutations are found in approximately 10 % of CRCs, with the most common being the *BRAF* V600E mutation in exon 15 (>90 % of cases) [144]. This mutation is a known negative prognostic factor in metastatic CRC, and some studies reported its association with a lower response to EGFR-targeted treatments [145–147]. In these patients, the BEACON study reported a prolonged survival with the combination of encorafenib (a *BRAF* V600E inhibitor) plus cetuximab compared to cetuximab plus chemotherapy as a second/third line of therapy [148,149], and a recent phase 3 trial also reported significantly longer OS and PFS in patients treated with encorafenib, cetuximab and chemotherapy compared to standard care in the first-line setting [150].

Although the *BRAF* V600E mutation can be detected by IHC in the context of universal screening for LS [151,152], the eligibility of patients with unresectable/metastatic CRC for targeted treatments should be assessed by PCR or NGS techniques, along with *NRAS* and *KRAS* testing [46,153].

HER2 overexpression occurs in approximately 2 % of CRCs [154], and HER2-targeted therapies are mainly adopted in the refractory setting [9,155,156].

HER2 expression can currently be assessed using two classification systems: the HERACLES criteria and those adopted in GC [154]. According to the HERACLES criteria, tumors with 3+ IHC staining intensity in at least 50 % of neoplastic cells are considered positive, while cases with 3+ staining intensity in <10 % of neoplastic cells, 2+ staining intensity in <50 % of neoplastic cells or 0/1+ staining intensity are considered negative [157,158]. Confirmation by ISH is required for cases with 2+ staining in ≥50 % of tumor cells or 3+ staining in 10 %–49 % of tumor cells [157,158]. The criteria adopted in GC require ISH confirmation only in tumors with 2+ staining in ≥10 % of neoplastic cells, while cases with 3+ staining in 10 %–49 % of cells are regarded as positive [105]. Unlike GC, there is no validated method for assessing HER2 in CRC biopsy specimens. Both classification systems can therefore be used (provided that the system used is specified in the diagnostic report). A recent study reported a good level of concordance between HER2 overexpression in 10 % to 49 % of neoplastic cells and ISH positivity, therefore, ISH could be unnecessary in this setting [159].

When available and cost-effective, multigene tumor NGS can be performed to detect *KRAS* G12C and *BRAF* V600E mutations, *ERBB2* amplifications, MSI status, and *POLE* mutations [8], the latter associated with high tumor mutational burden and good response to immunotherapy in pMMR tumors [160]. NGS can also be used to detect other tumor-agnostic biomarkers such as *NTRK1/2/3* and *RET* fusions, and tumors with high TMB [8].

4. Conclusion

The evolution of precision medicine has led to the development of personalized therapeutic strategies for a growing number of patients. This is particularly relevant in the advanced/metastatic stage, when patients are not eligible for surgery. In this setting, however, only small amounts of tissue are available for characterizing prognostic and predictive biomarkers, the number of which is constantly increasing. In GI oncology, pathologists often need to diagnose and evaluate biomarkers on small biopsy samples, which may be affected by pre-analytical and analytical issues. Moreover, in the metastatic setting, inadequate clinical information can result in waste of tissue in an attempt to define the primary tumor or, worst of all, in the investigation of the ‘wrong’ prognostic

and predictive biomarkers. For these reasons, a ‘critical’ approach to the diagnosis and the characterization of biomarkers is advised, according to Guidelines and evidence-based clinical practice, with particular reference to appropriate tissue handling.

Author contributions

Conceptualization: PP, FG, AV, LM, MF, Methodology: PP, FG, MF, Project administration: PP, FG, AV, LM, MF, Supervision: LM, MF, Validation: all authors, Writing - original draft: all authors, and Writing - review & editing: PP, FG, LM.

Declaration of competing interest

The authors have no conflicts of interest to disclose.

Acknowledgements

Paola Parente and Luca Mastracci are supported by grants from the Italian Health Ministry/Ricerca Corrente Program 2022–2024. The funding agencies had no role in the design and performance of the study.

References

- Fassan M, Scarpa A, Remo A, et al. Current prognostic and predictive biomarkers for gastrointestinal tumors in clinical practice. *Pathologica* 2020;112(3):248–59.
- Fassan M. Molecular diagnostics in pathology: time for a next-generation pathologist? *Arch Pathol Lab Med* 2018;142(3):313–20.
- Angerilli V, Galuppini F, Pagni F, et al. The role of the pathologist in the next-generation era of tumor molecular characterization. *Diagnostics (Basel)* 2021;11(2):339.
- Chalabi M, Verschoor Y L, Tan P B, et al. Neoadjuvant Immunotherapy in Locally Advanced Mismatch Repair-Deficient Colon Cancer. *N Engl J Med* 2024;390(21):1949–58.
- Hofheinz R-D, Fokas E, Benhaim L, et al. Localised rectal cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Ann Oncol* 2025;36(9):1007–24.
- Parente P, Grillo F, Vanoli A, et al. A charming molecular profile in the wrong tissue: the pathologist in the era of molecular diagnostics in the gastrointestinal tract. *Expert Rev Mol Diagn* 2023;23(12):1045–7.
- Chen L-T, Vogel A, Hsu C, et al. Pan-Asian adapted ESMO Clinical Practice Guidelines for the diagnosis, treatment and follow-up of patients with biliary tract cancer. *ESMO Open* 2024;9(8):103647.
- Mosele MF, Westphalen CB, Stenzinger A, et al. Recommendations for the use of next-generation sequencing (NGS) for patients with advanced cancer in 2024: a report from the ESMO Precision Medicine Working Group. *Ann Oncol* 2024;35(7):588–606.
- Cervantes A, Adam R, Roselló S, et al. Metastatic colorectal cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Ann Oncol* 2023;34(1):10–32.
- Shitara K, Fleitas T, Kawakami H, et al. Pan-Asian adapted ESMO Clinical Practice Guidelines for the diagnosis, treatment and follow-up of patients with gastric cancer. *ESMO Open* 2024;9(2):102226.
- Conroy T, Pfeiffer P, Vilgrain V, et al. Pancreatic cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Ann Oncol* 2023;34(11):987–1002.
- Vogel A, Bridgewater J, Edeline J, et al. Biliary tract cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Ann Oncol* 2023;34(2):127–40.
- Compton CC, Robb JA, Anderson MW, et al. Preanalytics and precision pathology: pathology practices to ensure molecular integrity of cancer patient biospecimens for precision medicine. *Arch Pathol Lab Med* 2019;143(11):1346–63.
- Agrawal L, Engel KB, Greytak SR, et al. Understanding preanalytical variables and their effects on clinical biomarkers of oncology and immunotherapy. *Semin Cancer Biol* 2018;52(Pt 2):26–38.
- Barberà A, González J, Martín M, et al. Impact of prolonged ischemia on the immunohistochemical expression of programmed death ligand 1 (PD-L1). *Appl Immunohistochem Mol Morphol* 2023;31(9):607–12.
- Yildiz-Aktas IZ, Dabbs DJ, Bhargava R. The effect of cold ischemic time on the immunohistochemical evaluation of estrogen receptor, progesterone receptor, and HER2 expression in invasive breast carcinoma. *Mod Pathol* 2012;25(8):1098–105.
- Annaratone L, Marchiò C, Sapino A. Tissues under-vacuum to overcome sub-optimal preservation. *N Biotechnol* 2019;52:104–9.
- Bass BP, Engel KB, Greytak SR, et al. A review of preanalytical factors affecting molecular, protein, and morphological analysis of formalin-fixed, paraffin-embedded (FFPE) tissue: how well do you know your FFPE specimen? *Arch Pathol Lab Med* 2014;138(11):1520–30.
- Hicks DG. The impact of pre-analytic variables on tissue quality from clinical samples collected in a routine clinical setting: implications for diagnostic evaluation, drug discovery, and translational research. In: Potts SJ, Eberhard DA, Wharton Jr Keith A, editors. *Molecular histopathology and tissue biomarkers in drug and diagnostic development*. New York, NY: Springer; 2015. p. 259–70.
- Grillo F, Ali M, Paudice M, et al. Impact of formalin fixation on mismatch repair protein evaluation by immunohistochemistry. *Virchows Arch* 2023;483(5):677–85.
- Marchiò C, Berrino E, Scarpino S, et al. Part I - pre-analytical phase. *Pathologica* 2025;117:S5–S17 (2(Suppl.1)).
- Nano E, Gambella A, Paudice M, et al. Be bold, start cold! cold formalin fixation of colorectal cancer specimens granted superior DNA and RNA quality for downstream molecular analysis. *Histochem Cell Biol* 2024;162(6):541–50.
- Bussolati G, Annaratone L, Medico E, et al. Formalin fixation at low temperature better preserves nucleic acid integrity. *PLoS One* 2011;6(6):e21043.
- Do H, Dobrovic A. Sequence artifacts in DNA from formalin-fixed tissues: causes and strategies for minimization. *Clin Chem* 2015;61(1):64–71.
- Douglas MP, Rogers SO. DNA damage caused by common cytological fixatives. *Mutat Res* 1998;401(1–2):77–88.
- Arreaza G, Qiu P, Pang L, et al. Pre-analytical considerations for successful next-generation sequencing (NGS): challenges and opportunities for formalin-fixed and paraffin-embedded tumor tissue (FFPE) samples. *Int J Mol Sci* 2016;17(9):1579.
- Hewitt SM, Lewis FA, Cao Y, et al. Tissue handling and specimen preparation in surgical pathology: issues concerning the recovery of nucleic acids from formalin-fixed, paraffin-embedded tissue. *Arch Pathol Lab Med* 2008;132(12):1929–35.
- Bussolati G, Annaratone L, Maletta F. The pre-analytical phase in surgical pathology. *Recent Results Cancer Res* 2015;199:1–13.
- Grillo F, Bruzzone M, Pigozzi S, et al. Immunohistochemistry on old archival paraffin blocks: is there an expiry date? *J Clin Pathol* 2017;70(11):988–93.
- Grillo F, Pigozzi S, Ceriolo P, et al. Factors affecting immunoreactivity in long-term storage of formalin-fixed paraffin-embedded tissue sections. *Histochem Cell Biol* 2015;144(1):93–9.
- He J, Wang X, Cai L, et al. Effect of storage time of paraffin sections on the expression of PD-L1 (SP142) in invasive breast cancer. *Diagn Pathol* 2023;18(1):131.
- Yanagihara K, Nagata K, Yamakawa T, et al. Decline of PD-L1 immunoreactivity with storage duration in Formalin-fixed paraffin-embedded breast cancer specimens: implications for diagnostic accuracy and immunotherapy eligibility in triple-negative breast cancer. *Cancers* 2025;17(19):3103.
- Angerilli V, Fassan M, Parente P, et al. A practical approach for PD-L1 evaluation in gastroesophageal cancer. *Pathologica* 2023;115(2):57–70.
- Lawson NL, Dix CI, Scorer PW, et al. Mapping the binding sites of antibodies utilized in programmed cell death ligand-1 predictive immunohistochemical assays for use with immuno-oncology therapies. *Mod Pathol* 2020;33(4):518–30.
- Lawson NL, Scorer PW, Williams GH, et al. Impact of decalcification, cold ischemia, and deglycosylation on performance of programmed cell death ligand-1 antibodies with different binding epitopes: comparison of 7 clones. *Mod Pathol* 2023;36(9):100220.
- Kim S-W, Jeong G, Ryu M-H, et al. Comparison of PD-L1 immunohistochemical assays in advanced gastric adenocarcinomas using endoscopic biopsy and paired resected specimens. *Pathology* 2021;53(5):586–94.
- Yeong J, Lum HYJ, Teo CB, et al. Choice of PD-L1 immunohistochemistry assay influences clinical eligibility for gastric cancer immunotherapy. *Gastric Cancer* 2022;25(4):741–50.
- Fassan M, Kuwata T, Matkowskyj KA, et al. Claudin-18.2 immunohistochemical evaluation in gastric and gastroesophageal junction adenocarcinomas to direct targeted therapy: a practical approach. *Mod Pathol* 2024;37(11):100589.
- Grillo F, Fassan M, Ceccaroli C, et al. The reliability of endoscopic biopsies in assessing HER2 status in gastric and gastroesophageal junction cancer: a study comparing biopsies with surgical samples. *Transl Oncol* 2013;6(1):10–16.
- Grillo F, Fassan M, Fiocca R, et al. Heterogeneous Her2/neu expression in gastric and gastroesophageal cancer. *Hum Pathol* 2016;48:173–4.
- Gullo I, Grillo F, Molinaro L, et al. Minimum biopsy set for HER2 evaluation in gastric and gastro-esophageal junction cancer. *Endosc Int Open* 2015;3(2):E165–70.
- Businello G, Angerilli V, Lonardi S, et al. Current molecular biomarkers evaluation in gastric/gastroesophageal junction adenocarcinoma: pathologist does matter. *Updates Surg* 2023;75(2):291–303.
- Pellino A, Brignola S, Riello E, et al. Association of CLDN18 protein expression with clinicopathological features and prognosis in advanced gastric and gastroesophageal junction adenocarcinomas. *JPM* 2021;11(11):1095.
- Grillo F, Gambella A, Bozzano S, et al. Number/quality of endoscopic biopsy samples in gastrointestinal cancers for biomarker testing: All that glitters is not gold. *Dig Liver Dis* 2026;58(2):265–72.

- [45] Grillo F, Paudice M, Gambella A, et al. Evaluating mismatch repair deficiency in colorectal cancer biopsy specimens. *Histochem Cell Biol* 2023;160(2):113–25.
- [46] Fassan M, Grillo F, Parente P, et al. The current success of precision oncology relies on precision diagnostics: how quality and quantity of endoscopic biopsies impact cancer patient outcome. *Dig Liver Dis* 2025;57(9):1826–9.
- [47] Bartley AN, Washington MK, Colasacco C, et al. HER2 Testing and Clinical decision making in gastroesophageal adenocarcinoma: guideline from the College of American Pathologists, American Society for Clinical Pathology, and the American Society of Clinical Oncology. *J Clin Oncol* 2017;35(4):446–64.
- [48] Marx AH, Tharun L, Muth J, et al. HER-2 amplification is highly homogenous in gastric cancer. *Hum Pathol* 2009;40(6):769–77.
- [49] Bozzetti C, Negri FV, Lagrasta CA, et al. Comparison of HER2 status in primary and paired metastatic sites of gastric carcinoma. *Br J Cancer* 2011;104(9):1372–6.
- [50] Kim MA, Lee H-J, Yang H-K, et al. Heterogeneous amplification of ERBB2 in primary lesions is responsible for the discordant ERBB2 status of primary and metastatic lesions in gastric carcinoma. *Histopathology* 2011;59(5):822–31.
- [51] Fassan M, Ludwig K, Pizzi M, et al. Human epithelial growth factor receptor 2 (HER2) status in primary and metastatic esophagogastric junction adenocarcinomas. *Hum Pathol* 2012;43(8):1206–12.
- [52] Tsapralis D, Panayiotides I, Peros G, et al. Human epidermal growth factor receptor-2 gene amplification in gastric cancer using tissue microarray technology. *World J Gastroenterol* 2012;18(2):150–5.
- [53] Fusco N, Rocco EG, Del Conte C, et al. HER2 in gastric cancer: a digital image analysis in pre-neoplastic, primary and metastatic lesions. *Mod Pathol* 2013;26(6):816–24.
- [54] Cho EY, Park K, Do I, et al. Heterogeneity of ERBB2 in gastric carcinomas: a study of tissue microarray and matched primary and metastatic carcinomas. *Mod Pathol* 2013;26(5):677–84.
- [55] Kochi M, Fujii M, Masuda S, et al. Differing deregulation of HER2 in primary gastric cancer and synchronous related metastatic lymph nodes. *Diagn Pathol* 2013;8:191.
- [56] Selcukbiricik F, Erdamar S, Buyukunal E, et al. Is her-2 status in the primary tumor correlated with matched lymph node metastases in patients with gastric cancer undergoing curative gastrectomy? *Asian Pac J Cancer Prev* 2014;15(24):10607–11.
- [57] Ieni A, Barresi V, Rigoli L, et al. HER2 Status in premalignant, early, and advanced neoplastic lesions of the stomach. *Dis Markers* 2015;2015:1–10.
- [58] Ieni A, Barresi V, Caltabiano R, et al. Discordance rate of HER2 status in primary gastric carcinomas and synchronous lymph node metastases: a multicenter retrospective analysis. *Int J Mol Sci* 2014;15(12):22331–41.
- [59] Gumusay O, Benekli M, Ekinci O, et al. Discordances in HER2 status between primary gastric cancer and corresponding metastatic sites. *Jpn J Clin Oncol* 2015;45(5):416–21.
- [60] Peng Z, Zou J, Zhang X, et al. HER2 discordance between paired primary gastric cancer and metastasis: a meta-analysis. *Chin J Cancer Res* 2015;27(2):163–71.
- [61] Creemers A, Ter Veer E, De Waal L, et al. Discordance in HER2 status in gastro-esophageal adenocarcinomas: a systematic review and meta-analysis. *Sci Rep* 2017;7(1):3135.
- [62] Amato M, Perrone G, Righi D, et al. HER2 Status in gastric cancer: comparison between primary and distant metastatic disease. *Pathol Oncol Res* 2017;23(1):55–61.
- [63] Angerilli V, Callegarin M, Govoni I, et al. Heterogeneity of predictive biomarker expression in gastric and esophago-gastric junction carcinoma with peritoneal dissemination. *Gastric Cancer* 2025;28(4):569–78.
- [64] Gao Y, Li S, Xu D, et al. Prognostic value of programmed death-1, programmed death-ligand 1, programmed death-ligand 2 expression, and CD8(+) T cell density in primary tumors and metastatic lymph nodes from patients with stage T1-4N+M0 gastric adenocarcinoma. *Chin J Cancer* 2017;36(1):61.
- [65] Svensson MC, Borg D, Zhang C, et al. Expression of PD-L1 and PD-1 in chemoradiotherapy-naïve esophageal and gastric adenocarcinoma: relationship with mismatch repair status and survival. *Front Oncol* 2019;9:136.
- [66] Liu DHW, Grabsch HJ, Gloor B, et al. Programmed death-ligand 1 (PD-L1) expression in primary gastric adenocarcinoma and matched metastases. *J Cancer Res Clin Oncol* 2023;149(14):13345–52.
- [67] Coati I, Lotz G, Fanelli GN, et al. Claudin-18 expression in oesophagogastric adenocarcinomas: a tissue microarray study of 523 molecularly profiled cases. *Br J Cancer* 2019;121(3):257–63.
- [68] Pectasides E, Stachler MD, Derks S, et al. Genomic heterogeneity as a barrier to precision medicine in gastroesophageal adenocarcinoma. *Cancer Discov* 2018;8(1):37–48.
- [69] Alsina M, Arrazubi V, Diez M, et al. Current developments in gastric cancer: from molecular profiling to treatment strategy. *Nat Rev Gastroenterol Hepatol* 2023;20(3):155–70.
- [70] Brannon AR, Vakiani E, Sylvester BE, et al. Comparative sequencing analysis reveals high genomic concordance between matched primary and metastatic colorectal cancer lesions. *Genome Biol* 2014;15(8):454.
- [71] Bhullar DS, Barriuso J, Mullamitha S, et al. Biomarker concordance between primary colorectal cancer and its metastases. *EBioMedicine* 2019;40:363–74.
- [72] Parente P, Malapelle U, Angerilli V, et al. MMR profile and microsatellite instability status in colorectal mucinous adenocarcinoma with synchronous metastasis: a new clue for the clinical practice. *J Clin Pathol* 2023;76(7):492–6.
- [73] Penault-Llorca F, Kerr KM, Garrido P, et al. Expert opinion on NSCLC small specimen biomarker testing - part 1: tissue collection and management. *Virchows Arch* 2022;481(3):335–50.
- [74] Vilkin A, Halpern M, Morgenstern S, et al. How reliable is immunohistochemical staining for DNA mismatch repair proteins performed after neoadjuvant chemoradiation? *Hum Pathol* 2014;45(10):2029–36.
- [75] Parente P, Grillo F, Vanoli A, et al. The day-to-day practice of MMR and MSI assessment in colorectal adenocarcinoma: what we know and what we still need to explore. *Dig Dis* 2023;41(5):746–56.
- [76] Bao F, Panarelli NC, Rennert H, et al. Neoadjuvant therapy induces loss of MSH6 expression in colorectal carcinoma. *Am J Surg Pathol* 2010;34(12):1798–804.
- [77] Gullo I, Grillo F, Mastracci L, et al. Precancerous lesions of the stomach, gastric cancer and hereditary gastric cancer syndromes. *Pathologica* 2020;112(3):166–85.
- [78] Grillo F, Mastracci L, Saragoni L, et al. Neoplastic and pre-neoplastic lesions of the oesophagus and gastro-oesophageal junction. *Pathologica* 2020;112(3):138–52.
- [79] Fassan M, Mastracci L, Grillo F, et al. Early HER2 dysregulation in gastric and oesophageal carcinogenesis. *Histopathology* 2012;61(5):769–76.
- [80] Lee S, de Boer WB, Fermoy S, et al. Human epidermal growth factor receptor 2 testing in gastric carcinoma: issues related to heterogeneity in biopsies and resections. *Histopathology* 2011;59(5):832–40.
- [81] Fassan M, Brignola S, Pennelli G, et al. PD-L1 expression in gastroesophageal dysplastic lesions. *Virchows Arch* 2020;477(1):151–6.
- [82] Angerilli V, Lonardi S, Farinati F, et al. Mismatch repair status and gastro-oesophageal dysplasia: need for a dedicated gastrointestinal pathologist? *Histopathology* 2022;80(7):1138–40.
- [83] Angerilli V, Pennelli G, Galuppi F, et al. Molecular subtyping of gastroesophageal dysplasia heterogeneity according to TCGA/ACRG classes. *Virchows Arch* 2022;481(4):545–52.
- [84] Fornaro L, Lonardi S, Catanese S, et al. Concordance of microsatellite instability and mismatch repair status in paired biopsies and surgical specimens of resectable gastroesophageal adenocarcinoma: time for a call to action. *Gastric Cancer* 2023;26(6):958–68.
- [85] Chen H, Luthra R, Goswami RS, et al. Analysis of pre-analytic factors affecting the success of clinical next-generation sequencing of solid organ malignancies. *Cancers (Basel)* 2015;7(3):1699–715.
- [86] Malapelle U, Angerilli V, Pepe F, et al. The ideal reporting of RAS testing in colorectal adenocarcinoma: a pathologists' perspective. *Pathologica* 2023;115(3):137–47.
- [87] de Bruin EC, van de Pas S, Lips EH, et al. Macrodissection versus microdissection of rectal carcinoma: minor influence of stroma cells to tumor cell gene expression profiles. *BMC Genom* 2005;6:142.
- [88] Malapelle U, Parente P, Pepe F, et al. Impact of pre-analytical factors on MSI test accuracy in mucinous colorectal adenocarcinoma: a multi-assay concordance study. *Cells* 2020;9(9):2019.
- [89] Torous VF, Cuda JM, Manucha V, et al. Cell blocks in cytology: review of preparation methods, advantages, and limitations. *J Am Soc Cytopathol* 2023;12(2):77–88.
- [90] Dai J, Zheng H, Jin J, et al. Claudin18.2 expression and clinicopathological features in cytology effusion specimens from gastric adenocarcinoma: a comparative study with tissue specimens. *Cancer Cytopathol* 2023;131(6):365–372.
- [91] Jacobi EM, Landon G, Broaddus RR, et al. Evaluating mismatch repair/microsatellite instability status using cytology effusion specimens to determine eligibility for immunotherapy. *Arch Pathol Lab Med* 2021;145(1):46–54.
- [92] Tian SK, Killian JK, Rekhtman N, et al. Optimizing workflows and processing of cytologic samples for comprehensive analysis by next-generation sequencing: memorial Sloan Kettering Cancer Center experience. *Arch Pathol Lab Med* 2016;140(11):1200–5.
- [93] Fielding D, Dalley AJ, Bashirzadeh F, et al. Diff-quick cytology smears from endobronchial ultrasound transbronchial needle aspiration lymph node specimens as a source of DNA for next-generation sequencing instead of cell blocks. *Respiration* 2019;97(6):525–39.
- [94] Obermannová RL, Leong T. ESMO Clinical Practice Guideline interim update on the treatment of locally advanced oesophageal and oesophagogastric junction adenocarcinoma and metastatic squamous-cell carcinoma. *ESMO Open* 2025;10(2):104134.
- [95] Obermannová R, Alsina M, Cervantes A, et al. Oesophageal cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Ann Oncol* 2022;33(10):992–1004.
- [96] Mastracci L, Grillo F, Parente P, et al. PD-L1 evaluation in the gastrointestinal tract: from biological rationale to its clinical application. *Pathol - J Ital Soc Anat Pathol Diagn Cytopathol* 2022;114:352–64.
- [97] Doki Y, Ajani JA, Kato K, et al. Nivolumab combination therapy in advanced esophageal squamous-cell carcinoma. *N Engl J Med* 2022;386(5):449–462.
- [98] Sun J-M, Shen L, Shah MA, et al. Pembrolizumab plus chemotherapy versus chemotherapy alone for first-line treatment of advanced oesophageal cancer (KEYNOTE-590): a randomised, placebo-controlled, phase 3 study. *Lancet* 2021;398(10302):759–71.
- [99] Kojima T, Shah MA, Muro K, et al. KEYNOTE-181 Investigators. Randomized Phase III KEYNOTE-181 Study of Pembrolizumab Versus Chemotherapy in Advanced Esophageal Cancer. *J Clin Oncol* 2020;38:4138–48.

- [100] Liu C, Fang F, Kong Y, et al. Tumor Area Positivity (TAP) score of programmed death-ligand 1 (PD-L1): a novel visual estimation method for combined tumor cell and immune cell scoring. *Diagn Pathol* 2023;18(1):48.
- [101] Yamashita K, Iwatsuki M, Harada K, et al. Can PD-L1 expression evaluated by biopsy sample accurately reflect its expression in the whole tumour in gastric cancer? *Br J Cancer* 2019;121(3):278–80.
- [102] Gambella A, Grillo F, Parente P, et al. Critical insights on real-life PD-L1 histopathological workflow and assessment in esophageal, esophagogastric junction, and gastric carcinoma in Italy. *ESMO Open* 2025;10(10):105846.
- [103] Lordick F, Carneiro F, Cascinu S, et al.; ESMO Guidelines Committee. Gastric cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Ann Oncol* 2022;33(10):1005–20. doi:10.1016/j.annonc.2022.07.004. <https://www.esmo.org/living-guidelines/esmo-gastric-cancer-living-guideline>.
- [104] Smyth EC, Kim KM, Rha SY, et al. FGFR2b protein overexpression: An emerging biomarker in gastric and gastroesophageal junction adenocarcinoma. *Cancer Treat Rev* 2025;139:102971.
- [105] Rüschoff J, Hanna W, Bilous M, et al. HER2 testing in gastric cancer: a practical approach. *Mod Pathol* 2012;25(5):637–50.
- [106] Hofmann M, Stoss O, Shi D, et al. Assessment of a HER2 scoring system for gastric cancer: results from a validation study. *Histopathology* 2008;52(7):797–805.
- [107] Kulangara K, Zhang N, Corigliano E, et al. Clinical utility of the combined positive score for programmed death ligand-1 expression and the approval of Pembrolizumab for treatment of gastric cancer. *Arch Pathol Lab Med* 2019;143(3):330–7.
- [108] Fernandez AI, Robbins CJ, Gaule P, et al. Multi-institutional study of pathologist reading of the programmed cell death ligand-1 combined positive score immunohistochemistry assay for gastric or gastroesophageal junction cancer. *Mod Pathol* 2023;36(5):100128.
- [109] Moehler M, Yoon HH, Wagner D-C, et al. Concordance between the PD-L1 tumor area positivity score and combined positive score for gastric or esophageal cancers treated with Tislelizumab. *Mod Pathol* 2025;38(9):100793.
- [110] Klempner SJ, Cowden ES, Cytryn SL, et al. PD-L1 immunohistochemistry in gastric cancer: comparison of combined positive score and tumor area positivity across 28-8, 22C3, and SP263 assays. *JCO Precis Oncol* 2024;8:e2400230.
- [111] Ye M, Huang D, Zhang Q, et al. Heterogeneous programmed death-ligand 1 expression in gastric cancer: comparison of tissue microarrays and whole sections. *Cancer Cell Int* 2020;20:186.
- [112] Luchini C, Bibeau F, Ligtenberg MJL, et al. ESMO recommendations on microsatellite instability testing for immunotherapy in cancer, and its relationship with PD-1/PD-L1 expression and tumour mutational burden: a systematic review-based approach. *Annals of Oncology* 2019;30(8):1232–43.
- [113] The Cancer Genome Atlas Research Network. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature* 2014;513(7517):202–9.
- [114] Pietrantonio F, Randon G, Di Bartolomeo M, et al. Predictive role of microsatellite instability for PD-1 blockade in patients with advanced gastric cancer: a meta-analysis of randomized clinical trials. *ESMO Open* 2021;6(1):100036.
- [115] Aiyer KTS, Doleman T, Ryan NA, et al. Validity of a two-antibody testing algorithm for mismatch repair deficiency testing in cancer; a systematic literature review and meta-analysis. *Mod Pathol* 2022;35(12):1775–83.
- [116] Rha SY, Zhang Y, Elme A, et al. Prevalence of FGFR2b protein overexpression in advanced gastric cancers during prescreening for the phase III FORTITUDE-101 trial. *JCO Precis Oncol* 2025;9:e2400710.
- [117] Kim HS, Kim JH, Jang HJ, et al. Pathological and prognostic impacts of FGFR2 overexpression in gastric cancer: a meta-analysis. *J Cancer* 2019;10(1):20–7.
- [118] Ahn S, Lee J, Hong M, et al. FGFR2 in gastric cancer: protein overexpression predicts gene amplification and high H-index predicts poor survival. *Mod Pathol* 2016;29(9):1095–103.
- [119] Hierro C, Alsina M, Sánchez M, et al. Targeting the fibroblast growth factor receptor 2 in gastric cancer: promise or pitfall? *Ann Oncol* 2017;28(6):1207–16.
- [120] Wainberg ZA, Kang Y-K, Lee K-W, et al. Bemarituzumab as first-line treatment for locally advanced or metastatic gastric/gastroesophageal junction adenocarcinoma: final analysis of the randomized phase 2 FIGHT trial. *Gastric Cancer* 2024;27(3):558–70.
- [121] Smyth EC, Chao J, Muro K, et al. Trial in progress: phase 3 study of bemarituzumab + mFOLFOX6 versus placebo + mFOLFOX6 in previously untreated advanced gastric or gastroesophageal junction (GEJ) cancer with FGFR2b overexpression (FORTITUDE-101). *J Clin Oncol* 2022;40:TPS4164.
- [122] Overman MJ, Hu C-Y, Kopetz S, et al. A population-based comparison of adenocarcinoma of the large and small intestine: insights into a rare disease. *Ann Surg Oncol* 2012;19(5):1439–45.
- [123] Pedersen KS, Raghav K, Overman MJ. Small bowel adenocarcinoma: etiology, presentation, and molecular alterations. *J Natl Compr Canc Netw* 2019;17(9):1135–41.
- [124] Pedersen KS, Foster NR, Overman MJ, et al. ZEBRA: a multicenter Phase II study of Pembrolizumab in patients with advanced small-bowel adenocarcinoma. *Clin Cancer Res* 2021;27(13):3641–8.
- [125] Schrock AB, Devoe CE, McWilliams R, et al. Genomic profiling of small-bowel adenocarcinoma. *JAMA Oncol* 2017;3(11):1546–53.
- [126] Vanoli A, Colella T, Parente P, et al. HER2, HER3, and mismatch repair protein expression in stage IV small bowel adenocarcinoma: results from a multicenter series. *Mod Pathol* 2025;38(11):100825.
- [127] Bhamidipati D, Colina A, Hwang H, et al. Metastatic small bowel adenocarcinoma: role of metastasectomy and systemic chemotherapy. *ESMO Open* 2021;6(3):100132.
- [128] Marabelle A, Le DT, Ascierto PA, et al. Efficacy of Pembrolizumab in Patients With Noncolorectal High Microsatellite Instability/Mismatch Repair-Deficient Cancer: Results From the Phase II KEYNOTE-158 Study. *J Clin Oncol* 2020;38(1):1–10.
- [129] Quaquarini E, Grillo F, Gervaso L, et al. Prognostic and predictive roles of HER2 status in non-breast and non-gastroesophageal carcinomas. *Cancers (Basel)* 2024;16(18):3145.
- [130] Meric-Bernstam F, Makker V, Oaknin A, et al. Efficacy and safety of Trastuzumab Deruxtecan in patients with HER2-expressing solid tumors: primary results from the DESTINY-PanTumor02 Phase II trial. *J Clin Oncol* 2024;42(1):47–58.
- [131] Wang J, Zhu X, Chen J, et al. Successful treatment with trastuzumab plus chemotherapy as the first-line regimen in advanced small bowel adenocarcinoma harboring HER2 amplification: a report of two cases. *Oncol Lett* 2024;27(2):64.
- [132] National Comprehensive Cancer Network (NCCN). NCCN Clinical Practice Guidelines in oncology (NCCN Guidelines®) for small bowel adenocarcinoma V.4.2025.
- [133] Pascual J, Attard G, Bidard F-C, et al. ESMO recommendations on the use of circulating tumour DNA assays for patients with cancer: a report from the ESMO Precision Medicine Working Group. *Ann Oncol* 2022;33(8):750–768.
- [134] Stjepanovic N, Moreira L, Carneiro F, et al. Hereditary gastrointestinal cancers: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2019;30(10):1558–71.
- [135] Tibiletti MG, Carneiro I, Calò V, et al. Universal testing for MSI/MMR status in colorectal and endometrial cancers to identify Lynch syndrome cases: state of the art in Italy and consensus recommendations from the Italian Association for the Study of Familial Gastrointestinal Tumors (A.I.F.E.G.). *Eur J Cancer Prev* 2022;31(1):44.
- [136] Yurgelun MB, Hampel H. Recent Advances in Lynch Syndrome: Diagnosis, Treatment, and Cancer Prevention. *Am Soc Clin Oncol Educ Book* 2018;23:101–9.
- [137] Argilés G, Tabernero J, Labianca R, et al. Localised colon cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2020;31(10):1291–305.
- [138] Andre T, Elez E, Van Cutsem E, et al. Nivolumab plus Ipilimumab in microsatellite-instability-high metastatic colorectal cancer. *N Engl J Med* 2024;391(21):2014–26.
- [139] André T, Shiu K-K, Kim TW, et al. Pembrolizumab in microsatellite-instability-high advanced colorectal cancer. *N Engl J Med* 2020;383(23):2207–18.
- [140] Ludford K, Ho WJ, Thomas JV, et al. Neoadjuvant pembrolizumab in localized microsatellite instability high/deficient mismatch repair solid tumors. *J Clin Oncol* 2023;41(12):2181–90.
- [141] Cercek A, Lumish M, Sinopoli J, et al. PD-1 blockade in mismatch repair-Deficient, locally advanced rectal cancer. *N Engl J Med* 2022;386(25):2363–76.
- [142] Schirripa M, Nappo F, Cremolini C, et al. KRAS G12C metastatic colorectal cancer: specific features of a new emerging target population. *Clin Colorectal Cancer* 2020;19(3):219–25.
- [143] Fakih MG, Salvatore L, Esaki T, et al. Sotorasib plus Panitumumab in refractory colorectal cancer with mutated KRAS G12C. *N Engl J Med* 2023;389(23):2125–39.
- [144] Clarke CN, Kopetz ES. BRAF mutant colorectal cancer as a distinct subset of colorectal cancer: clinical characteristics, clinical behavior, and response to targeted therapies. *J Gastrointest Oncol* 2015;6(6):660–7.
- [145] Loupakakis F, Ruzzo A, Cremolini C, et al. KRAS codon 61, 146 and BRAF mutations predict resistance to cetuximab plus irinotecan in KRAS codon 12 and 13 wild-type metastatic colorectal cancer. *Br J Cancer* 2009;101(4):715–721.
- [146] Angerilli V, Sabella G, Centonze G, et al. BRAF-mutated colorectal adenocarcinomas: pathological heterogeneity and clinical implications. *Crit Rev Oncol/Hematol* 2022;172:103647.
- [147] Pietrantonio F, Petrelli F, Coiro A, et al. Predictive role of BRAF mutations in patients with advanced colorectal cancer receiving cetuximab and panitumumab: a meta-analysis. *Eur J Cancer* 2015;51(5):587–94.
- [148] Kopetz S, Grothey A, Yaeger R, et al. Encorafenib, Binimetinib, and Cetuximab in BRAF V600E-mutated colorectal cancer. *N Engl J Med* 2019;381(17):1632–43.
- [149] Tabernero J, Grothey A, Van Cutsem E, et al. Encorafenib plus Cetuximab as a new standard of care for previously treated BRAF V600E-mutant metastatic colorectal cancer: updated survival results and subgroup analyses from the BEACON study. *J Clin Oncol* 2021;39(4):273–84.
- [150] Elez E, Yoshino T, Shen L, et al. Encorafenib, Cetuximab, and mFOLFOX6 in BRAF-mutated colorectal cancer. *N Engl J Med* 2025;392(24):2425–37.
- [151] Grillo F, Paudice M, Pigozzi S, et al. BRAFV600E immunohistochemistry can reliably substitute BRAF molecular testing in the Lynch syndrome screening algorithm in colorectal cancer. *Histopathology* 2024;84(5):877–87.
- [152] Galuppini F, Pennelli G, Loupakakis F, et al. BRAF p.V600E-specific immunohistochemical assessment in colorectal cancer endoscopy biopsies is consistent with the mutational profiling. *Histopathology* 2017;71(6):1008–11.
- [153] Malapelle U, Angerilli V, Intini R, et al. Detecting BRAF mutations in colorectal cancer in clinical practice: an Italian experts' position paper. *Crit Rev Oncol Hematol* 2025;206:104574.

- [154] Ahcene Djaballah S, Daniel F, Milani A, et al. HER2 in Colorectal Cancer: The Long and Winding Road From Negative Predictive Factor to Positive Actionable Target. *Am Soc Clin Oncol Educ Book* 2022;42:1–14.
- [155] Siena S, Di Bartolomeo M, Raghav K, et al. Trastuzumab deruxtecan (DS-8201) in patients with HER2-expressing metastatic colorectal cancer (DESTINY-CRC01): a multicentre, open-label, phase 2 trial. *Lancet Oncol* 2021;22(6):779–89.
- [156] Strickler JH, Cercek A, Siena S, et al. Tucatinib plus trastuzumab for chemotherapy-refractory, HER2-positive, RAS wild-type unresectable or metastatic colorectal cancer (MOUNTAINEER): a multicentre, open-label, phase 2 study. *Lancet Oncol* 2023;24(5):496–508.
- [157] Valtorta E, Martino C, Sartore-Bianchi A, et al. Assessment of a HER2 scoring system for colorectal cancer: results from a validation study. *Mod Pathol* 2015;28(11):1481–91.
- [158] Sartore-Bianchi A, Trusolino L, Martino C, et al. Dual-targeted therapy with trastuzumab and lapatinib in treatment-refractory, KRAS codon 12/13 wild-type, HER2-positive metastatic colorectal cancer (HERACLES): a proof-of-concept, multicentre, open-label, phase 2 trial. *Lancet Oncol* 2016;17(6):738–46.
- [159] Evans MG, Krause HB, Xiu J, et al. Evidence for unified assessment criteria of HER2 immunohistochemistry in colorectal carcinoma. *Mod Pathol* 2025;38(2):100654.
- [160] Rousseau B, Bieche I, Pasmant E, et al. PD-1 blockade in solid tumors with defects in Polymerase Epsilon. *Cancer Discov* 2022;12(6):1435–48.